# The Role of the Buffalo in Rural Development in Asia

SAREC/NARESA Buffalo Research and Development Programme
Peradeniya, Sri Lanka

Publication No. 3

# ROLE OF THE BUFFALO IN RURAL DEVELOPMENT IN ASIA

Proceedings of a Regional Symposium
Organized by the Buffalo Research Programme
of the Swedish Agency for Research Cooperation with
Developing Countries (SAREC) and the Natural Resources,
Energy and Science Authority of Sri Lanka (NARESA)
held 10-15 December 1995 in Peradeniya, Sri Lanka

Edited by

B.M.A.O. Perera
J.A. de S. Siriwardene
N.U. Horadagoda
M.N.M. Ibrahim

NARESA Press, Colombo, Sri Lanka

**Library of Congress Cataloging-in-Publication Data**

---

SAREC/NARESA Regional Symposium on the
Role of the Buffalo in Rural Development on Asia
1995, Peradeniya, Sri Lanka

Incudes bibiliographical references and index.
ISBN 955-590-007-8

1.Buffalo- Regional Symposium. 2.Rural Development - Asia
3. Buffalo - Rural Development I. Perera, B.M.A.O.
II. Siriwardene, J.A. de S. III. Horadagoda, N.U.
IV. Ibrahim, M.N.M.

---

Proceedings of the SAREC/NARESA Regional Symposium on the Role of the Buffalo in Rural Development in Asia, held 11-15 December 1995 Peradeniya, Sri Lanka


47/5 Maitland Place
Colombo 7
Sri Lanka

ISBN 955-590-007-8



Printed in Sri Lanka

## Preface

Despite the importance of the buffalo in the rural economy of Sri Lanka, serious scientific attention was directed towards improving the productivity of this animal only after 1980, as a sequel to a national workshop organized under the sponsorship of the Swedish Agency for Research Cooperation with Developing Countries (SAREC). This workshop identified gaps in knowledge which hampered better utilization of this animal's potential. Subsequently, SAREC provided funding through the Natural Resources, Energy and Science Authority (NARESA) for a five year research programme from 1983. During this first phase, 19 research projects were undertaken on fundamental aspects of nutrition, reproduction, diseases and socio-economics. The results were presented at a symposium held in Kandy in 1989. Subsequently, SAREC provided funds for a second phase comprising 34 projects covering a wide range of topics, including applied studies to develop new technologies to improve the productivity of buffaloes under rural farming conditions. This programme also contributed significantly towards the building up of infrastructure in the participating institutions and provided opportunities for postgraduate training of young researchers. Supplementary support was also provided to this programme by other donor agencies, chiefly the International Atomic Energy Agency (Vienna), the Australian Centre for International Agricultural Research, the Overseas Development Administration of the UK and the Government of the Netherlands.

In order to disseminate the knowledge gained and to transfer the technologies developed to the end-users (the village farmers), SAREC is now supporting a third phase of two years, during 1995 and 1996. The main objective of Phase 3 is to demonstrate the applicability and effectiveness of the new technologies and thereby popularize buffalo rearing through intensification of management practices. All activities of this phase are being conducted in close collaboration with the various institutions in the country dealing with research, education, training, extension and development relating to animal production and health.

This publication contains the research papers presented by Sri Lankan scientists at a Regional Symposium on the Role of the Buffalo in Rural Development in Asia held in December 1995. Also included are keynote lectures delivered by seven eminent foreign scientists from the Asian region and abstracts of some of the theses presented by postgraduate students who participated in the programme. The symposium provided an opportunity to review the work done under the second phase of the SAREC/NARESA buffalo research and development programme and to share experiences with leading scientists from Asian countries. It provided a forum for discussion of strategies for dissemination of technical knowledge to rural farmers. A summary of the discussions together with conclusions and recommendations are also included in these proceedings.

The project management team wishes to thank SAREC and NARESA for their excellent support, the scientists and collaborating institutes for their contribution to the programme, the innovative farmers who agreed to test the new technologies, and all those who helped to make this Symposium possible.

## CONTENTS

## Welcome Address by Dr. Oswin Perera (Project Representative)

Honourable Mr. Bernard Soysa, Minister of Science, Technology and Human Resources Development; His Excellency Mr. T. Akesson, Charge d' Affaires for Sweden; Professor Priyani Soysa, Director General of the Natural Resources, Energy and Science Authority of Sri Lanka (NARESA); Dr. Afzal Sher, representative of the Swedish Agency for Research Cooperation with Developing Countries (SAREC/Sida); distinguished keynote speakers, foreign and Sri Lankan guests, participants, ladies and gentlemen.

On behalf of the Project Staff of the SAREC/NARESA Buffalo Research and Development Programme in Sri Lanka, it is indeed a great privilege and pleasure to welcome you most cordially to the opening of this Regional Symposium on "*The Role of the Buffalo in Rural Development in Asia*".

The starting point of this programme was the holding of a National Workshop, in 1980, to review all aspects of buffalo research conducted up to that time, and to identify gaps in knowledge which impeded better utilization of this animal's potential by rural farmers. Based on the recommendations of the workshop, SAREC provided funding through NARESA for a five year research programme from 1983. This first phase focused on fundamental aspects of nutrition, reproduction, diseases and socio-economics. A large scale field survey was conducted to gather base-line information on the management and productivity of indigenous buffaloes, and a monograph, now recognized as the standard reference work, was published. Two buffalo research farms were also established.

The results of these studies were presented at a symposium held in Kandy in 1989, and lead to a second phase in which SAREC provided funds for a further five year programme. This second phase included more applied studies to develop technologies which can be used at the field level to improve productivity under rural farming conditions.

In order to disseminate the knowledge gained, and to transfer the technologies developed to the end-users (i.e. the village farmers), SAREC is now supporting a third phase of two years, during 1995 and 1996. The activities being undertaken are five-fold. The first is establishment of Field Projects in villages, to demonstrate the applicability and effectiveness of the new technologies. Selected farmers in three regions are provided assistance and technical advice to upgrade their holdings to serve as "Model-farms". These farms will in turn serve as demonstration sites and training locations for other farmers. Secondly, a Buffalo Information Centre is being established, to serve as a repository and resource base for future research and development. Thirdly, a series of Publications is under preparation, including a book for scientists and students, and a handbook for extension workers, to document and disseminate the scientific, technical and practical knowledge that has been gained. Fourthly, a Public Awareness programme is being launched, through linkages with on-going agricultural programmes on television and radio, to broadcast the potential of the buffalo and to popularize buffalo farming based on sound principles. Finally, a programme of Continuing Education is being implemented, to update the knowledge and skills of farmers, extension workers, field officers and administrators in appropriate technologies which can maximize the utilization of this valuable livestock resource in rural development.

All these activities are conducted in close collaboration with the main governmental and non-governmental institutions responsible for livestock development in Sri Lanka. These include the Department of Animal Production and Health (DAPH), the Veterinary Research Institute (VRI), the Provincial Directorates of Livestock in the project locations, the National Livestock Development Board (NLDB), the Livestock Development Division of the Mahaweli Authority of Sri Lanka (MASL), the Coconut Triangle Milk Producers' Cooperative Union (CTMU), and the Hector Kobbekaduwa Agrarian Research and Training Institute (HARTI). The project draws heavily on the expertise of research workers at the University of Peradeniya and is coordinated by a small Secretariat.

I also wish to gratefully acknowledge the supplementary support provided to the overall programme by other international and bilateral donor agencies. Chief among these are the International Atomic Energy Agency (IAEA) in Vienna, the Australian Centre for International Agricultural Research (ACIAR), the Overseas Development Administration (ODA) of the UK and the Government of the Netherlands.

The Regional Symposium which we are here to inaugurate today has several objectives. It will critically review the work done under the second phase of the SAREC/NARESA buffalo research and development programme, and share these experiences with eminent scientists in other Asian countries. It will also draw on their experiences in the application and dissemination of technical knowledge to rural farmers in their own countries, and discuss strategies for wider application of selected technologies. Finally, it will identify potential areas for further study and technology transfer. Clearly, an important challenge for all of us in the future is to develop innovative management packages, utilizing locally available resources, which will be sustainable under the more intensive land use systems that are evolving in most parts of the Asian continent.

Ladies and Gentlemen, Thank you for your attention.

## Address by Prof. Priyani E. Soysa (Director General, NARESA)

Honourable Minister, Charge d' Affaires for Sweden, Dr. Afzal Sher, Dr. Abeygunawardena, Dr. Oswin Perera, researchers, guests and visitors. This seminar marks the termination of phase II of the SAREC Buffalo Research Programme and experts from the Asian region have been invited to review the research results and share their experiences. Research on the water buffalo has been generously supported by SAREC since 1983. This aid which was channelled through NARESA, has amounted to nearly 75 million rupees over the past 12 years. SAREC has also provided assistance to the National Aquatic Resources Authority (NARA) and the Ruhunu University; the grand total being nearly a hundred million rupees. Sri Lanka is indeed grateful to Sweden for its interest in research in this country. The research programme had the support of a group of senior scientists from the University, the Department of Animal Production and Health and other agencies. Honourable Minister Bernard Soysa on assumption of duties questioned me on the progress of this research programme and also expressed his wish to share the results of research and the implications to the farmer with his colleague, the Honourable Mr. Thondaman, Minister of Livestock Development and Rural Industries. The latter in turn was excited about the need of buffaloes for draught and now, in the development of milk production. Sri Lanka imports millions of rupees worth of milk food and I myself am concerned about this vast expenditure. How could the buffaloes help in this situation? Work has been done to improve the potential of the local buffalo breed by better efforts in reproduction, nutrition, reducing calf mortality and also morbidity among the adults of this species.

The research has produced a better understanding of viral, parasitic and other disease conditions that can be prevented and treated. Two research farms in Peradeniya and Narangalla have been developed to support research. These activities must now be taken over by either the VRI or the Department of Animal Production and Health. Several young scientists have visited collaborating research institutes in Sweden thus helping Sri Lanka to build research capabilities and develop human resources. The Project supported research training and the output from this exchange training programme was 8 PhD's and 17 Master's qualified scientists. The funding for research has now ceased and final reports were expected by end of September. SAREC is now funding Phase III of the project which amplifies the usefulness of the research and the need for dissemination of the research results. A buffalo information centre has been established for this purpose and recently we engaged the services of a consultant from Thailand to advice on setting up of this centre. The duration of third phase is two years. NARESA hopes that the Department of Animal Production & Health will maintain this centre thereafter. The exercise of producing information in the form of books for scientists and the handbooks and leaflets for farmer is in progress. The proceedings of this workshop should be available in a few months, perhaps also incorporating the proceedings of a previous workshop held in 1988/89. The farmers have to be appraised of useful scientific progress, breaking some ineffective traditional practices. The urea molasses mineral brick has been made available in the project areas, its usefulness has been demonstrated along with other intensive farming strategies to increase lactation. We have to learn lessons from India where milk is cheap and consumption is popular. In Sri Lanka milk is expensive and is becoming more and more expensive.

We need to commercialize the production of the urea molasses nutrient brick. However, I understand that farmers outside the project area are also enthusiastically purchasing this brick. NARESA is now working with the National Engineering Research Division (NERD) hopefully to stimulate the commercial production of this brick.

At this seminar participants should discuss whether the research has significance for wider practical application and planned intervention for the greater good of our rural people. Perhaps the gaps in the local scientific knowledge and research will be filled by the exchange of ideas at this regional seminar. You have my best wishes for a lively exchange of views and I thank you for your patient hearing.

## Address by Dr. Afzal Sher
## (Representative, SAREC/Sida)

Honourable Minister, Bernard Soysa, Director General NARESA, Professor Priyani Soysa, Charge d' Affaires for Sweden, Dr. Perera, Dr. Abeygunawardena, distinguished scientists, ladies and gentlemen. I feel very honoured to be invited to address such a distinguished gathering at the opening ceremony of the regional Symposium on "the Role of Buffalo in Rural Development in Asia" a topic on which, I must confess, I am no expert. The Symposium programme reveals that some of the most eminent foreign scientists on the water buffalo will be sharing their experiences with the Sri Lankan counterparts.

In the history of mankind, civilization has risen and fallen, cultures of great sophistication have developed in all continents. Societies offering great promise at one time have failed to sustain progress and turned into decay leaving behind only fragments of past glories. We marvel at the intricacy and the precision of the asterisk calendar and the wisdom of confucion. They represent cultural achievements of a society once at the forefront but now long since left behind. Man has always been striving towards a better life but progress has been uneven to say the least. In many instances, changes for the better come to a halt or even regress. What are the driving forces behind progress and why some cultures are unable to sustain forces of progress remains to be a question. There are many theories. Some authors associate strong religious and ideological influences associated with stagnation and decay, while others would rather interpret the fall of mighty powers as a consequence of expressive exploitation of scarce resources, human or material as the case may be. I would like to argue that science in general and basic sciences in particular have been and must certainly continue be the decisive factor for achieving development. This is in no way an original idea but it has often been forgotten in the context of aid for developing countries. Before proceeding on to matters of this Symposium, let me say a few words regarding this SIDA-SAREC confusion. SAREC was an independent research financing agency of Sweden created in 1975 under the Ministry of Foreign Affairs. As of 1st

July 1995, the Swedish government decided to merge all the five Swedish aid agencies into one agency. SIDA that is familiar to you is written in capital letters, but the new agency is also called Sida, with only the first letter in capital. More importantly, it represents a completely different agency. The earlier SIDA was the Swedish International Development Authority, but the new agency created by merging all aid agencies is referred to as the Swedish International Development Cooperation Agency.

Most of you are aware that SAREC has supported the buffalo research programme since 1982. In 1994, when I participated in a discussion with the water buffalo research and advisory committee, it was mentioned that during the 10 years of research, some 90 research projects on different aspects of the water buffalo were approved. Apart from creating a conducive research environment and establishing the necessary infrastructure the project has trained a good number of researchers. There has been 8 PhD and 17 MSc programmes completed and some 100 scientific papers published. Thus it was suggested that the programme should now enter into a phase of dissemination of information. The water buffalo research committee thus proposed to SAREC that a final two year grant be awarded to the programme with the main aim of (a) finalising the ongoing research projects, and (b) translating the results and knowledge gained into practical recommendations and guidelines which shall be transferred to implementing agencies and farmers through workshops, publications and comprehensive programme reports. This Symposium is a link in the chain of activities to achieve the aim, I just mentioned. I hope the result of this programme can be implemented for the development and prosperity of the Sri Lankan people. I would also take this opportunity to congratulate the Water Buffalo Research Team, NARESA, Mr. Asoka de Silva, the former Deputy Director General of NARESA who was attached to this programme right from its inception, my predecessor Carl Guvtsson Thormstorm and all those people who have worked very hard to make this programme a success. As I am aware that you have a tough schedule ahead of you, I would not like to take more of your valuable time. I wish you all the best in your Symposium and may God bless you.

## Address by Mr. Bernard Soysa (Hon. Minister for Science, Technology and Human Resources Development)

Madam chairperson, professors, research workers distinguished visitors, ladies and gentlemen, as mentioned by the Director General of NARESA, this particular project with regard to the buffalo was one that was pointed out to me as of great importance when I assumed duties as minister one year ago. It is true that the importance of the buffalo in the rural economy of this country has not been appreciated over the years. Even farmers fail to appreciate the role of this animal in their own lives in sufficient measure. The replacement of the buffalo with the tractor was resisted by the farmer not due to any set of causes but due to the normal conservatism of the rural people. I remember when the late Mr. Philip Gunawardena was Minister of Agriculture, in his attempts to reorganise paddy cultivation in this country, he brought forward the paddy lands bill that required the participation of the cultivating peasants in managing rural committees. There was some difficulty in getting farmers to co-operate and accept this new structure. As you are aware, this was an attempt to cut across the semi-feudal relationships which existed with regard to land holdings and cultivation under the "Ande' System". It was difficult to persuade the cultivators. I remember a very enthusiastic friend of mine and I attempted to convert the rural people in Tissamaharama to accept this new structure and participate in the committees. We told them that they could pool their resources to buy a tractor, but the response was "why do we need a tractor, we have the buffalo". My friend argued for half an hour to try to prove to them the superiority of the tractor. The peasants were unconvinced and one of them said he would think about it and come back the next day with his answer. When we met him the next day his face was alight and he said he had the answer to all the arguments, which was that the tractor cannot be bred, and therefore the buffalo was superior. While rice farmers were prepared to defend the buffalo not much has been done to preserve the buffalo in the economy. The use of the buffalo is known to them but not the care of the buffalo in the same way. I was discussing this matter with a person from the AMUL Project in India which has been so very successful in increasing milk production. He admitted that the availability of traditional grazing land for the buffalo in India too is diminishing. Artificial feeding and other innovations had to be devised. He said that if that is the problem facing India, it is bound to be a problem in Sri Lanka too. If we are to face such a problem then what are the answers? A research output of the SAREC/NARESA Project is the use of the urea molasses mineral brick. We have been trying to persuade the Minister of Livestock Development to try to sponsor its commercial production through involvement of the private sector. There are many other aspects for which the biotechnologist must find solutions. The whole question of upgrading the local buffalo with imported buffalo breeds have been tried several times. I do not know how far the experiments have succeeded. I am told that in order to increase the productivity of the buffalo, attempts have been made to use artificial insemination and that so far it has not proved to be successful. Therefore the whole question of how to save the buffalo is still a baffling problem. We need to share our experiences in Asia because Malaysia, Vietnam, Thailand, Burma, India and Pakistan have the buffalo as a very important element in the economy of those countries, particularly to the agricultural economy and therefore if we pool the research done in all these countries, I am sure we can benefit by one another's experiences.

The problems may not be the same in each country but while variations exists, these can be understood and appropriate measures adopted. The important work that you have to do is to share the experiences during the course of this Symposium. I do not want to say much more because my knowledge on the buffalo is extremely limited. My acquaintance with the buffalo has not always been a very pleasant one. When I have attempted to cross fields and go up mountain sides, even up Piduruthalagala, I have had most hostile encounters with the buffalo. However, I do not bear a grudge against the animal on that account but the fact remains that I am not competent to speak about its welfare or what is required for the development of the buffalo here. I leave that to the scientists who have been working in the field. I wish your cooperative efforts in sharing knowledge will prove beneficial not only to Sri Lanka and the rural population of this country but also to neighbouring countries as well. I am happy that as a minister I was asked to come to the inauguration of deliberations today, to see what can be done at the Ministerial level to help you to get the necessary collaboration with other people and to see that where there is any lack of resources that might be rectified. On that score as far as the ministry is concerned, I am very happy to see that organisations such as NERD and NARESA which are under my ministry have stepped into the programme and are prepared and anxious to co-operate in getting this project going. I wish your deliberations every success and trust that at the end of the deliberations we will have a situation where everything has not gone up in hot air and discussion, but implementation may be possible through some collaborative mechanism which can bring the cultivating peasants into the programme, without whom all such discussions would be futile.

The dichotomy between research and implementation is a very dangerous one; we can have a lot of valuable research which remains barren because it is not implemented. Therefore the question of how to translate the valuable results of research into practical implementation mechanisms at the grass roots level is a problem. However, I think it is necessary for SAREC and NARESA to give their minds along with scientists to the problem of bridging this gap and allowing the peasant to come on to the information highway of today. This is all I have to say to you, I wish your deliberations all success and I want to thank NARESA and SAREC for inviting me to come here today and may your deliberations bring fruitful results for all countries concerned. Thank you very much.

## Vote of Thanks by Dr. H. Abeygunawardena

Distinguished guests, ladies and gentlemen, it is my pleasure to propose a vote of thanks on behalf of the Organizing Committee of this Regional Symposium. Mr. Bernard Soysa, the Honourable Minister for Science, Technology and Human Resources Development, we are most grateful to you Sir, for accepting our invitation to grace this occasion, amidst a very busy schedule. Your Excellency, Charge d' Affaires for Sweden, thank you very much for being with us at the opening of this Regional Symposium. Professor Priyani E. Soysa, the Director General of NARESA, we wish to thank you madam, for the constant encouragement and stimulation extended towards the staff of the buffalo research programme in organizing this symposium.

I wish to thank you all for accepting our invitation and being present with us this morning. Your presence is indeed a great source of encouragement and inspiration. Despite the importance of the water buffalo in rural farming communities, this species had not received indepth scientific investigations in Sri Lanka until SAREC sponsored a comprehensive research programme which commenced in the early 1980's. We wish to acknowledge the generous contribution of SAREC and it is a pleasure to have with us today, Dr. Afzal Sher and Dr. Ronnie Duel, the representatives of SAREC. We are also pleased to have in our midst Professor Ingemar Settergren from Sweden who has been associated with this programme as a reviewer and promoter from its inception.

On behalf of the organizing committee, I wish to express our gratitude to all the eminent scientists from the Region and the representatives of international agencies for accepting our invitation and being present here with us. We are certain that you will inspire our researchers by sharing your experiences and play an important role during the discussions and in the formulation of future research directions.

I wish to acknowledge the cooperation extended by the Department of Animal Production & Health, National Livestock

Development Board, the Mahaweli Authority of Sri Lanka, University of Peradeniya, Hector Kobbekaduwa Agrarian Research and Training Institute and the Coconut Triangle Milk Producers Union, to the buffalo research programme and in organizing this symposium. I also wish to express our gratitude to the commercial organizations who have contributed in various ways towards the symposium. A special word of thanks to the Director of the Plant Genetic Resources Centre (PGRC) and his staff for providing this excellent facility for the symposium.

The current phase of the Buffalo Research Programme, as mentioned earlier is primarily aimed at disseminating information and we are indebted to the participating scientists, farmers, extension workers, research and technical assistants for their cooperation. We are optimistic that our untiring efforts will succeed in delivering the new knowledge to the end-user, the rural farmer.

# BUFFALO PRODUCTION SYSTEMS IN SOUTH EAST ASIA AND POSSIBILITIES FOR TRANSFER OF APPROPRIATE TECHNOLOGIES TO IMPROVE PRODUCTIVITY: AN OVERVIEW

V. G. Momongan

*Institute of Animal Science, College of Agriculture*
*University of the Philippines at Los Baños*
*College Laguna 4031*
*THE PHILIPPINES*

**Abstract**: Swamp buffaloes which are predominant in Southeast (SE) Asia are raised by smallholder farmers primarily for draught. Buffalo production in SE Asia has always been an integral component of crop production. While 96.5% of the world buffalo population are in Asia, only 12% are in SE Asia. Decreasing trends in buffalo populations are occurring in Brunei, Malaysia, Philippines and Thailand. Increasing trends in the use of tractors to replace or combine with buffalo draught power are occurring throughout SE Asia. Thailand and Indonesia had 337% and 279% increase in the number of tractors, respectively, in 1993 compared to that in 1979-81. Inspite of the increasing use of the tractor for tillage, buffalo is predicted to remain with smallholder farmers in SE Asia because of distinct advantages appreciated by farmers.

The different buffalo production systems are briefly discussed. Buffalo beef production had increased substantially in 1994 compared to that during 1979-81, contributing 26% of the total beef production in Asia and SE Asia. However, the contribution of buffalo beef to total meat production was very small, indicating a tremendous potential for the buffalo meat to take a greater share of the market for meat products. The contribution of buffalo milk to total milk production was 40% in Asia and only 11% in SE Asia, supporting the observation that swamp buffaloes are not primarily used for milking. Appropriate technologies to improve the productivity of swamp buffaloes are also discussed.

**Keywords:** Buffalo, production systems, SE Asia, appropriate technologies, productivity.

## INTRODUCTION

The predominant water buffalo in Southeast Asia is the swamp type, of which 95 to 99% are raised by smallholder farmers as an integral part of their farming system. In Thailand, the smallholder farmer usually has 1 - 3 buffaloes per household (Konanta and Intaramongkol, 1994); in Lao PDR, 2 - 4 buffaloes (Bouahom, 1994); in the Philippines, 1 - 2 carabaos; and in Indonesia, it ranges from 2 (Central Java) to 14 (South Sulawesi), with a mean of 6 buffaloes per family (Toelihere, 1980). In Vietnam, 1 - 2 buffaloes are kept per family in the delta areas and 5 - 7 in the central highlands (Cuong and Hien, 1989). Farmers regard the buffalo as a form of security which can be sold when there is a dire need for cash.

Most of the swamp buffaloes are raised primarily for draught, and secondly, for meat and milk. In the Philippines, 99% are raised by smallholder farmers mainly for draught purposes. In Vietnam, 97% are kept by smallholder farmers (Nguyen *et al.*, 1994), and 65 to 68% of the total buffalo population are draught animals (Cuong and Hien, 1989). In Lao PDR, about 95% of the traction power for land preparation in rice cultivation comes from buffalo (Bouahom, 1994). Buffaloes are also used for transport in pulling carts, sledges, bamboos or logs; for riding or as pack animals; and in extracting juice from sugar cane, or in lifting water for irrigation.

## THE BUFFALO POPULATION IN SOUTHEAST ASIAN COUNTRIES

Of the 148.8 million buffaloes in the world, 96.5% or 143.6 million are in Asia. However, only about 12% or 17.9 million are in Southeast (SE) Asia (Table 1). The buffalo populations in Brunei, Malaysia, Philippines and Thailand have decreased compared to their respective populations in 1979-81. Those in the rest of the SE Asian countries showed an increase, with Cambodia and Lao PDR registering 120% and 57% increases respectively, over the last 15 years. Also, the least number of people sharing a buffalo is in Lao PDR, Cambodia and Thailand with about 4, 12 and 14 people/buffalo, respectively. Although the buffalo population in Indonesia had increased by 42.8% in 1994 and ranks second in SE Asia, yet the number of people sharing per buffalo (55.4/buffalo) is even greater than that in Brunei (28/buffalo). Malaysia had the greatest number of people per buffalo, at 106 persons/buffalo.

**Table 1.** World, Asian and Southeast Asian human and buffalo populations.

| Geographical locations | 1994 Popln. (x 1,000) | | No. of people per buffalo | % Change in buffalo popln. 1994 over '79-'81 |
|---|---|---|---|---|
| | Human | Buffalo | | |
| World | 5,630,240 | 148,798 | 37.8 | +22.30 |
| Asia | 3,333,188 | 143,640 | 23.2 | +21.60 |
| SE Asia | 474,978 | 17,871 | 26.6 | + 5.50 |
| Brunei | 280 | 10 | 28.0 | -23.10 |
| Cambodia | 9,868 | 829 | 12.0 | +120.50 |
| Indonesia | 194,615 | 3,512 | 55.4 | +42.80 |
| Lao PDR | 4,742 | 1,308 | 3.6 | +57.40 |
| Malaysia | 19,695 | 186 | 105.9 | -32.60 |
| Myanmar | 45,555 | 2,130 | 21.4 | +12.30 |
| Philippines | 66,188 | 2,630 | 25.2 | -7.40 |
| Singapore | 2,821 | ---- | ---- | --- |
| Thailand | 58,183 | 4,257 | 13.7 | -28.30 |
| Vietnam | 72,931 | 3,009 | 24.2 | +30.20 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

## BUFFALO PRODUCTION SYSTEMS

In general, buffalo raising in SE Asian countries is not considered a distinct enterprise, but has always been an integral part of the crop production system in smallholder farms. Buffaloes provide draught power and manure for maintaining soil fertility and utilize crop residues and farm by-products as feeds. Only in few areas can you find buffaloes being raised for milk or meat production as the primary concern. In most cases, buffalo meat is considered a by-product of the draught or milking animal. Milk is obtained mainly for consumption in the locality.

## Draught Buffalo Production

Production of draught buffalo is mostly associated with rice farming systems, especially in rain-fed rice paddies, although they are utilized also in other types of farming systems, such as sugar cane, coconut, oil palm and other upland crops or a combination thereof. In the Philippines, a carabao is used for 84 working days annually, although in rice-based farming systems carabaos work for 98 days yearly (Alviar, 1986). Thai buffaloes work 20 - 146 days yearly (Chantalakhana and Bunyavejchewin, 1989). In Vietnam, buffaloes work for 3 - 4 months yearly in mountainous areas and 5 - 6 months in the delta region (Cuong and Hien, 1989).

In recent years in SE Asia, there has been a slow increase in farm mechanization replacing the draught buffalo. There was an increase of 138% in the number of tractors in 1993 compared to that in the 1979-81 period, although, the increase in areas cultivated with rice was only about 12% over the same period of time (Table 2). Indonesia and Myanmar had the highest increase in land area under rice while Philippines, Lao PDR and Malaysia showed a decrease in land area under rice in 1993 compared to that in 1979-81.

Thailand had the highest increase in the number of tractors (336.7%) in 1993, followed by Indonesia (278.7%), compared to those in the 1979-81 period. Bunyavejchewin *et al.* (1994) reported that some of the farmer's primary reasons for purchasing tractors to replace or combine with buffalo draught power were: (1) speed in tillage, (2) suitability for ploughing clay soil, (3) shortage of family labour due to the migration of working age members of the family to industrial cities, and

**Table 2.** Changes in the hectarage planted with rice in relation to the changes in the number of tractors and buffalo population in the World, Asia and Southeast Asia.

| Geographical locations | Changes in area planted with rice in 1993 over 1979-81 | | Changes in the number of tractors and buffaloes in 1993 over 1979-81 | | | |
|---|---|---|---|---|---|---|
| | | | Tractor | | Buffalo | |
| | Area (1000ha) | % | No. | % | (1000 head) | % |
| World | +3,164 | +2.2 | +3,916,928 | +18.0 | +26,800 | +22.0 |
| Asia | +2,454 | +1.9 | +2,338,573 | +70.0 | +24,984 | +21.2 |
| SE Asia | +4,049 | +11.8 | +109,775 | +138.2 | +1,055 | +6.2 |
| Brunei | -1 | -50.0 | +3 | +4.3 | -3 | -23.1 |
| Cambodia | +670 | +58.0 | +15 | +1.1 | +428 | +113.8 |
| Indonesia | +1,950 | +21.5 | +25,757 | +278.7 | +991 | +40.3 |
| Lao PDR | -183 | -25.3 | +364 | +69.2 | +336 | +40.4 |
| Malaysia | -54 | -7.5 | +4,724 | +60.8 | -90 | -32.6 |
| Myanmar | +803 | +17.1 | +2,262 | +25.9 | +214 | +11.3 |
| Philippines | -231 | -6.6 | +932 | +8.8 | -280 | -9.9 |
| Singapore | --- | --- | +21 | +47.7 | --- | --- |
| Thailand | +174 | +1.9 | +61,525 | +336.7 | -1,187 | -20.0 |
| Vietnam | +921 | +16.5 | +14,172 | +62.1 | +646 | +28.0 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

(4) as a means for quick transportation. However, buffalo raising will still remain an integral part of the farming systems of the smallholder farmers because of some of the advantages the farmer derives in keeping buffaloes. A mature buffalo may void 5-7 MT of fresh manure per annum which could fertilize the soil. Raising buffaloes will not entail extra expense to the farmer but may even help to utilize excess family labour and farm waste products more efficiently. Moreover, even with the use of a hand tractor for tillage, the buffalo is still used to plough the rice paddy close to the dike. Management of buffaloes under rice-based farming systems is considered a very simple, less strenuous task and it is usually assigned to family members like children, women or old folks who cannot do hard work. The buffaloes are tethered and allowed to graze along roadsides, vacant lots or communal grazing lands and in paddy fields after the harvest of primary or secondary crops, to utilize rice straw, corn stover, groundnut hay, soybean hay and other fodder or grasses as feed.

In coconut, oil palm or sugar cane-based farming systems, buffaloes are used to transport coconuts, bunches of fresh fruit, oil palm nuts or sugar cane stalks to loading areas. Castrated bulls are usually preferred for such tasks because of their size, strength and docility. In Thailand, buffalo bulls are usually castrated at the age of 2.5 to 3.0 years (Chantalakhana and Bunyavejchewin, 1989); and in the Philippines, at a mean age of 3.3 years with a range of 1.8 - 4.8 years (De Guzman and Perez, 1981 cited by Momongan, 1992). When not used for work, buffaloes are tethered or allowed to graze under coconut or oil palm trees. Under sugar cane-based farming systems, buffaloes are tethered under trees or under shelter and fed with sugar cane tops or young sugar cane plants or other crop residues.

Mating occurs in communal grazing areas, usually after the crop harvest. As a result the farmer may not know which buffalo bull mated the cow. With the onset of the rainy season when the paddy fields are prepared and planted for the next cropping season, the buffaloes are tied near or under the house of the farmer and fed rice straw or freshly-cut grasses. Concentrate supplements are not normally given, except table or mineral salts. Occasionally, rice bran or other farm by-product concentrates may be given especially to the nursing or milking dam.

The health care for buffaloes is minimal. Farmers do not buy vaccine to vaccinate their buffaloes, especially against haemorrhagic septicemia and foot and mouth disease. They depend on the free vaccination program of the government which is not conducted regularly. Deworming of buffaloes is not normally performed by farmers, resulting in high calf mortality which may range from 10 to 40%. Weaning of calves is not usually done and it is not uncommon to see a one year old calf by the side of its dam.

## Buffalo Meat Production

Traditionally, when the buffalo is no longer fit for draught or for milk production, the animal is slaughtered for meat. The meat from such carcasses is tough and fibrous, and the dressing percentage varies from 40 - 50%. This is the reason why some consumers consider buffalo beef inferior to bovine beef. However, if the buffalo is slaughtered at a young age similar to that of cattle, the quality of buffalo meat is just as good as beef (Calub *et al.*, 1971; Joshi, 1988; Ibarra, 1988; Zava, 1991; Oliveira *et al.*, 1991).

In general, the total buffalo beef production increased substantially in 1994 compared to that during the 1979-81 period by 67.2% in Asia and 22.4% in SE Asia. However, in terms of buffalo beef per caput, the figure in SE Asia decreased (-7.5%), while that in Asia increased by 31.5% (Table 3). If we examine the trend in consumption in various SE Asian countries, buffalo beef per caput in 1994 increased in Cambodia, Lao PDR, Philippines and Vietnam; and decreased in Indonesia, Malaysia, Myanmar and Thailand as compared to their respective figures in 1979-81.

Table 4 shows the percentage of buffalo beef production to the total beef production in 1994.

**Table 3.** World, Asia and Southeast Asia buffalo beef production and human population in 1994 in relation to production and human population in 1979-81.

| Geographical locations | Human popln. in 1980 (x 1000) | Total buffalo beef prodn. in 79-81 (x 1000MT) | Buffalo beef availability per caput (kg) | Human popln. in 1994 (x 1000) | Total buffalo beef prodn. in 1994 (x 1000MT) | Buffalo beef per caput (kg) |
|---|---|---|---|---|---|---|
| World | 4,444,331 | 1,542 | 0.35 | 5,630,240 | 2,532 | 0.45 |
| Asia | 2,586,528 | 1,409 | 0.54 | 3,333,188 | 2,356 | 0.71 |
| SE Asia | 359,599 | 241 | 0.67 | 474,978 | 295 | 0.62 |
| Brunei | 193 | --- | --- | 280 | --- | --- |
| Cambodia | 6,498 | 6 | 0.92 | 9,968 | 13 | 1.30 |
| Indonesia | 150, 958 | 42 | 0.28 | 194,615 | 52 | 0.27 |
| Lao PDR | 3,205 | 5 | 1.56 | 4,742 | 8 | 1.69 |
| Malaysia | 13,763 | 7 | 0.51 | 19,695 | 4 | 0.20 |
| Myanmar | 33,821 | 18 | 0.53 | 45,555 | 22 | 0.48 |
| Philippines | 48,317 | 28 | 0.58 | 66,188 | 44 | 0.66 |
| Singapore | 2,415 | --- | --- | 2,821 | --- | --- |
| Thailand | 46,718 | 73 | 1.56 | 58,183 | 58 | 1.00 |
| Vietnam | 53,711 | 62 | 1.15 | 72,931 | 94 | 1.29 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

**Table 4.** World, Asia and Southeast Asia buffalo beef and bovine beef production in 1994.

| Geographical locations | Beef production | | | |
|---|---|---|---|---|
| | Buffalo | Cattle | Total beef | Percentage of buffalo beef |
| | -------------(x1000 MT)------------- | | | |
| World | 2,532 | 50,509 | 53,041 | 4.8 |
| Asia | 2,356 | 6,619 | 8,975 | 26.2 |
| SE Asia | 295 | 820 | 1,115 | 26.4 |
| Brunei | --- | 2 | 2 | 0.0 |
| Cambodia | 13 | 21 | 34 | 38.2 |
| Indonesia | 52 | 271 | 323 | 16.1 |
| Lao PDR | 8 | 6 | 14 | 57.1 |
| Malaysia | 4 | 14 | 18 | 22.2 |
| Myanmar | 22 | 90 | 112 | 19.6 |
| Philippines | 44 | 89 | 133 | 33.1 |
| Singapore | --- | --- | --- | --- |
| Thailand | 58 | 248 | 306 | 19.0 |
| Vietnam | 94 | 79 | 173 | 54.3 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

While buffalo beef accounted for only 4.8% of the total beef production in the world, in Asia and SE Asia, the figure was 26%. In Lao PDR and Vietnam, buffalo meat accounted for more than 50% of the total beef production in 1994 and about one-third of the beef production in the Philippines and Cambodia. However, the percentage of buffalo beef to the total meat production is very small (Table 5), indicating a tremendous potential for the buffalo meat to increase its share of the market for meat products. With increasing use of tractors in place of draught buffaloes, more animals may be available for buffalo meat production.

In SE Asian countries, there are isolated cases where buffaloes are raised primarily for meat. In Indonesia, this occurs mainly in the marshland of Kalimantan where they multiply naturally. The animals are caught only

**Table 5.** World, Asia and Southeast Asia buffalo beef production in relation to total meat production and human population in 1994.

| Geographical locations | Human popln. (x 1000) | Total meat prodn. (x 1000 MT) | Total buff. beef prodn. (x 1000 MT) | Total meat per caput (kg) | % buffalo beef to total meat |
|---|---|---|---|---|---|
| World | 5,630,240 | 191,564 | 2,532 | 34.02 | 1.32 |
| Asia | 3,333,188 | 67,390 | 2,356 | 20.22 | 3.51 |
| SE Asia | 474,978 | 7,213 | 295 | 15.19 | 4.08 |
| Brunei | 280 | 7 | --- | 25.00 | --- |
| Cambodia | 9,968 | 139 | 13 | 13.94 | 9.32 |
| Indonesia | 194,615 | 1,654 | 52 | 8.50 | 3.12 |
| Lao PDR | 4,742 | 69 | 8 | 14.55 | 11.62 |
| Malaysia | 19,695 | 894 | 4 | 45.39 | 0.44 |
| Myanmar | 45,555 | 312 | 22 | 6.85 | 7.01 |
| Philippines | 66,188 | 1,239 | 44 | 18.72 | 3.52 |
| Singapore | 2,821 | 151 | --- | 53.53 | --- |
| Thailand | 58,183 | 1,492 | 58 | 25.64 | 3.90 |
| Vietnam | 72,931 | 1,256 | 94 | 17.22 | 7.49 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

when they are ready to be marketed for meat. Also, in Tana Toraja, Central South Sulawesi, the piebald or black and white buffalo is kept only for meat production and slaughtered at traditional burial ceremonies (Satari *et al.*, 1994). In Brunei, the swamp buffalo is mainly used for meat and in limited cases, for work (Cho, 1981). The average liveweight of the mature Brunei buffalo is 401.2 ± 49.9 kg (range: 259-450 kg). Most of these buffaloes are to be found in the districts of Brunei/Muara (62%) and Tutong (30%).

### Buffalo Milk Production

More than 40% of the milk in Asia comes from water buffalo (Table 6). However, in SE Asia, buffalo contributes only about 11% to the total milk production, supporting the observation that swamp buffalo is not normally milked but primarily raised as a draught animal. Most of the milk obtained in SE Asia comes from river type buffaloes. In Malaysia, the entire river type buffalo population is confined to Perak and Selangor, the animals are mostly owned by Indian farmers and the average herd size being 5-25 animals (Jainudeen, 1989). In the Philippines, buffalo milk production is mainly from Murrah and river-swamp crossbreds which are confined in the provinces of Bulacan, Nueva Ecija, Rizal, Pampanga and Laguna, and contributed about 52% of the 29,000 MT of milk produced in 1994. Buffalo milk contributed more than 40% to the total milk production in Vietnam while Cambodia, Indonesia and Lao PDR do not produce substantial amounts of buffalo milk.

Studies showed that Murrah-swamp crossbreds produce significantly more milk than the swamp type buffalo (Yongzuo and Weiming, 1991; Momongan *et al.*, 1994; Ly, 1994; Shrestha and Parker, 1994).

## TECHNOLOGIES TO IMPROVE PRODUCTIVITY

It has been the common observation that the swamp type buffalo has low productivity, particularly in its reproductive efficiency. It is known to

**Table 6.** World, Asia and Southeast Asia buffalo and cattle milk productions in 1994 *vs* 1979-81.

| Geographical locations | Milk production | | | | | | Percent buffalo milk of total milk prodn. in 1994 |
|---|---|---|---|---|---|---|---|
| | Buffalo | | | Cattle | | | |
| | *1994* | *1979-81* | *Percent change* | *1994* | *1979-81* | *Percent change* | |
| | -----(x 1000MT)----- | | | -----(x 1000MT)----- | | | |
| World | 48,190 | 27,754 | +73.6 | 458,645 | 420,785 | +9.0 | 9.5 |
| Asia | 46,470 | 26,410 | +76.0 | 67,125 | 36,955 | +81.6 | 40.9 |
| SE Asia | 157 | 106 | +48.1 | 1,252 | 465 | +169.2 | 11.1 |
| Brunei | --- | --- | --- | --- | --- | --- | --- |
| Cambodia | --- | --- | --- | 19 | 14 | +35.7 | 0.0 |
| Indonesia | --- | --- | --- | 425 | 79 | +438.0 | 0.0 |
| Lao PDR | --- | --- | --- | 12 | 6 | +100.0 | 0.0 |
| Malaysia | 10 | 13 | -23.1 | 34 | 25 | +36.0 | 22.7 |
| Myanmar | 97 | 54 | +79.6 | 441 | 283 | +55.8 | 18.0 |
| Philippines | 15 | 17 | -11.8 | 14 | 13 | +7.7 | 51.7 |
| Singapore | --- | --- | --- | --- | --- | --- | --- |
| Thailand | 6 | 7 | -14.3 | 265 | 19 | +1,249.7 | 2.2 |
| Vietnam | 29 | 15 | +93.3 | 42 | 26 | +61.5 | 40.8 |

Adapted from: 1994 Production Yearbook (FAO, 1995)

be late maturing, with a low pregnancy rate and calf crop, long service period and calving interval, and low milk production. Many of these productivity traits are influenced by the management systems. Research has shown that proper selection of breeding animals, nutrition, and care and management can improve rate of growth, attainment of sexual maturity, conception rate, postpartum ovarian activity and milk production.

## Nutrition

Many of the buffaloes under the care of smallholder farmers are fed with low quality roughages and farm by-products (rice straw, corn stover, sugarcane tops, etc.) without supplementation except table and/or mineral salts in some instances. With this kind of feeding system, animals cannot be expected to perform well. However, many studies have shown that supplementation of feed with concentrates, and/or even urea-molasses-mineral block (UMMB) can improve the productivity of the buffalo (Leng, 1984; Leng, 1994; Neric *et al.*, 1984; Abelilla and Oliveros, 1995).

## Genetic Improvement

It has been demonstrated in Thailand that strict selection of breeding swamp buffalo bulls through performance testing greatly improved their reproductive performance at the Surin Livestock Breeding Station. Konanta and Intaramongkol (1994) reported that the age of first calving of Thai buffaloes was reduced from 5.26 $\pm$ 0.94 years in 1976-77 to 3.61 $\pm$ 0.26 years in 1991, and calving interval, from 587.6 $\pm$ 108.9 days in 1981 to 493.7 $\pm$ 100.1 days in 1991. Conception rate was increased from 67.5% in 1976-81 to 72.7% in 1984-89, and calving rate from 66.6% in 1976 to 69.2% in 1989.

Studies on crossbreeding of Murrah and swamp buffaloes showed significant improvement in body weight and milk production as well as the reproductive performance of the $F_1$ crossbreds over those of the swamp buffalo (Jainudeen, 1989; Yongzuo and Weiming, 1991; Sitamorang and Sitepu, 1991; Ly, 1994; Momongan *et al.*, 1994). The use of oestrus synchronization as a tool for the application of artificial insemination (AI) under village conditions has made possible the rapid production of Murrah-swamp crossbreds in the Philippines.

The development of biotechnology is a potential tool for the rapid genetic improvement in water buffalo. Superovulation of genetically superior heifers/dams and transferring the recovered embryos to synchronized recipients may hasten genetic improvement through efficient progeny testing and shorten the generation interval. *In vitro* oocyte maturation (IVM) and fertilization (IVF) could be utilized to maximize the genetic potential of a high-prized female buffalo. After the animal has passed its usefulness and has been slaughtered, its ovaries can be recovered and the oocytes collected for IVM, IVF and *in vitro* culture (IVC) until ready for transfer to recipients or can be cryopreserved for future transfers. Cloning of fertilized cells would further increase beyond imagination, the multiplication of genetic materials.

## Care and Management

The majority of smallholder farmers allow the calf to be with the dam until milk secretion has dried up, thus prolonging the recurrence of postpartum oestrus, resulting in extended service periods. Studies have shown that early weaning or restricted suckling induced the dam to return to early postpartum oestrus (Jainudeen and Sharifuddin, 1984; Wongsrikeao *et al.*, 1990; Nordin and Jainudeen, 1991). Data at the Philippine Carabao Research and Development Center (PCRDC, 1981-92) indicated that calf mortality under village conditions was about 25% in areas where regular deworming of calves was not practised and only 10% in areas where PCRDC administered regular deworming of calves every three months. El-Garhy *et al.* (1991) observed that calf mortality is an indicator of the level of management under which the herd is reared.

Very often, the low conception rate and poor calf crop could be traced to the lack of breeding bulls and/or AI services in the community. The situation is further aggravated by the common practice of castrating the big and strong bulls used for draught purposes. A responsive extension network which may include education of the farmers on appropriate buffalo management practices and extensive health care and bull dispersal/AI services which should be anchored to farmers' organized groups or cooperatives have been shown to be effective in advanced countries.

**Acknowledgments**

The author extends his profound gratitude to Dr. Annabelle S. Sarabia for the assistance in the preparation of this manuscript and to the Organizing Committee for the invitation given to him to participate in the 1995 SAREC/NARESA Regional Symposium.

**References**

Abelilla, N.C. and Oliveros, B.A. (1995) Performance of growing crossbred carabaos fed rice straw supplemented with concentrate and/or urea-molasses-mineral-block. *Proceedings of 32nd PSAS Annual Convention*, 26-27 October 1995, PICC, Metro Manila, Philippines. Vol. 1, pp 261-272.

Alviar, N.G. (1986) Socioeconomics of swamp buffalo raising in the Philippines. In: *International Seminar on Prospects and Problems of Asian Buffalo Development*, 10-11 April 1986, PCARRD, Los Baños, Laguna, Philippines.

Bouahom, B. (1994) Buffalo improvement in Lao PDR. In: *Long-term Genetic Improvement of the Buffalo.* Bunyavejchewin, P., Chantalakhana, C. and Sangdid, S., Eds. Buffalo and Beef Production Research and Development Center, Bangkok, Thailand. pp 85-88.

Bunyavejchewin, P., Sangdid, S. and Chantalakhana, C. (1994) Socio-economic conditions affecting the use of draught buffalo versus two-wheeled tractor in some villages of Surin province. In: *Long-term Genetic Improvement of the Buffalo.* Bunyavejchewin, P., Chantalakhana, C. and Sangdid, S., Eds. Buffalo and Beef Production Research and Development Center, Bangkok, Thailand. pp 28-41.

Calub, A.D., Castillo, L.S., Madamba, J.C. and Palo, L.P. (1971) The carcass quality of carabaos and cattle fattened in feedlot. *Phil. J. Anim. Sci.* **89**, 69-78.

Chantalakhana, C. and Bunyavejchewin, P. (1989) Buffalo production research and development in Thailand. In: *Seminar on Buffalo Genotypes for Small Farms in Asia.* Vidyadaran, M.K., Azmi, T.I. & Basrur, P.K., Eds. Center for Tropical Animal Production and Disease Studies: Serdang, Malaysia. pp 117-125.

Cho, T.L.K. (1981) Buffalo production and development in Brunei. In: *Recent Advances in Buffalo Research and Development.* FFTC Book Series No. 22. Food and Fertilizer Technology Center. Taipei, Taiwan. pp 46-56.

Cuong, L.X. and Hien, N.X. (1989) Buffalo production in Vietnam. In: *Seminar on Buffalo Genotypes for Small Farms in Asia.* Vidyadaran, M.K., Azmi, T.I. and Basrur, P.K., Eds. Center for Tropical Animal Production and Disease Studies, Serdang, Malaysia. pp 153-158.

De Guzman, Jr., M.R. and Perez, Jr., C.B. (1981) Feeding and management practices, physical characteristics and uses of carabaos (swamp bufffaloes) in the Philippines. In: *Recent Advances in Buffalo Research and Development.* FFTC Book Series No. 22. Food and Fertilizer Technology Center. Taipei, Taiwan. pp 104-115.

El-Garhy, M.M., Metias, K.M., El-Rashidy, A.A., Kholeaf, Z.M. and Tawfik, M.S. (1991) A study on losses among newly born buffalo calves in a large dairy herd in Egypt. *Third World Buffalo Congress*, 13-18 May 1991, Varna, Bulgaria. Vol. IV, pp 1049-1054.

Ibarra, P.I. (1988) Buffalo meat: Quantitative and qualitative aspects. *Second World Buffalo Congress*, 12-16 December 1988, New Delhi, India. Vol. II, Part II, pp 514-523.

Jainudeen, M.R. (1989) Buffalo farming in Malaysia: A country report. In: *Seminar on Buffalo Genotypes for Small Farms in Asia.* Vidyadaran, M.K., Azmi, T.I. and Basrur, P.K., Eds. Center for Tropical Animal Production and Disease Studies, Serdang, Malaysia. pp 145-151.

Jainudeen, M.R. and Sharifuddin, W. (1984) Postpartum anoestrus in the suckled swamp buffalo. In: *The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vienna. pp 29-41.

Joshi, D.C. (1988) Meat production in buffaloes. *Second World Buffalo Congress*, 12-16 December 1988, New Delhi, India. Vol. II, Part II, pp 491-498.

Konanta, S. and Intaramongkol, J. (1994) Buffalo selection schemes in Thailand. In: *Long-term Genetic Improvement of the Buffalo.* Bunyavejchewin, P., Chantalakhana, C. and Sangdid, S., Eds. Buffalo and Beef Production Research and Development Center, Bangkok, Thailand. pp 71-84.

Leng, R. (1984) The potential of solidified molasses-based blocks for the correction of multinutritional deficiencies in buffaloes and other ruminants fed low-quality agro-industrial byproducts. In: *The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vienna. pp 135-150.

Leng, R. (1994) Nutritional strategies applicable in small farm enterprises to increase buffalo production. *Proceedings of the First Asian Buffalo Association Congress*, 17-21 January 1994, Khon Kaen, Thailand. pp 29-38.

Ly, L.V. (1994) Buffalo research and production development in Vietnam. *Proceedings of the First Asian Buffalo Association Congress*, 17-21 January 1994, Khon Kaen, Thailand. pp 90-97.

Momongan, V.G. (1992) Carabao draught power. In: *Carabao Production in the Philippines.* Ranjhan, S.K. and Faylon, P.S., Eds. PHI/86/005 Field Document No. 13. Book Series No. 126/1992. Los Baños, Laguna. pp 165-182.

Momongan, V.G., Parker, B.A. and Sarabia, A.S. (1994) Crossbreeding of buffaloes in the Philippines. In: *Long-term Genetic Improvement of the Buffalo.* Bunyavejchewin, P., Chantalakhana, C. and Sangdid, S., Eds. Buffalo and Beef Production Research and Development Center, Bangkok, Thailand. pp 57-67.

Neric, S.P., Aquino, D.L., Dela Cruz, P.C. and Ranjhan, S.K. (1984) Effect of urea-molasses-mineral block lick on the growth performance of caracows kept on themeda pasture of central Luzon during the wet season. In: *The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vienna. pp 127-133.

Nguyen, B.X., Ty, L.V., Duc., N.H. and Uoc, N.T. (1994) Biotechnology for buffalo genetic improvement in Vietnam. In: *Long-term Genetic Improvement of the Buffalo.* Bunyavejchewin, P., Chantalakhana, C. and Sangdid, S., Eds. Buffalo and Beef Production Research and Development Center, Bangkok, Thailand. pp 89-95.

Nordin, Y. and Jainudeen, M.R. (1991) Effect of suckling frequencies on postpartum reproductive performance of swamp buffaloes. *Third World Buffalo Congress*, 13-18 May 1991, Varna, Bulgaria. Vol. III, pp 737-743.

Oliveira, A. de L., Veloso, L. and Schalch, E. (1991) Carcass characteristics and yield of zebu steers compared with buffalo. *Third World Buffalo Congress*, 13-18 May 1991, Varna, Bulgaria. Vol IV, pp 1019-1026.

Satari, G., Suradisastra, K., Lubis, A. and Nasir, C. (1994) The role of buffalo in Indonesia: Myth and reality. *Proceedings of the First Asian Buffalo Association Congress*, 17-21 January 1994, Khon Kaen, Thailand. pp 285-293.

Shrestha, N.P. and Parker, B.A. (1994) Heterosis for growth and milk production in Phil-Murrah $F_1$ hybrids. *Phil. J. Vet. Anim. Sci.* **20**, 26-32.

Situmorang, P. and Sitepu, P. (1991) Comparative growth performance, semen quality and draught capacity of the Indonesian swamp buffalo and its crosses. In: *Buffalo and Goats in Asia: Genetic Diversity and Its Application*, 10-14 February 1991. Tulloh, N.M., Ed. ACIAR Proceedings No. 34, pp 102-112.

Toelihere, M.R. (1980) Buffalo production and development in Indonesia. In: *Buffalo Production for Small Farms.* Tetangco, M.H., Ed. FFTC Book Series No. 15. ASPAC Food and Fertilizer Technology Center: Taipei, Taiwan. pp 39-53.

Wongsrikeao, W., Boon-ek, L., Wanapat, M. and Taesakul, S. (1990) Influence of nutrition and suckling patterns on the postpartum cyclic activity of swamp buffaloes. In: *Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vienna. pp 121-131.

Yongzuo, X. and Weiming, Z. (1991) Swamp buffaloes and their improving objectives in Sichuan of China. *Third World Buffalo Congress*, 13-18 May 1991, Varna, Bulgaria. Vol. II, pp 355-366.

Zava, M. (1991) Buffalo's meat production evaluation. *Third World Buffalo Congress*, 13-18 May 1991, Varna, Bulgaria. Vol. IV, pp 1011-1018.

NA- 143- 19

# SAREC/NARESA PROJECT ON DISSEMINATION OF INFORMATION ON IMPROVED BUFFALO PRODUCTION SYSTEMS TO SMALL FARMERS

J.A. de S. Siriwardene
*Project Coordinator*

*SAREC/NARESA Buffalo Information Dissemination Project*
*Getambe, Peradeniya*
*SRI LANKA*

The SAREC/NARESA Buffalo Research and Development Programme initiated in 1983 was originally conceived as a research oriented project, based on the recommendations of the National Workshop held in 1980. However, at the end of the first two phases, the emphasis was changed from research to information dissemination. SAREC funding was provided initially for a period of five years from 1983 to 1989. The objectives of Phase I were to generate the baseline information on production systems and uses, and to identify the constraints to buffalo production in Sri Lanka. A Symposium was held at the end of 1989 to review the outcome of the research studies, and SAREC agreed to fund the research programme for a further 5 year period. During Phase II, an interdisciplinary approach was introduced and studies were carried out on a wide range of subjects which included physiology and biochemistry, management and production, disease, reproduction and nutrition. In addition, it was also expected that the researchers would identify the major constraints that limited buffalo production and work towards the development of measures to overcome some of these constraints. It was also evident from the surveys conducted that the buffalo still remained an important source of farm power associated with rice cultivation and that the animal was increasingly being used as a source of milk and meat. It was also evident that despite its low productivity, the animal will continue to play a vital role in the rural agricultural economy in the future.

The research programme made outstanding contributions to knowledge, bringing about a greater understanding of the physiology, biochemistry and reproduction in the buffalo. Information on utilization of feed resources and methods for improving the quality and digestibility of these resources have been made available. Studies carried out on feed resources and methods for improving the quality and digestibility of these resources have been made available. Studies carried out on disease conditions have thrown more light on the epidemiology of diseases and were useful in the development of improved prophylactic measures to control some diseases of economic importance. New innovations for utilization of crop residues and supplementary feeds have been developed.

Through the project, SAREC also provided funds for postgraduate training of several researchers and for the development of field research stations at Narangalla and Mawala and a field laboratory at Narangalla.

This Symposium, the second in the series, will review the research studies which were completed during Phase II. A number of important and useful findings will be reported in the next few days. The information generated will have a three fold application. Firstly, it will contribute to knowledge among the scientific community. Secondly, it with provide useful information for updating material for training of professionals and extension personnel. Thirdly, the information will find application in the development of management innovations for field application. Some of this information has already been incorporated into the management packages currently being extended to the field.

Towards the end of 1994, after a review of the research work completed up to that time, SAREC concluded that adequate research information had been generated during phases I and II. Appreciating the value of the findings for field application, SAREC agreed to fund a third phase for transferring the research findings to the end users.

Phase III commenced activities in January, 1995 and its main objectives are:

(1) to set up a Buffalo Information Centre for Sri Lanka
(2) to disseminate the research information generated in Phases I & II
(3) to popularize intensive buffalo farming for draught and milk production with emphasis on dairying

It was evident that the realization of the third objective depended on the success of the extension effort in removing the constraints that cause production losses. The earlier two phases had identified these constraints as

(1) the low genetic potential of the local buffalo
(2) the dwindling land availability
(3) the seasonal fluctuation in feed availability
(4) the high incidence of disease
(5) the lack of knowledge and inability of farmers to change their management practices to cope with the changing social environment

The project taking into consideration these constraints, developed a plan of activities designed to make available the current knowledge on buffalo production and to develop management packages to cater to the need for a gradual move towards intensification of management. These were:

(1) setting up of the Buffalo Information Centre
(2) dissemination of information to end users
(3) demonstration of the concept and feasibility of intensification of management practices
(4) improvement of the genetic potential of the local buffalo for milk and other production traits
(5) facilitating the production of urea-molasses-mineral supplements
(6) popularization of consumption of buffalo products

The project has initiated the implementation of many of these activities during the course of this year.

## Buffalo Information Centre

The available information on buffalo production in Sri Lanka is not easily accessible, particularly to extension personnel. The need to collect and lodge all documented material in one location was keenly felt. Since extension and training is a function of the Department of Animal Production and Health (DAPH), the Project with the collaboration of the DAPH took steps to establish this Centre at the Central Library of the DAPH, which is appropriately located at the Institute of Continuing Education in Animal Husbandry (ICEAH). The Project obtained the services of a Consultant, the Director of the International Buffalo

Information Centre (IBIC) in Bangkok, to assist in the setting up of this Centre. A section of the Library has been partitioned off for this purpose. Books, journals and audio-visual material will be provided by the Project. The Centre will work closely with the publication unit of DAPH in the task of disseminating extension information.

The Centre will be the repository of information on buffalo production. A computerized information storage and retrieval system will be put in place. It is expected that this Centre will also maintain close liaison with the IBIC and other Centres and have access to the Agrinet information system.

## Dissemination of Information

Several approaches have been initiated under this activity. A Compendium of Research Publications relating to work on buffalo production carried out in Sri Lanka, is under preparation. This will contain titles of papers, the bibiliographic information and an abstract of each publication. Much of the preparatory work has been completed. The Compendium is expected to be published in late 1996.

A book on Buffalo Production designed for use by scientists, professionals, trainers and extension staff is also under preparation. It will provide information relevant to Asia but with special reference to Sri Lanka. The book will cover four major areas, namely, production systems and uses, nutrition, reproduction and health. A number of scientists are assisting in this task.

A Handbook on Buffalo Husbandry Practices for use by extension staff is also under preparation. This will serve as a practical guide for dissemination of information to farmers. In addition, training material and extension leaflets are being produced to meet the extension needs.

In the area of training, the project is collaborating closely with the ICEAH of the DAPH. A Workshop with farmer participation was held at Kuliyapitiya in August this year to assess the training needs of farmers. It was encouraging to note that farmers who attended the workshop gave high priority to the need for training on maximizing production on limited land. Training material is being developed in line with the priority training needs identified by farmers. A number of training programmes for farmers have been conducted at Kuliyapitiya and Thambuttegama. The Project also conducted a training course on nutrition for extension staff at the ICEAH in August this year. The services of an expatriate Consultant were obtained to assist with this course.

A programme for creating public awareness on buffalo production has been launched in collaboration with the University of Peradeniya, DAPH and the Department of Agriculture. The Project has plans to carry programmes on television and radio during the next year. A schools radio quiz programme is scheduled to be held next year. Training videos will also be produced for use in farmer training programmes.

## Demonstration of Intensive Management of Buffaloes

Rural farmers particularly in the dry zone, who have depended to a large extent on communal grazing lands for maintenance of their stock, are finding it increasingly difficult to maintain their buffaloes and cattle in the face of diminishing availability of grazing land. Traditionally, these animals were reared in large herds to ensure the social security of farmers. Animals are generally sold for meat in times of financial need. With increasing alienation of land for agricultural purposes, farmers are compelled to reduce the size of herds, which will mean a decline in their potential source of income. Moreover, there is a trend towards migration of children of farmers away from their homes, in search of more lucrative and prestigious occupations. Parents are finding it increasingly difficult to manage large herds of animals, due to a gradual reduction in family labour. The tendency is for farmers to opt for smaller herds of dual purpose stock.

In order to encourage farmers to adopt more intensive management practices through demonstration of intensive management methods, the Project has established 8 demonstration farms at Thambuttegama and 15 more in the Kuliyapitiya, Bingiriya and Pannala Veterinary ranges in the Kurunegala District. At Thambuttegama, the

*Project on dissemination of information*

Project selected both experienced and inexperienced farmers for the purpose of using their farms as demonstration centres. Farmers new to dairying were less receptive to new interventions than traditional farmers. Learning from this experience, only those currently engaged or who have had experience in dairying were selected as demonstration farmers in the Kurunegala District. The project provided crossbred milking buffaloes to these farmers and assistance to introduce intensive management packages in these farms. These farms will in turn serve as demonstration sites and training locations for other farmers who wish to learn from the experiences of their colleagues.

**Breed Improvement**

This activity is scheduled for next year and will be limited to selected areas in the Kurunegala District. Since AI in buffaloes is not widely practised in this country the Project will provide assistance to Provincial DAPH to popularize Artificial Insemination in buffaloes. A sizeable proportion of the buffalo population are of the indigenous type. Cross breeding to exotic milking breeds will be introduced in keeping with Government's breeding policy.

**Production of Feed Supplements**

Research studies have identified undernutrition and imbalances in the diet as the primary cause of low productivity in ruminants in this country. The Project has given priority to training farmers on measures to correct these deficiencies. It has launched a programme to popularize the feeding of a urea-molasses-mineral block (UMMB) supplement. Assistance has been provided to the Livestock Development Division of the Mahaweli Authority of Sri Lanka to improve the UMMB production facility at the Livestock Farm at Girandurukotte. A concrete mixer and a set of moulds have been provided. Assistance for construction of a molasses storage tank and improvement of a building to house the production facility has been provided. The unit is now providing the requirements of the Project and in addition has commenced marketing the supplements to the Coconut Triangle Milk Union (CTMU) and Milk Industries of Lanka Company Limited (MILCO). Reports from CTMU are encouraging. During the first month of introduction of the UMMB, the CTMU had sold nearly 500 blocks to members of the Union. The Project plans to set up a second production facility in Kuliyapitiya in the near future.

Information on the value of UMMB as a feed supplement, provided to the Ministry of Livestock Development and Rural Industries has evoked much interest. The Ministry is currently studying the feasibility of promoting the setting up of a large scale production plant.

The Project scientists and staff have worked hard to achieve the objectives of the programme. However, as the duration of the current phase is only two years, the time constraint will be a serious limitation. As those involved in extension are fully aware, extending new innovations to traditional rural farmers is a very difficult and time consuming task. Although two years is a very short time in which to make an impact in the field, the project is determined to transfer the new technologies to at least a core group of innovative farmers in selected areas.

The project staff are looking forward to listen to the presentations of the researchers during the next few days. We have with us very eminent foreign scientists who are specialists in their own fields and also a galaxy of eminent local scientists participating in this Symposium. The Symposium provides an excellent forum for Sri Lankan scientists to share experiences with scientists from other Asian countries on the application and dissemination of technical knowledge to rural farmers. It provides an opportunity to discuss and develop guidelines for wider application of selected technologies and to identify potential areas for further study and technology transfer. Clearly, an important challenge for the future will be to develop innovative management packages, utilizing locally available resources, which will be sustainable under the intensive land use systems that are evolving in most countries in Asia. I hope there will be active participation of all who are here and that discussions will be fruitful.

# DISCUSSION

**Q:** C. Devendra - Thank you Dr. Siriwardene for a very enlightening presentation giving the background to Phase III of the Buffalo Research Programme. I want to pose a quick question on the information centre, I am a little bit concerned and need a little clarification. Do I understand that the information centre to be set up is a national one because we have the International Buffalo Information Centre (IBIC) in Bangkok which was set up some 10-12 years ago. I am not sure whether you are focusing on Sri Lanka or on the region, and on the other side we also have the PCC, the Philippine Carabao Centre which might one day want to do the same. Therefore we need to be sure of the boundaries and hopefully mutually reinforcing, but nevertheless focusing on the buffalo. I think it is important that we define our boundaries.

**A:** J.A. de S. Siriwardene - You are right. This will be a local centre which will cater to the local scientists and extension staff and also be a source of information for training at the Institute of Continuing Education. This will therefore be a national endeavour.

**Q:** S.K. Ranjhan - I wonder whether in preparing the research and development programme for Phase III you have kept in mind the farmers perceptions about research and development with regard to this species. You mentioned that the buffalo population in Sri Lanka is going down, that means in the eyes of the farmer, this animal is not as important as cattle. If it is important, naturally the population will increase. So while preparing the research and development project for the next two years I think it would be relevant to know what the farmer perception is with regard to the transfer of technologies that are generated by the institutions, be it crossbreeding or the use of the urea molasses block.

**A:** J.A.de S. Siriwardene - In some areas, particularly in the North Central Province, where very large herds of may be 300 to 400 animals have been maintained, the availability of grazing land has been diminishing. Maintenance of large herds would not be possible in the context of the changing scenario. Due to the unavailability of land for grazing, the population has dwindled to some extent in some areas. I think I am not wrong when I say that there has been a decrease in the population. There is however, a great deal of interest among farmers to keep buffaloes for two reasons (1) because the higher fat content in buffalo milk brings more income and (2) buffalo curd is a very popular delicacy in this country and fetches a very high price compared to liquid milk. The phase III programme has taken into consideration these factors as well as the need to provide information to suit the changing socio-economic conditions of farmers.

**Q:** N. H. Shah - The swamp buffalo is generally used for draught purposes and I would like to know whether both male and female animals are used for draught purposes.

**A:** V.G. Momongan - Yes, both male and female animals are used for draught.

**Q:** N. H. Shah - Have you made any study to evaluate the working capacity of the male and the female buffaloes.

**A:** V.G. Momongan - This study has focused on the male and not on the female animal. In other countries such as Indonesia they have studied the working capacity of the female.

**Q:** S.K. Ranjhan - With increased mechanization of agriculture which is bound to come in 20 years, what do you think will be the role of the swamp buffalo in South East Asia. To give you an example from India, twenty years back draught animal power accounted for around 70 per cent, but today it has come down to 26 percent. The rest are going for mechanized agriculture. If we restrict the functions of the swamp buffalo for draught purposes alone the data from many countries like Malaysia and Philippines will indicate a decrease in the animal population. What would be the future scenario in the South East Asian countries with increased mechanization if the swamp buffalo is not developed as a milk and meat animal?

**A:** V.G. Momongan - With increased mechanization I would predict that more buffaloes would be utilised for milk production and beef production. It is for this reason that we in the Philippines have started a crossbreeding programme with Murrah, and the F1 has been shown to produce much more milk than the native animal. However, we have a difficulty in the *inter se* mating of the F1 because the F2 would have a very variable result and it could discourage the farmer. For example, a farmer having a good female would breed it to a crossbred male and get a small F2 calf which is very discouraging and the farmer may not readily understand why it is so. Therefore to prevent the misconception that this crossbreeding programme is not good we would only maintain the F1 crossbreds and backcross these either to native animals if the farmer wants it for draught or to the Murrah if the farmer is more inclined for milk production. We would also go for castration of all F1 bulls to prevent *inter se* mating under village conditions.

**Q:** D.K. Singh - If the female buffalo is used for draught power naturally this will have adverse effects on milk production. In my opinion, the female should be used for milk and finally meat when the animal is old. Is there any adverse effects on the breeding efficiency of the crossbred between swamp and riverine buffaloes?

**A:** V.G. Momongan - I have already mentioned that *inter se* breeding has an adverse effect and that is why it is discouraged in the Philippines.

**Comment:** D.K. Singh - We have data that the chromosome numbers are different in the two types of buffaloes and the crossbred of the F1 generation will have breeding defects which should be explained before conducting a crossbreeding programme.

**Comment:** V.G. Momongan - Yes, we have some data on testicular abberation in F1 bulls, particularly in the sertoli cells but whether this is sufficient to make the F1 bulls sterile or infertile is not really proven. Anyway we do not use F1 bulls for breeding.

**Comment:** D.K. Singh - My comment is that breeding the swamp buffalo with Murrah will not give the future breeding stock in your country. There are chromosome abberations.

**Comment:** S.K. Ranjhan - In most of the workshops this question arises about crossing swamp with Murrah. I think there is sufficient evidence with the PCRDC in the Philippines. Virtually, there is no reproductive effect of course in F1 where the chromosome number is 49, because riverine has 50 and swamp 48. When gametogenesis takes place the chromosome number comes to 24 and 25 therefore, in the F2 generation when there is *inter se* mating you get a population segregation as mentioned by Dr. Momongan, there is a variation in the chromosome number in the population. You get 48, 49 or 50. This has been proved in the PCRDC and the information has been published . By and large there is no reproductive problem with the F1 population. The national policy in a country is a matter for each country to decide on. In the Philippines, they discouraged *inter se* mating and that is a policy they have adopted and Prof. Momongan has given justification for that policy.

# RELEVANCE AND IMPORTANCE OF CROP-ANIMAL SYSTEMS IN ASIA

C. Devendra

*Senior Associate, International Livestock Research Institute*
*P. O. Box: 30709, Nairobi,*
*KENYA*

**Abstract:** The relevance and importance of crop-animal systems in Asia is discussed in the context of considerable opportunities to increase the role and contribution of animals in these systems in target agroecological zones (AEZ). Maximizing this contribution is especially important at the present time in order to respond to the twin challenges of rapidly increasing human populations and the demand for more food of animal origin, and scarcity of land. The genesis of mixed farming systems and types and characteristics of crop-animal systems in different AEZ in Asia is described. Attention is drawn to the contribution of animals in these systems. The justification for the development of rainfed lowland and upland areas is discussed with reference to the presence of relatively large concentrations of 96.8% cattle and of 83.0% goats and sheep as percentages of the total individual populations in Asia. The impact of crop-animal systems in terms of demonstrable evidence to the contribution of sustainable agriculture and environmental integrity is considerable. The benefits of the adoption of technological options are summarized. The final part of the paper discusses opportunities for research and development with specific reference to priority AEZ, production systems, commodity (animal species) emphasis, and the need for a concerted systems approach that can focus on the totality of farming systems involving interdisciplinarity to address complex problems.

**Keywords:** Crop-animal systems, types, characteristics, impact

## INTRODUCTION

Crop-animal systems are traditional farming systems throughout Asia. They are characteristic of activities in the preponderance of small farm systems, specific features of which include small size of holdings, low input use, low level of efficiency, diversification of agriculture and the involvement of several millions of resource-poor farmers. In these small farms, both ruminants (buffaloes, cattle, goats and sheep) and non-ruminants (chickens, ducks and pigs) are found, and combinations are reared, depending on the farmers' objectives and prevailing marketing opportunities (Devendra, 1983).

Maximizing the contribution from animals in the crop-animal systems represents a particularly compelling challenge at the present time because of the twin reasons of rapidly increasing human population and therefore demand for more animal products, and land scarcity. ADB (1991) projections for example, based on past trends, suggest that for an annual growth rate and growth in real incomes of 1.9% and 2.0% respectively, total meat supplies will have to double and milk production increased by 2.5 times in the year 2006. Likewise the demand for egg consumption is also projected to increase by 2.9 times. The prospects for interregional and open market-oriented trade thus appears to be good especially for low cost producers with a strong and cost-effective production base. Thailand and Indonesia are good examples of this situation and are already forging ahead with expanding productivity.

In terms of agroecological zone, agricultural development in the past has tended to concentrate its efforts in the mainly lowland irrigated areas with outstanding success, especially through the "Green Revolution". Similar success with livestock development has been unfortunately very limited, with the exception of "Operation Flood" and dairy development in India, and intensive pig and chicken production in peri-urban areas.

This paper discusses the relevance and importance of crop-animal systems, describes their genesis and characteristics in the context of opportunities for increasing the contribution from animals in these systems in Asia.

C. Devendra

# CROP-ANIMAL SYSTEMS

Crop-animal systems or more commonly mixed farming systems are varied. They are especially relevant and extremely important in Asian agriculture. The term mixed farming is more commonly used, however, it is too generic, and the reference to crop-animal systems is more appropriate. The latter is also consistent with the approach to integrated natural resource use and management. Emphasis on these systems has attracted global attention recently, and this is reflected also in the mission goals of the newly created International Livestock Research Institute (ILRI), based in Nairobi, Kenya:-

i) Animal performance through research and conservation of animal genetic resources.
ii) Sustainable productivity of major livestock and crop-livestock systems.
iii) Technical and economic performance of the livestock sector, and
iv) The development, transfer and utilization of research-based technology to national programmes and client farmers.

## Relevance

The relevance and potential importance of integrated systems is associated with the complementarity of the crop and animal subsystems resulting in increased total productivity. In this context there are eight major advantages of integrated systems:

- Diverse and efficient resource use;
- Reduced risk;
- Better use of farm labour for higher productivity and increased income;
- Efficient use of biological and chemical energy in the system and less dependence on external inputs;
- Development of sustainable systems that use recycling, involve no pollution and are consistent with environmental protection;

- Increased economic output; and
- Development of stable farm households.

## Importance

Crop-animal systems are particularly important production systems and especially challenging in agriculture. They involve a variety of both crops and animals owned by farmers invariably within the same location and agroecological zones. In Asia, they form the main pattern of farming systems in the preponderance of small farms. These systems are likely to be increasingly more important in the future in the context of their contribution to the following:

- Increasing food production and food security;
- Contribution to the development of sustainable agriculture through efficient and integrated use of biodiversity and ecosystems, and
- Positive effects on the environment.

The projected population increase in Asia of 4.9 billions by the year 2025 (World Resources Institute, 1992) is of overriding concern. Of this, South-East Asia will account for about 716 million people, an increase of approximately 74% over the 1993 population data. Continuing poverty will be apparent; if food production is going to rely only on the use of existing lands, which are already overstretched, environmental degradation is inevitable. The pressure on land use is also compounded by existing and projected increases of large animal populations, which further justify efficiency in natural resource use to maximize productivity and enhance the environment (Devendra, 1993).

Systems are also very important in their potential contribution to the development of sustainable agriculture, and positive effects on the environment. This development is associated with the following beneficial effects:

- Improved efficiency in the integrated management and use of natural resources (land, crops, animals and water).

- Demonstration of environmentally sound, low-input integrated systems.
- Alleviation of rural poverty.
- Nutrient recycling, and
- Positive environmental effects that have economic, ecological and sociological benefits.

## Priority

These considerations lead to the conclusion that the importance of crop-animal systems is therefore far reaching, and justify increased research and development attention in the future. In terms of the production resources available for integrated and sustainable use across ecosystems, development opportunities for increasing productivity and the contribution by animals, and positive effects on the environment, these systems merit higher priority compared to other livestock production systems.

## GENESIS OF MIXED FARMING SYSTEMS

Mixed farming systems, essentially crop-animal systems have evolved and developed over many centuries. The principal determinants of the type of crop and animal system in a particular location are the agroecological conditions (Duckham and Masefield, 1970; Spedding, 1975; Ruthernberg, 1980; Sere and Steinfeld, 1994).

Climate and to a lesser extent, soil affect natural vegetation and determine what crops can be grown. These in turn determine the feed base and its quantity, quality and distribution. The feed base, together with the disease challenge, governs the development of potential animal production systems. Feed resources provide a direct link between crops and animals and the interaction of the two largely dictates the development of such systems (Figure 1).

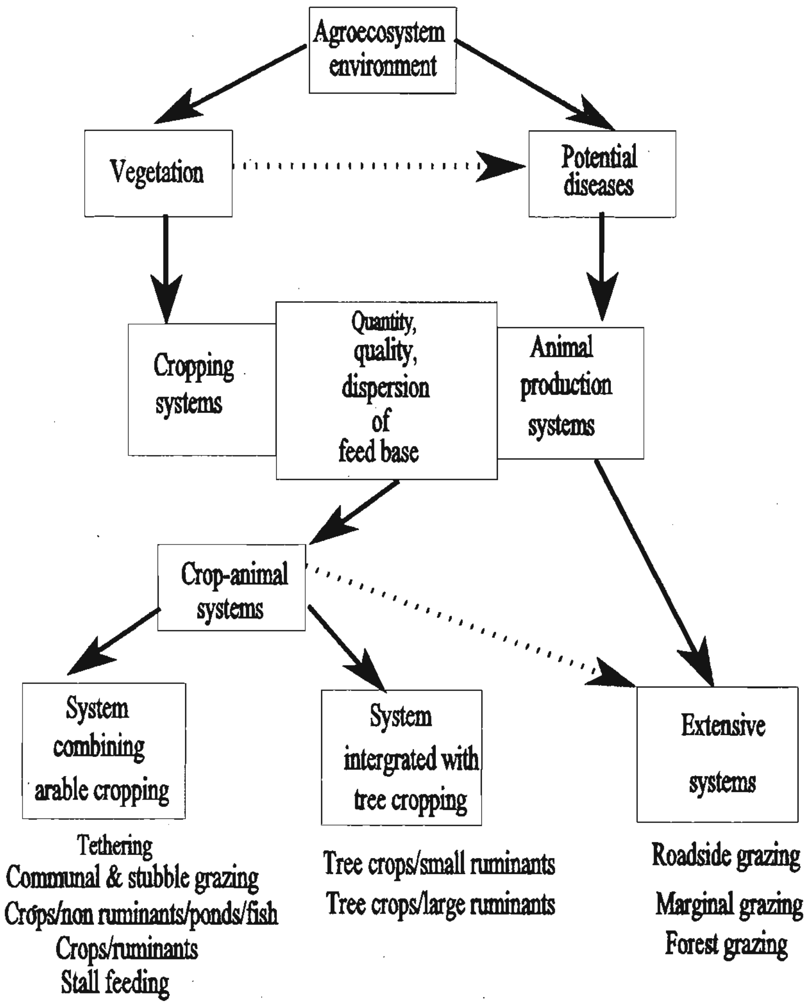



**Figure 1.** Genesis and types of animal production systems in Asia.

Mixed farming systems in the Asian region are varied. Farming practices have developed as a response to environmental dictates, especially temperature, rainfall, altitude, type and intensity of animal production and human intervention. Diversification of resource use spreads risk and provide stability. Farmers consciously diversify the use of the resources to produce a mix of activities that are economically rewarding. Within this broad variety of agricultural activities, opportunities are created that enable switching of practices within and between crops and animals. Diversification rather than specialization is the primary consideration. The inclusion of animals is based on the consideration that they provide power, food, a supplementary income, insurance and a safe investment. Seldom are more than two species of ruminants reared together. Among the agroecosystems, feed supplies are most abundant in the humid areas and here ruminants are valuable in converting feed to animal products that include meat, milk, fibre and power.

## TYPES AND CHARACTERISTICS OF CROP-ANIMAL SYSTEMS

Sere and Steinfeld (1994) have recently described the world livestock production systems. These include mixed farming systems in which more than 10% of the dry matter fed to animals comes from crop by-products and stubble, or more than 10% of the total value of production comes from non-livestock farming activities. Within these, they have subdivided the systems into rainfed and irrigated ones, and further into temperate, humid and arid subsystems. One system that they failed to address and describe concerns the uplands which are very important in Asia. This system is discussed in detail in this paper to expand and add clarity to what has been reported, with specific reference to mixed farm systems in Asia.

Crop-animal systems and their intensification has recently been discussed (Devendra, 1995a). Two broad types of systems are identified:-

- Systems combining animals and annual cropping in which there are two further subtypes:
    - systems involving non-ruminants, ponds and fish
    - systems involving ruminants

- Systems combining animals and perennial cropping in which there are again two subtypes:
    - systems involving ruminants
    - systems involving non-ruminants

These systems are the backbone of Asian agriculture (Devendra, 1983). These systems have many distinctive features including:

- Diversification in the use of production resources
- Reduction in, and spread of risk
- Small size of most farms
- Large populations of ruminants (buffalo, cattle, goats and sheep) and non-ruminants (chicken, ducks and pigs)
- Integration of crops and animals
- Multipurpose roles of crops and animals
- Low use of inputs and traditional systems
- Location in the three main agroecosystems (highlands, arid and semi-arid tropics, and humid/subhumid tropics).

Six distinct types of mixed farming systems are found in Asia in the various agroecological zones. Table 1 gives a broad description of these. An important element of mixed farming systems, and especially the contribution of animals to viability and stability, is the use of draught power. In the Philippines and Thailand, of particular significance is the fact that for farm sizes of up to 2 ha both manure and animal power were important, but the role of the latter was more significant than the former. Only 2.3% of farms use mechanical power (Table 2).

## CONTRIBUTION OF ANIMALS within CROP-ANIMAL SYSTEMS

Animals are primarily important for food production, but they also serve a variety of other useful functions. They also provide an important entry point for rural development, but demonstrable success stories about this role are few and far between. This is especially the situation with ruminants, the reasons for which are linked to over

**Table 1**. Agroecological zones and types of mixed farming systems in Asia

| Agroecological zone | Growing period (days) | Crops | Animals | Mixed farming benefits |
|---|---|---|---|---|
| Rainfed temperate and highlands | < 110 | Barley, millet, mustard, potatoes, fruits | Yak, cattle, sheep | Traction, transport, manure (fuel and soil fertility), reduced risk, survival |
| Rainfed humid and sub-humid uplands | 180-270 | Maize, rice, wheat, root crops, plantation crops | Cattle, pigs, chickens | Traction, transport, income, manure (fuel and soil fertility), crop residues |
| Rainfed humid and sub-humid lowlands | 180-300 | Maize, rice, wheat, root crops, sugar cane, mungbean | Buffalo, cattle, pigs, chickens, ducks | Traction, transport, income, manure (fuel and soil fertility), crop residues |
| Irrigated humid/sub-humid lowlands | 180-365 | Maize, rice, cassava, sweet potatoes | Buffalo, cattle, pigs, chickens, ducks | Traction, transport, income, manure (fuel and soil fertility), crop residues |
| Rainfed arid and semi-arid lowlands, unirrigated | 60-120 | Sorghum, millet, groundnut, soya bean, pigeon pea, cotton | Camels, donkeys, cattle, goats, sheep, chickens | Traction, transport, income, manure (fuel and soil fertility), reduced risk, survival |
| Irrigated arid/semi-arid lowlands | 75-180 | Millet, groundnut, pigeon pea, cotton | Cattle, pigs, chickens | Income, manure (fuel and soil fertility), reduced risk, survival |

**Table 2.** Estimates of farm power sources for different size farms in the Philippines and Thailand (RAPA/FAO, 1989).

| Farm size (ha) | Farms with manual power | | Farms with animal power | | Farms with mechanical power | | Farms with mechanical and animal power | |
|---|---|---|---|---|---|---|---|---|
| | '000s | % | '000s | % | '000s | % | '000s | % |
| 1 | 355 | 40 | 495 | 55 | 19 | 2 | 27 | 3 |
| 1-2 | 263 | 19 | 1026 | 75 | 23 | 2 | 62 | 4 |
| 2-5 | 267 | 14 | 1484 | 76 | 40 | 2 | 166 | 8 |
| 5 | 76 | 7 | 852 | 74 | 31 | 3 | 190 | 16 |
| Total | 961 | 18 | 3857 | 72 | 113 | 2 | 445 | 8 |

emphasis on mainly dairy development with variable success, failure to look at the totality and use of the available animal resources and promotion of balanced development, and a systems-oriented thrust that can provide for increased food production and sustainable development. In this context, it is especially imperative to keep in perspective the broad functions of animals (Devendra, 1993).

(a) Diversification of resources and reduction of socio-economic risks
(b) Promotion of linkages between system resource components (land, water and crops)
(c) Generation of value-added products (e.g. utilization of fibrous crop residues to produce meat and milk), and
(d) Contribution to sustainability in integrated systems.

Although animals are widely recognized as a valuable component of the natural resources, serious doubts exist about the efficiency of existing production systems and the utilization of resources to support them. The overriding issue is inadequate productivity, and in some countries the increasing cost of food imports. Questions are therefore being raised about the availability and application of technological options, and the capacity of the available resources to meet future food needs of humans.

The situation is serious and is being exacerbated by the following facts: rapid human population increase; economic growth and rising incomes; increased demand for food; resource degradation; inadequate technology application; failure of livestock projects (ADB, 1991); and inadequate demonstrable evidence of the role of livestock in sustainable development. Resource degradation is reflected in overgrazing in the semi-arid tropics of South Asia, and in South East Asia in the overcultivation of crop lands, especially in lowland irrigated systems. Rice yields for example, have been declining and the decrease in growth yields is associated with declining factor-productivity and further exploitation of the yield gap is considered uneconomical (Pingali, 1992). Maximizing the contribution from animal production systems represents therefore a particularly compelling challenge at the present time.

## JUSTIFICATION FOR THE DEVELOPMENT OF RAINFED LOWLAND AND UPLAND AREAS

Agricultural development in the past has overemphasized the use of the lowland irrigated area with considerable success. However, these have been associated with resource degradation: overgrazing in the semi-arid areas of South Asia and also of Mongolia, deforestation, shifting agriculture and salinisation in South East Asia.

Since arable land for expanded agricultural development is scarce, attention now needs to shift to the rainfed, lowland and upland areas. Concerning the development and contribution of animals, there is an even greater justification for concerted research and development in the rainfed lowland and upland areas. Table 3 provides data on the magnitude and relative concentrations of cattle and small ruminants (goats and sheep) in the rainfed lowland and upland areas. These populations are large and account for as much as 96.8% and 83.0% of the total populations of cattle and small ruminants in Asia. In the rainfed lowland areas, cattle for beef, milk and draught power, and also goats and sheep are considerable. These sizeable populations depend exclusively on the available feed resources which result from mainly crop production. In the more humid and high rainfall areas of South East Asia, both forage availability and crop residues are generally more abundant and adequate to meet dietary needs. The problem however, is the seasonality of production and drought periods of variable lengths which seriously hamper productivity. In many situations therefore, strategic supplementation to ensure productivity from animals is essential.

**Table 3.** Relative size of cattle and small ruminant (goat and sheep) populations in the lowland and upland areas of Asia (TAC, 1992).

| Ecoregion | Cattle | Small ruminants (goats and sheep) |
|---|---|---|
| Rainfed: arid and semi-arid tropics and subtropics | 179 | 221 |
| Rainfed humid/sub-humid tropics and sub-tropics | 186 | 265 |
| Total | 365 | 486 |
| As % of total population in Asia | 96.8 | 83.0 |

Ruminants in these areas provide a variety of important products, mainly beef, milk, goat meat and mutton. Skins and hides are very valuable by-products. Sustained and high production from these animals necessitate high managerial efficiency of the production systems in which the strategy is to ensure all year round feeding.

## Rainfed Humid/ Sub-humid Lowlands

The rainfed humid and sub-humid lowlands are contiguous with the lowland irrigated systems, and continue into the uplands. Rainfall is unreliable and the soils are poor. Crop production is mainly dependent on the extent of rainfall. With rapid population increase, crops such as rice and wheat are generally grown on land with high productive potential, but with export potential, have moved somewhat into lowland areas. By contrast, the production of roots, tubers and pulses which are mostly grown on land with low potential have stagnated or declined.

Crop production is not as intensive as in the lowland irrigated areas, but rice with a variety of cash crops are grown. Usually one rice crop and occasionally two crops of rice are grown and the cash crops include maize, groundnuts, sugarcane, roots, tubers, pulses, fruits, chillies and pepper. These crops produce a variety of crop residues which together with available wayside grazing provide the main feeds for the animal production. With pigs, they depend to a large extent on feeds from root crops with very little use of purchased concentrates.

Depending on the access to water from rivers, streams or ponds, a common production system involves the integration of pigs, fish and vegetables. The synergistic interactions of these components have a greater total effect than the sum of the individual effects (Edwards *et al.*, 1988), in which ecological and economic sustainability is achieved. In these circumstances, ducks and to a lesser extent chickens also play a valuable part in the integrated systems. Research experience in Thailand indicates that the use of low inputs for poultry-fish integration for small farmers is more feasible for such systems and intensification has many difficulties. By comparison, ruminant-fish integrated systems appears to have more potential because of less reliance on purchased feeds (Edwards and Little, 1995).

Throughout the rainfed lowlands and to a lesser extend in the uplands, native poultry production for meat and eggs is very important, and is an underestimated animal production activity. At the village level, there is organized production mainly by women and children in which there is significant income generation. This is associated often, with relatively higher prices for these birds for the reason that they taste better and the lean content is higher. The income generated makes an important contribution to the stability as well as the nutrition of farm households. Unfortunately there has been very little research and development effort to improve the system and considerable opportunities exist for improving and increasing productivity from native chickens. Specific areas which warrant such effort include genetic improvement, animal health, improved feeding and nutrition, socio-economics and marketing.

## Rainfed Humid/Sub-humid Uplands

The uplands like the lowlands, include unirrigated areas with unreliable rainfall and areas with steep slopes or poor soils up to about 1100m above sea level. The area is generally very fragile and environmental degradation is greater than in the lowland areas.

Both the rainfed lowland and upland areas are generally low producing areas, and house several millions of poor people who prefer these areas and even perceive healthier living. These people are poorer than those in the irrigated areas who live close to forest areas. These areas are increasingly being used for crop production and are also associated with increased human and livestock population pressure. One crop of rice is grown together with root crops like cassava and sweet potatoes as well as cash crops like mung bean (Devendra, 1995b).

Farming activities in this area involve some form of shifting cultivation. This is mainly due to the unavailability of, and lack of access to, agricultural inputs, credit and markets. Thus the farmer has little choice but to move on as the fertility of the land cultivated becomes depleted. Shifting cultivation is a major survival strategy of communities of poor farmers, who cultivate root crops or grain as their major source of subsistence. It involves modifying the forest by clearing, burning, selective cultivating and weeding, and also some hunting and gathering. Most lands that are cleared fail to remain productive because the nutrients are held in the biomass and not in the soil, leading to infertile land. The land cultivated is neither permanent or continuous and is usually followed by a fallow. Often herds of cattle are maintained which are grazed on the forest margins and then shifted such as is done in Laos and Cambodia.

## IMPACT OF CROP-ANIMAL SYSTEMS

*Demonstrable evidence:* Although demonstrable evidence of the benefits of crop-animal systems concerning especially the contribution of animals is limited, the available evidence clearly indicates that these systems have considerable merit. It is not proposed to discuss the available information as this has already been reviewed (Devendra, 1993; 1995a).

By way of example however, one case study is identified appropriate to Sri Lanka. In the upland areas of the mid country in Sri Lanka, crop production involves tree crops (coconuts and fruits), root crops and herbs in stratified layers. Animals are integrated into about 20% of these farms, mainly cattle for dairying, goat and poultry production.

Economic performance for the period 1985 - 1992 for three sizes of farmer-managed farms (0.5, 10 and 2 acres) showed that dairying contributed to most of the total gross profits of 31, 63 and 69% for the three types of farms respectively. This was followed by crops (29, 37 and 19%), poultry (22, 0 and 9%) and goats (18, 0 and 3%) respectively. Among the animals, dairy cattle and goats gave the greatest income returns (Table 4). Animals also significantly contributed to the improvement of soil fertility through manure and biogas production to replace domestic fuel needs (de Jong *et al.*, 1994).

*Environmental impacts:* The positive environmental impacts resulting from crop-animal interactions are numerous. These include *inter alia*:

- Improved tillage and soil conservation through use of animal draught power compared to mechanical power.
- The supply of dung and urine to improve soil fertility.

**Table 4.** Average economic performance per year, per acre and per person of the farm activities of the three MLDC Farms - 1985 - 1992 (de Jong *et al.*, 1994).

| Item | Half acre | | One acre | | Two acre | |
|---|---|---|---|---|---|---|
| | Rs/year | Rs/acre | Rs/year | Rs/acre | Rs/year | Rs/acre |
| Total revenue | 55,483 | 110,966 | 39,780 | 39,780 | 70,455 | 35,227 |
| Total direct cost | 36,937 | 78,874 | 12,611 | 12,611 | 33,401 | 16,700 |
| Total gross margin | 18,546 | 37,092 | 27,169 | 27,169 | 37,054 | 18,527 |
| As % of 0.5 acre farm | 100 | 100 | 147 | 73 | 200 | 50 |
| Gross margin/LU[1] | 8,243 | (2.25 LU) | (2.5LU) | (4.25LU) | | |
| Gross margin/(person) | 12,364 | (1.5 p) | 18,113 | (1.5 p) | 21,174 | (1.75 p) |
| As % of 0.5 acre farm | 100 | | 147 | | 171 | |
| Gross margin per Rs 1,000 investment | 387 | | 594 | | 599 | |

[1] Livestock Unit

- Development of food-feed systems results in increased feeds for animals for longer periods, increased crop yields and soil fertility, and
- Demonstrable sustainable systems that integrate the management and use of natural resources and environmental protection.

*Major conclusions from case studies*: A number of major conclusions can be made from the results of the 10 case studies cited:

(a) In all cases, without exception, mixed farming systems involving crops (annual and perennial) and animals (ruminants and non-ruminants) and their interactions were positive and beneficial.

(b) The benefits resulting from positive crop-animal interactions were directly associated with increased productivity from the integration, increased income and demonstration of sustainable systems involving ecological and environmental attributes.

(c) The main parameters used as indicators of sustainability were yield and net returns, simply because these are the main ones used by farmers and others in farming system performance. In one case, soil fertility and soil erosion indicators were also used. Future projects will need to include in the methodologies, more vigorous indicators of sustainability such as total factor productivity as well as assessment of environmental impacts. The latter include both environmental (e.g. soil status) and economic (eg. food security and farm income) impacts.

(d) Exciting opportunities exist for extending wider use of a variety of technology development initiatives in both the rainfed, lowland and upland agroecological zones. Priorities need to be established to focus on the development of these agroecological zones in tandem with national goals, appropriate use of animal species and available natural resources.

(e) An overriding constraint in the rainfed lowlands and uplands concerns feed resources. The problems involved are firstly increased feed production to alleviate existing deficits, and secondly, improved efficiency in feed utilization. Animals in these systems subsist on very low planes of nutrition and droughts. Meeting their nutrient needs through the development of all year round feeding is a most important strategy.

(f) Application of the potentially important technologies and the development of others has the distinct possibility of expanding the use of underutilised rainfed lowlands and uplands, increase food production from more efficient use of the natural resources, and also concurrently demonstrate the important role of animals in sustainable agriculture.

(g) A major shift of development activities to the more complex rainfed lowland and upland systems will need strong policy and institutional support to deal with issues such as land tenure, common property rights and marketing.

*Summary of Benefits:* In cognisance of the case studies presented and the data therein, Table 5 presents a summary of the positive impacts resulting from the available technologies that are associated with sustainable agriculture. These relate to both socio-economic and environmental benefits.

**Table 5.** Benefits of some technological options in crop-animal systems and demonstrable sustainable agriculture in Asia.

| Technology | Soil conservation | Soil fertility | Increased animal performance | Increased crop yields | Increased food security | Increased income/household |
|---|---|---|---|---|---|---|
| 1) Supplementation | | | + | | + | + |
| 2) Draught animal power | + | + | | + | + | + |
| 3) Legumes (feed, green manure, hedges in rice bunds) | + | + | + | + | | + |
| 4) Food-feed systems | + | + | + | + | + | + |
| 5) Tree strata forage system | + | + | + | + | + | + |
| 6) Alley cropping | + | + | + | + | | + |
| 7) Sloping agricultural land technology (SALT) | + | + | | + | + | + |
| 8) Manure availability | + | + | + | + | | |
| 9) Rice-fish integration | + | + | | + | + | + |
| 10) Ruminants-tree crop integration | + | + | + | + | + | + |

# OPPORTUNITIES FOR RESEARCH AND DEVELOPMENT

Considerable opportunities exist for research and development on animals in crop-animal systems. In this context the priority agroecological zones, production systems, and commodity emphasis are as follows:-

## Priority Agro-ecological Zones

*South East Asia*

(a) Rainfed humid/Sub humid lowlands
(b) Rainfed humid/Sub humid uplands
(c) Rainfed semi-arid humid lowlands (Thailand and Indonesia)

*South Asia*

(a) Unirrigated arid/semi-arid lowlands
(b) Irrigated arid/semi-arid lowlands
(c) Temperate and tropical highlands (Hindu-Kush Himalayan region)

## Priority Production Systems

*South East Asia*

A. Rainfed humid/sub-humid lowlands
(a) Rice/livestock/integration
(b) Rice/buffaloes/cattle
(c) Rice/ducks
(d) Crops/ poultry

B. Rainfed humid/sub-humid uplands
  (a) Tree crops/ small ruminants systems
  (b) Tree crops/cattle systems

*South Asia*

A. Unirrigated rainfed arid/semi-arid lowlands
  (a) Goat and sheep production, including landless systems

B. Irrigated rainfed arid/semi-arid lowlands
  (a) Rice-wheat/buffalo
  (b) Rice-wheat/cattle

C. Tropical highlands
  (a) Maize-barley/cattle
  (b) Goat/sheep production

**Commodity Emphasis**

In relation to the priority AEZ and production systems, the commodity emphasis to increase productivity, often through the use of multipurpose animals is as follows:

*South East Asia*

Cattle - Beef, milk and some draught power
Buffaloes - Beef and draught power
Goats - Goat meat
Sheep - Mutton
Ducks - Meat and eggs
Pigs (native) - Pig meat
Chickens (native) - Poultry meat and eggs

*South Asia*

Buffaloes - Milk and some draught power
Cattle - Draught power and some milk
Goats - Goat meat and some milk
Sheep - Mutton

Throughout Asia, large scale poultry and pig production are very well advanced, capital - intensive industries in which the private sector is heavily involved. On the other hand, there is an ever increasing role through policy and regulatory mechanisms, to ensure that increased efficiency of production can be enhanced through integrated production involving management, improved feeding and nutrition, animal health, product handling and use.

**Systems Approach**

Because of the complexity of research and development in these difficult areas, resource management will have to be more holistic. The costs are therefore higher, but the payoffs for the contribution of livestock are likely to be much more in the future.

An important prerequisite to the systems approach is commitment to inter-disciplinarity, integrated natural resource use and management and sustainable development. This is especially important for research and development of crop-animal systems in specific ecosystems. Also, the formulation of research programmes must involve both production and post-production components, and community-based participation in response to the real needs of farmers.

The components of the systems approach involve five successive stages:

- Description or diagnosis
- Design
- Interventions and testing

## References


ADB (1991) *Sector paper on livestock.* Agric. Dept. Staff Paper No. 4, Manila, Philippines, 225 pp

de Jong, R., Kuruppu, L.G., Jayawardena, Q.W. and Ibrahim, M.N.M. (1994) Performance of small livestock crop demonstration cum training farms in Sri Lanka. *Asian-Australian J. Anim. Sci.* **7**, 571-582.

Devendra, C. (1983) Small farm systems combining crops and animals. *Proc. V World Conf. Anim. Prod.* **1**, 173-191.

Devendra, C. (1991) The potential for integration of small ruminants and tree cropping systems in South and South East Asia. *World Anim. Rev.* (FAO) **66**, 13-22.

Devendra, C. (1993) Sustainable animal production from small farm systems in South East Asia. *FAO Anim. Prod. and Health Paper* No, 106, Rome, Italy.

Devendra, C. (1995a) Mixed farming and intensification of animal of production in Asia. *Proc. ILRI/FAO Roundtable Livestock Development in Low Income Countries,* Addis Ababa, Ethiopia, p. 133-144.

Devendra, C. (1995b) Environmental characterisation of crop-animal systems in rain-fed lowland and upland areas. In: *Proc. of International Workshop on Crop-animal Interactions.* Devendra, C. and Sevilla, C.C. (Eds). International Rice Research Institute, Los Banos, Philippines, p. 43-64.

Duckham, A.M. and Masefield, O.D. (1970) *Farming Systems of the World.* Chatto and Windus: London, UK.

Edwards, P., Pullin, R.S.V. and Gartner, J.A. (1988) Research and education for the development of crop-livestock-fish farming systems in the tropics. *International Centre for Land Use and Resource Management Studies and Reviews* No: 16, Manila, Philippines, 53 pp.

Edwards, P. and Little, D.C. (1995) Integrated crop-fish-livestock improvements in South East Asia. *Proc. Consultation for the South East Asia Region.* (Eds. C. Devendra and P. Gardiner), ILRI, Nairobi, Kenya. p. 65-75.

FAO/RAPA (1989) *Livestock Development in Asia-Pacific Region.* Publ. 1891/1, Food and Agriculture Organisation, Bangkok, Thailand, 55 pp.

Ruthenberg, H. (1980) *Farming Systems in the Tropics* (2nd Ed.). Clarendon Press: Oxford, UK, 424 pp.

Sere, C. and Steinfeld, H. (1994) World livestock production system: Current studies, issues and trends. *FAO Anim. Prod. and Health paper* (In press).

Spedding, C.R.W. (1975) *The Biology of Agricultural Systems.* Academic Press: London, UK.

TAC/CGIAR. (1992) *Review of CGIAR Priorities and Strategies.* Part 1. TAC Secretariat, Food and Agriculture Organization: Rome, Italy (Mimeograph, 250 pp.)

FAO/RAPA (1989) *Livestock Development in Asia-Pacific Region.* Publ. 1891/1, Food and Agriculture Organisation, Bangkok, Thailand, 55 pp.

Ruthenberg, H. (1980) *Farming Systems in the Tropics* (2nd Ed.). Clarendon Press: Oxford, UK, 424 pp.

Sere, C. and Steinfeld, H. (1994) World livestock production system: Current studies, issues and trends. *FAO Anim. Prod. and Health paper* (In press).

Spedding, C.R.W. (1975) *The Biology of Agricultural Systems.* Academic Press: London, UK.

TAC/CGIAR (1992) *Review of CGIAR Priorities and Strategies.* Part 1. TAC Secretariat, Food and Agriculture Organization: Rome, Italy (Mimeograph, 250 pp.)

Pigali, P.L. (1992) Diversifying Asian rice farming systems: a deterministic paradigm. In trends in Agricultural Diversification: Regional Perspective. *World Bank Technical Paper* 180, Washington D.C., USA., pp 107-126

World Resources Institute (1992) *Towards Sustainable Development.* World Resources Institute, Washington, D.C., USA. xiv + 3035 pp.

NA-145-55

# THE USE OF FEMALE BUFFALOES FOR ON-FARM WORK

P. Bunyavejchewin and C. Chantalakhana

*Buffalo and Beef Production Research and Development Center (BPRADEC) Suwanvajokkasikit Animal Research and Development Institute (SARDI) Kasetsart University, Bangkok THAILAND*

**Abstract:** In Thailand, draught animals remain an important source of farm power for crop production in most rural rain-fed areas. The distribution of draught animals varies in different locations relating directly to rain-fed rice growing areas where draught animal power (DAP) plays a significant role. With more use of small tractors, the purpose for keeping of buffaloes on-farm is not only for draught but also for sale, for manure and as the family's financial asset. Thereby, farmers tend to keep more female buffaloes than male animals because females can produce offspring which can be sold for additional income. Inevitably, cows including pregnant ones are also used for work during the cultivation season.

Pregnant buffaloes are used for work at various stages of pregnancy, ranging from 2-3 months to 6-8 months of pregnancy. For intense work during the regular rainy season, some pregnant cows work from 45-90 days or 225-450 hours mainly in first tillage. No incidence of health problems or abortion was observed. For first tillage performed by buffalo cows with average weight of 405-411 kg, the area that could be ploughed was approximately 405-420 $m^2/h$. For second tillage cows with average weight of 404-410 kg could plough 708-807 $m^2/h$.

It is foreseeable that buffalo cows will continue to play a significant role in the future in the supply of animal power for rural rain-fed farming. Although an increase in mechanization is evident, some kinds of work in soil preparation has to be done by animals. Thereby, more combined use of mechanical power and draught cows will be practical and economical under small farm systems in Northeast Thailand.

**Keywords:** Animal power, buffalo, female performance

## INTRODUCTION

Agriculture is an important sector in Thailand in which approximately 60-65% of the total Thai population (almost 60 millions) are currently engaged. An estimate of the extent of farm land in 1991 was 21.3 million hectares or 41.5% of total land (OAE, 1994). The extent of agriculture (about 85%) depend on rainfall and occupy most rural areas where draught animals are an important source of power for farming.

The distribution of animals used for draught power, mainly buffaloes, varies in different locations depending on the extent of rainfed rice growing land, where they play a prominent role. Due to advanced technology in mechanical power, the use of draught animal power is rated low in status and little attention is paid to it, though it is appropriate economically and socially in the circumstances of remote rural areas, where farmers depend on rainfall for crop production. With more use of two-wheeled tractors small farmers have come to limit the number as well as sex of buffaloes raised in their farms. Female buffaloes are more preferable to keep on-farm than male ones for some specific reasons. Thereby, buffalo cows are also used for work during the growing season (Chantalakhana *et al.*, 1991). That situation is confirmed in this paper which presents the results of an on-farm monitoring study in two villages of Northeast Thailand, conducted during the years 1991-1994. The paper is focused on three points; 1) the proportion of working female and male buffaloes in the studied villages, 2) the use of pregnant cows for work, and 3) draught capacity of female buffaloes.

## VILLAGE DESCRIPTION

Two villages in Surin province having topographical differences, one lowland and the other mixed lowland-upland, were selected to represent two typical farming systems prevalent in lower-northeast Thailand. Farmers in these two villages depend only on rainfall for crop production. In 1991 and 1992 the annual rainfall was 1411 and 1040 mm, the average annual temperature was 27.1$^0$ C and 26.9$^0$ C, and the average annual relative humidity was 70.1% and 70.9%, respectively. Normally the rainy season lasts from May to October.

*Lowland Village (LV):* In 1992, there were 121 households, of which 78.5% grew crops, mainly rice. Seventy-three per cent of the total number of households raised swamp buffaloes; the average was 2.2 head per household and the total number was 245. There were approximately 95 cattle and some pigs and poultry (chickens, ducks and geese) in the village. Besides income from crops and livestock, villagers also earned income from off-farm employment (Table 1).

**Table 1.** Characteristics of the two villages studied (Bunyavejchewin *et al.*, 1994a).

| Characteristics | LV | UV |
|---|---|---|
| Total no. of households (HH) | 121 | 96 |
| Average members per HH | 4.4 | 4.9 |
| HHs growing crops (No.) | 95 | 76 |
| (%) | 78.5 | 79.2 |
| Land holding, (rai)[1] | | |
| Homestead | 1.2(0.12-5)[2] | 1.6(0.25-5) |
| Cultivated area | 19.0(1-120) | 16.5(1-65) |
| Livestock raised, (No.)[3] | | |
| Buffaloes | 2.2(72.7)[4] | 3.0(79.2) |
| Cattle | 1.6(48.8) | 2.7(72.9) |
| Pigs | 0.8(24.8) | 0.8(46.9) |
| Ducks | 7.1(25.6) | 2.6(40.6) |
| Chickens | 14.4(90.9) | 18.6(94.8) |
| Family income source | On/off-farm | Mostly on-farm |
| Power source for tillage[5] | | |
| HHs using buffaloes, (No.) | 34 | 5 |
| (%) | (35.8)[6] | (6.6) |
| HHs using small tractors, (%) | 29(30.5) | 37(48.7) |
| No.of HH using both, (%) | 30(31.6) | 33(43.4) |

[1]- 1 rai = 0.16 ha [2] - Mean (range) [3]- Average from total number of households [4] - figure in brackets is the percentage of households raising buffaloes [5] - Only the households which had their own cropping activities [6] - figure in brackets is the percentage of total households growing crops

*Mixed Lowland-Upland Village (UV):* The number of households in this village in 1992 was 96, of which 79.2% grew crops the whole year round. The major crops which were the main income source of the household were rice, maize, Chinese radish and groundnut. Because of topographical variation in the cultivated area, farmers grew rice mostly in the lowland areas and the other crops in upland areas. Seventy-nine per cent of all households raised swamp buffalo, the average being 3 head per household. The total number of cattle in the village was 198, and there were also some pigs, chickens and ducks (Table1).

Comparing the data from the two villages in 1992 with those collected in the year 1988 (Chantalakhana *et al.*, 1991), some characteristics had changed. There were increases in the number of households by 5.2% and 6.7% in LV and UV respectively, and in the number of cattle, especially in UV.

## USE OF DRAUGHT BUFFALO COWS

The intensity of work for draught animals in Thailand varies between locations, seasons and farming systems. Traditionally, male animals are preferred for work, while females are kept for producing calves in order to gain additional income for farmers. Due to increasing shortage of draught animals during the recent decades both male and female buffaloes have been used for work including pregnant buffaloes.

Daily records on the use of individual buffaloes for farm work in the two villages, including ploughing, harrowing and carting, showed that the draught buffaloes were used on average of 51 to 67 days per year, ranging from 8 to 115 days (Chantalakhana *et al.*, 1991). Two to three draught buffaloes can take care of farm work on two to three hectares of farm area. From the same study it has been shown that buffaloes are used most intensely during July and August which are the months of most intense ploughing and planting.

There has been a trend in small farms to raise more female animals for work than males; females are preferred because they can produce calves which can be sold. The figures from three-consecutive years (Bunyavejchewin *et al.*, 1994b) revealed that both villages used more females than males for work (Table 2), but the range of ratios of working male to female in the mixed lowland-upland village (1:5-1:12) was much higher than that of the lowland village (1:2.6-1:3.4). In practice, most of the bigger males were castrated at the age of 2.5 to 3 years in order to reduce their aggressiveness and for ease of handling during training for work. Only a few intact bulls in the villages studied were used for work due to their individual tameness. This gave more chance for natural buffalo mating which mostly occurred during January to March when animals were in prime condition due to abundant feed supply in the post-harvest period from October to December. Since farmers kept more cows than bulls, pregnant cows were eventually used for work whenever needed. Calvings occurred mostly during November to January, resulting in many 4-6 month pregnant cows being worked during the cropping season (June to August).

**Table 2.** Numbers of working animals in two villages in northeast Thailand during 1992-1994 (Bunyavejchewin *et al.*, 1994b).

| Year | LV | | | UV | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total |
| **1992** | | | | | | |
| Total no. of buffaloes | 91 | 158 | 249 | 93 | 192 | 285 |
| Working buffaloes | 37 | 96 | 133 | 9 | 109 | 118 |
| **1993** | | | | | | |
| Total no. of buffaloes | 92 | 183 | 275 | 114 | 172 | 286 |
| Working buffaloes | 25 | 84 | 109 | 7 | 78 | 85 |
| **1994** | | | | | | |
| Total no. of buffaloes | 97 | 170 | 267 | 97 | 152 | 249 |
| Working buffaloes | 21 | 68 | 89 | 11 | 55 | 66 |

The distribution of different classes of buffaloes and the calving rates in the two villages are shown in Table 3.

**Table 3.** Animal numbers and calving rates of buffaloes under village conditions (Bunyavejchewin *et al.*, 1994b).

| Item | Calving season | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1991/92 | | | 1992/93 | | | 1993/94 | | |
| | LV | UV | Total | LV | UV | Total | LV | UV | Total |
| Total no. of buffaloes | - | - | - | 249 | 285 | 534 | 27593 | 28611 | 561 |
| - male | - | - | - | 91 | 93 | 184 | 18213 | 41721 | 206 |
| - female | - | - | - | 158 | 192 | 350 | 14932 | 31422 | 355 |
| Mature females | 121 | 120 | 241 | 129 | 131 | 260 | 20000 | 75000 | 262 |
| Mature bulls | - | - | - | 20 | 13 | 33 | 0 | 00 | 91 |
| Cows calving | 31 | 52 | 83 | 47 | 46 | 93 | | | 58 |
| - non-working pregnant cows | 4 | 6 | 10 | 13 | 13 | 26 | | | 20 |
| - working pregnant cows | 27 | 46 | 73 | 34 | 33 | 67 | | | 38 |
| Calving rate (%) | 25.6 | 43.3 | 34.4 | 34.4 | 35.1 | 35.8 | | | 22.1 |

## WORKING PREGNANT BUFFALO COWS

As mentioned it is often seen in the villages that pregnant females are used for work during the growing season. Thirty seven pregnant buffaloes were observed, 23 in the 1992/93 and 14 in the 1993/94 calving seasons by Bunyavejchewin *et al.* (1994c). Twelve out of 23 were used to work in the year 1992 cropping season (around May to August), while 6 out of 14 were worked in 1993. Due to different rainfall patterns between the two cropping seasons, working intensities were different. An unusually short rainy season with the small amount of rainfall in 1992 resulted in less intensive use of buffalo power compared to that in 1993 when rainfall distribution was normal. Table 4 presents the general working profiles of pregnant cows calving in different months of the calving season. It can be seen that pregnant buffaloes in this study were used to work at various stages of pregnancy, ranging from 2-3 months to 6-8 months in 1992. However, the number of working days were few, ranging from 2-5 days to 11 days during the cropping season, or 3 to 17 hours per animal per season. This workload is considered to be very light due to the drought conditions which prevailed during the 1992 cropping season.

Many farmers who used buffaloes for tillage turned to hiring a two-wheeled tractor. On the other hand, in the 1993 cropping season, rainfall was near normal and the buffaloes were used more intensely. Working days of six pregnant buffaloes ranged from 45-90 and the number of working hours per season were 225 to 450. The females were at different stages of pregnancy ranging from 2-3 months to 5-7 months of pregnancy. No incidence of adverse health conditions or abortion was recorded, though some were worked five hours a day during 2-3 months of the cropping season.

**Table 4.** Use of pregnant buffalo cows for work in two cropping seasons (Bunyavejchewin *et al.*, 1994c).

| No. pregnant cows observed | Month of calving | Avg. age (yr) (min-max) | Stages of pregnancy (mo) when worked | Period of work | | Type of work |
|---|---|---|---|---|---|---|
| | | | | Days (min-max) | Hours (min-max) | |
| 1992/93 calving season | | | | 1992 cropping season | | |
| 3 | Nov 92 | 7(4-9) | 6-8 | 2-6 | 3.5-7.5 | 1st tillage |
| 6 | Dec 92 | 10(4-19) | 5-7 | 2-5 | 3-8 | Mostly 1st tillage |
| 1 | Feb 93 | 7 | 2-3 | 11 | 14 | 1st tillage |
| 2 | Mar 93 | 14.5(8-21) | 2-5 | 4-11 | 5-17 | 1st tillage |
| 1993/94 calving season | | | | 1993 cropping season | | |
| 2 | Nov 93 | 8(4-12) | 5-7 | 60-90 | 360-450 | 1st, 2nd tillage and harrowing |
| 2 | Dec 93 | 8(7-9) | 4-6 | 45-70 | 270-420 | 1st tillage |
| 2 | Mar 94 | 7.5(6-9) | 2-3 | 45-60 | 225-300 | 1st tillage |

## WORK OUTPUT FROM DRAUGHT COWS

In an attempt to compare the work output of females with that of male buffaloes on-farm, measurements were made in terms of the area ploughed. Due to the fact that farmers used more females for work, there were only a few observations recorded in males (Table 5). It can be seen that the area ploughed on the second tillage was more than that in the first tillage by one and a half to two fold. Since there was a wide variation in the number of observations on draught performance between the two sexes as well as buffalo body weights, the difference between sexes could not be clearly determined. However, it appeared that the work output was dependent on body weight.

**Table 5.** Draught abililty of female buffaloes and a comparison with that of a limited number of males.

| Type of work | Soil type | Sex | Body weight (kg) ) | No | Area ploughed ($m^2/h$) | C.V. (%) |
|---|---|---|---|---|---|---|
| First tillage | Sandy loam | F | 405±34 | 25 | 405±143 | 35.0 |
| | | M | 537±70 | 3 | 503±265 | 52.7 |
| | Clay loam | F | 414±60 | 11 | 420±173 | 41.2 |
| | | M | - | - | - | - |
| Second tillage | Sandy loam | F | 410±47 | 26 | 807±501 | 62.1 |
| | | M | 448±89 | 3 | 891±552 | 61.9 |
| | Clay loam | F | 404±57 | 11 | 708±513 | 72.4 |
| | | M | 418±89 | 3 | 669±235 | 35.1 |

## FUTURE ROLE OF DRAUGHT BUFFALO COWS

In the foreseeable future buffalo cows will continue to play a significant role as a source of animal power in small farm systems in some areas especially in Northeast Thailand.

During the past two decades village buffalo herds generally consisted of 50% males and most of them were used for work and transportation. However, as evident in recent years, more female buffaloes are used for work instead of males in order to utilize the female for calf production and additional income, as well as for other purposes. Therefore village buffaloes will continue to serve as multi-purpose animals in the future, including a source of DAP. Some transition from draught buffalo to a small tractor or a hand tractor is increasingly seen under village circumstances where crop production depends on rainfall. The small tractor is also fashionable among rural farmers and has a faster working speed, but its cost-effectiveness is questionable (Bunyavejchewin *et al.*, 1994a). A study has found that the break-even point of the utilization of a small tractor was at 242.4 hours per year (Bunyaprawit, 1985). It is often seen that the small tractor is underutilized by some farmers due to many reasons, such as having small pieces of land for cultivation, lack of family labour for hiring out the small tractor during off-growing season period and the lack of knowledge in using the small tractor properly which impacts directly on user's health, etc. In addition, it is evident that some farmers revert to the use of the draught buffalo when the tractor breaks down (Priebprom and Sirichinda, 1994). On the other hand, some kinds of work such as furrowing or ploughing near dikes, are more conveniently done by buffalo. Thereby, more combined use of mechanical power and draught buffalo will be practical and economical under rural small farm systems in Thailand.

## References

Bunyaprawit, M. (1985) The economic values of tractors. *Appropriate Technology Journal* 5,: 24-29. (in Thai).

Bunyavejchewin, P., Sangdid, S. and Chantalakhana, C. (1994a) Socioeconomic conditions affecting the use of draught buffalo versus two-wheeled tractor in some villages of Surin province. In: *Proceedings of the First Asian Buffalo Association (ABA) Congress,* BPRADEC, Bangkok, Thailand. pp. 28-41.

Bunyavejchewin, P., Sangdid, S. and Chantalakhana, C. (1994b) Reproductive efficiency of draught buffalo under village conditions. Paper presented at the *Symposium on Improving Draught Capacity of the Multi-purpose Buffaloes in Small-farm Systems.* Kasetsart University, Bangkok, Thailand, 6-8 December 1994.

Bunyavejchewin, P., Chantalakhana, C., Sangdid, S. and Poondusit, T. (1994c) Factors affecting draught capacity of buffalo in two villages of Surin province. Paper presented at the *Symposium on Improving Draught Capacity of the Multi-purpose Buffaloes in Small-farm Systems.* Kasetsart University, Bangkok, Thailand, 6-8 December 1994.

Chantalakhana, C., Bunyavejchewin, P., Chotemetepirom, S. and Kaewsomprasong, K. (1991) Household characteristics and monitoring of year-round buffalo husbandry and activities in two villages in Northeast Thailand. In: *Draught Power from Swamp Buffaloes in Asia,* Bunyavejchewin, P. and C. Chantalakhana (Eds), Proceedings, BPRADEC, Kasetsart University, Bangkok, Thailand. pp 239-268.

Office of Agricultural Economics (1994) *Agricultural Statistics of Thailand Crop Year 1993/1994.* Center for Agricultural Statistics, MOAC, Bangkok, Thailand.

Priebprom, S. and Sirichinda, A. (1994) Economic assessment of role and contribution of buffaloes and two-wheel tractors in small-farm systems. Paper presented at the *Symposium on Improving Draught Capacity of the Multi-purpose Buffaloes in Small-farm Systems.* Kasetsart University, Bangkok, Thailand, 6-8 December 1994.

# TRANSFER OF TECHNOLOGY IN SMALLHOLDER INTENSIVE BUFFALO FARMING: RESULTS FROM A PILOT STUDY IN MAHAWELI SYSTEM 'H'

H. Abeygunawardena[1], D.H.A. Subasinghe[2], A.N.F. Perera[3], S.S.E. Ranawana[4], M.W.A.P. Jayatilake[5] and B.M.A.O. Perera[1]

*[1] Department of Veterinary Clinical Studies, University of Peradeniya [2] Department of Animal Production and Health, Peradeniya [3] Department of Animal Science, University of Peradeniya [4] Veterinary Research Institute, Gannoruwa, Peradeniya [5]Department of Agricultural Economics and Extension, University of Peradeniya, SRI LANKA*

**Abstract:** Buffalo and cattle farming has become increasingly difficult and uneconomic because of (1) increased pressure on agricultural land (2) seasonal fluctuations in feed availability and quality (3) low productive and reproductive efficiency and (4) unfavourable ratio between the price of milk and the price of feed. The conventional research and extension delivery system has failed to disseminate research information and to solve the problems faced by resource poor farmers. Therefore, this study based on the farming systems research approach was aimed at (a) developing a model for smallholder intensively managed buffalo farm units (SIMBU) incorporating technologies from research and (b) demonstrating the feasibility, and the cost and benefits of intensification of buffalo farming. The model for SIMBU was designed to maintain 3 adult dairy buffaloes. A feeding system consisting of native grass, tree fodder and rice straw supplemented with 1 kg/day of Urea-Molasses-Multinutrient Mixture (UMMM) was developed.

The testing and refinement of the feeding system was conducted in 4 successive phases, with roughage as the basal diet supplemented with, 3 kg of coconut poonac and 3 kg of rice bran (Diet 1), 1 kg/day of UMMM given as a lick for 4 months (Diet 2), 1 kg/day of UMMM fortified with 50 g of fish meal as a by-pass protein for 6 months (Diet 3), and 1 kg/day of UMMM given as a block (UMMB) containing 12% urea, 40% molasses, 30% rice bran, 10% cement, 5% minerals and 3% fish meal for 12 months (Diet 4). For Diets 2, 3 and 4, the mean (±SD) feed intake on DM basis was 10.0±3.5, 10.4±3.2 and 11.4±0.3 kg per day, respectively. During the four phases, the mean body weight (based on chest girth) was 441.3±95.9, 476.3±40.1, 467.2±45.9 and 464.1±27.1 kg and mean milk yield was 2.4±2.6, 3.9±1.1, 4.0±2.3 and 4.3±1.4 litres, respectively. The cost of supplementary feed per day declined from Rs.24.00 for Diet 1 to Rs.5.50 for Diet 4 (US $1=Rs.55.00). The annual calving rate during the first year under SIMBU was 62% and calving interval 584±80 days. The mean birth weight of calves (30.42±1.9 kg) and growth rates during the first 12 months (256±73 g/day) were similar to those recorded for Surti buffaloes on state farms. The costs borne by the farmers were for labour (of which the opportunity cost was very low), for maintenance of the sheds, collection of straw, cutting native pasture and fodder, milking, care of the animals and assisting the project personnel in keeping records of inputs and outputs. The benefits to the farmers were the income from milk for meeting daily cash needs, cushioning the losses from crop failures, availability of manure for home gardens as well as for rice fields, use of female buffaloes for draught and an increase in total assets with progressive increase in herd size.

**Keywords:** Buffalo farming, small holders, farming systems research, technology transfer

## INTRODUCTION

Crop-livestock mixed farming is a hallmark of agricultural land use systems in Sri Lanka. Cultivation of rice in lowlands and vegetables and other subsidiary crops in highlands and in home gardens have always been considered as the primary occupation of the majority of smallholder farmers. It is estimated that nearly one third of smallholders keep cattle and/or buffaloes as a secondary activity (de Silva *et al.*, 1985; Abeygunawardena *et al.*, 1994). These animals are mostly of the indigenous type and are managed as large herds with minimum inputs. They are fed on natural pasture and fodder in common lands such as state reservations, scrub land around the village, catchment areas of the village irrigation tanks and in paddy fields after the harvest. The animals provide multiple outputs, mainly draught power for paddy cultivation, meat and milk. Although the quantity of milk is small it provides a regular cash income. The sale of excess animals, mainly males and old animals also brings in a cash income. Besides, livestock farming cushions the risk associated with crop failures and it is also a method of building the asset base of the rural households (Ozawa *et al.*, 1978; Richards and Agalawatta, 1981; de Silva *et al.*, 1985; Abeygunawardena *et al.*, 1991; Abayawansa *et al.*, 1993).

Though cattle and buffalo farming has been an integral component of rural agrarian systems, its existence has been threatened with increased fragmentation of agricultural land by the rapidly increasing population and also by the alienation of lands, hitherto considered as communal grazing land, for irrigated agriculture. Consequently, common pasture grounds have dwindled and movement of animals had to be restricted. Damage to crops by free roaming cattle and buffaloes have increased resulting in frequent conflicts between crop farmers and animal owners (Abeygunawardena *et al.*, 1991; Abayawansa *et al.*, 1993). However, the value of livestock farming, particularly cattle and buffalo has been increasing, as the need for supplementing the meager income from on-farm and off-farm activities has never been so great. Another constraint has been the seasonal fluctuation of pasture and fodder availability, which is closely associated with the distinct seasonal bimodal rainfall pattern. Limitations in nutrient supply and fluctuations in the quality of forage have been shown to effect the productivity and reproduction of both cattle and buffaloes (de Silva *et al.*, 1985; Perera *et al.*, 1987; Abeygunawardena *et al.*, 1994). The solution to this is to provide concentrates, but at the current farm gate price of milk, feeding concentrates is beyond the capability of subsistence farmers.

In the face of vast and rapid changes occurring in the socio-economic sphere of the country, there is a need to generate appropriate technologies and indeed, transfer these to the end users, the rural subsistence farmers, to help them to overcome pressing problems. During the past few decades, a vast amount of knowledge and technologies have been generated by the national and international research systems to overcome nutritional constraints. In the use of crop residues as ruminant feeds, technologies such as treatment with alkalies (Owen and Jayasooriya, 1989), or urea (Nolan and Leng, 1989), provision of bypass proteins and manipulation of energy density of ruminant diets (Nolan and Leng, 1989), use of molasses as a rapidly fermentable carbohydrate and as a palatable carrier for essential nutrients (*e.g.* urea and minerals) and as a source of trace minerals (*e.g.* sulphur, calcium, potassium) and the use of urea-molasses multinutrient mixtures (UMMM) to enhance the digestibility of coarse forages and crop residues, have been introduced. These are particularly useful with forage low in nitrogen, to enhance microbial protein synthesis in the rumen (Preston and Leng, 1987; Ranjhan, 1992). Unfortunately, most of these have not been delivered to end users and attempts made by the conventional research and extension system have fallen far below expectations. The exception has been the widespread adoption of UMMM formulations in ruminant feeding (Preston and Leng, 1987). Other frontier developments were in the fields of reproduction and health. A considerable amount of experimentation has been done to explore methods for improving the reproductive performance of animals, both under large farm and smallholder situations. The value of limited suckling by the calf during the postpartum period in improving postpartum fertility and the use of exogenous hormones to induce oestrus in postpartum anoestrous animals have been well demonstrated in Sri Lanka (Abeygunawardena *et al.*, 1995a; 1995b) as well as in other countries (Odde, 1980; Randel, 1981; Bastidae *et al.*, 1984 ). As regards health, work done in Sri Lanka has shown that appropriate anthelminthics administered to young buffalo calves once, between 10 and 16 days significantly reduces the effects of the most pathogenic gastrointestinal nematode, *Toxocara vitulorum* (Roberts, 1989).

In general, the conventional research and extension delivery systems have failed to disseminate the new information generated by research. This led to the emergence of farming systems research where the end user becomes an active partner of the entire experimentation and transfer process (Chambers *et al.*, 1983; Starkey, 1989). The objectives of this study were (a) to adopt the farming systems approach (b) to develop smallholder intensively managed model buffalo farm units, by incorporating technologies from the research, both of national and international origin and (c) to demonstrate the feasibility and cost and benefits of intensification of buffalo farming in smallholder production systems.

## MATERIALS AND METHODS

I. *Review of current knowledge and perceptions on buffalo farming*: At the commencement of the study, a workshop on the "Transfer of Technology on the Water Buffalo to the End Users" was conducted with participation of research scientists, animal production and health officers (veterinarians) and extension workers. At this workshop, it was noted that the buffalo farming systems in the country ranged from extensive to semi-intensive and to intensive management systems (classified on the basis of feeding and management practices). These systems have primarily evolved as a result of a multitude of factors such as agro-ecology, availability of grazing lands and the type of cropping systems. The extensive system is the most prevalent in the dry zone and in the dry-intermediate zone, which extends across nearly two thirds of the country. The semi-intensive system is found in the wet zone and wet-intermediate zone, particularly in the coconut triangle area and some of the dry zone irrigated settlement areas. Conversely, the intensive management system is rare and has evolved as a result of the limitation of land in peri-urban areas. At this workshop it was also highlighted that the sustainability of all 3 systems, particularly in the coconut triangle area and in irrigated settlement schemes (*e.g.* Mahaweli Project areas) were doubtful owing to the increasing pressure on agricultural land. It was further highlighted that in most areas, opportunities for underemployed labour in economic activities were low. Rearing animals for dairying, particularly buffaloes, due to its multipurpose potential such as milk rich in fat, draught and possibly for meat, may be of value to supplement the meager farm incomes. The ability of the buffalo to utilize coarse forages (Cockrill, 1974) was also considered as an added advantage. Intensification of buffalo (along with cattle) farming was recognized as the only option available to farmers in all these areas.

Based on these considerations, the appropriateness of enhancing the sustainability of ruminant production systems through introduction of low cost feeding systems based on crop residues and agro-industrial byproducts was agreed upon. Many studies have demonstrated that most of the native forages and crop residues are low in nitrogen and minerals and of low digestibility. In such situations, the use of urea and molasses as a feed supplement has proved useful. Though the ability of various chemicals such as alkali and urea to increase the digestibility of such feeds has been demonstrated, and the value of non-protein nitrogen in enhancing rumen microbial digestion is also well known, attempts made to popularize the use of chemically treated crop residues (*e.g.* urea-treated or ensiled rice straw) among smallholder farmers fell far below expectations, not only in Sri Lanka but also in other countries (Jayasooriya, 1993). Preston and Leng (1987) have reported that the incorporation of urea into molasses, a rapidly fermentable carbohydrate, as a source of ruminant feed found greater acceptability among farmers than feeding of other forms of nitrogen and mineral supplements because of the convenience. In addition to feeding, other practices such as limited suckling and the use of exogenous hormones in optimizing postpartum reproductive performance, the practice of strategic worm treatment of calves between 10 and 16 days of age and the regular prophylactic vaccination against endemic diseases in maintaining optimum health conditions in animals, were also stressed. The Workshop concluded that it would be appropriate to develop smallholder intensively managed buffalo units (SIMBU) through introduction of aforementioned and other new technologies, with the objective of testing the feasibility, acceptability and cost and benefits of such a systems approach. A further objective was to popularize the intensive rearing of buffaloes among resource poor rural farmers using this model as a demonstration unit.

II. *Selection of location*: A rapid rural appraisal (RRA) was conducted in the coconut triangle (intermediate zone) and in Mahaweli Systems B, C and H (dry zone) regions, to identify areas where farmers faced pressing problems related to (1) limitation of land for grazing, (2) seasonal fluctuations in availability of pasture and fodder, (3) availability of underemployed or surplus labour with low opportunity cost[1]. On the basis of the RRA, two buffalo rearing areas, the North-Western Province (Kurunegala Divisional Secretariat area) and Mahaweli System H area (Thambuttegama) were selected to conduct a survey to identify farming system and farm household constraints. A questionnaire was developed to collect information on (a) farm households (land and labour use pattern), (b) livestock farming (species, breed types, herd size, management practices, *etc.*), (c) income from livestock, if any and (d) limitations to buffalo and cattle farming. Based on the results of the survey and RRA, a cluster of smallholder farmers (n=9) in the village of Suddassanagama at Thambuttegama in Mahaweli System H area was selected to locate on-farm trials.

III. *Basic criteria for 'small-holder intensively managed buffalo units' (SIMBU) and feeding system:* The RRA indicated that the animals to be included into the SIMBU must be suitable for both milk and draught and, for the units to be economically viable, the average daily milk yield per unit must be more than 5-6 litres per day at current prices of liquid milk. Further, the use of costly concentrate feeds (*e.g.* coconut poonac and rice bran) must be kept to a minimum, while the use of crop residues (*e.g.* rice straw) and locally available tree fodders must be promoted. It was therefore decided that in order to overcome the nutrient deficiencies that would result, a feeding system for SIMBU should be formulated based on the following guidelines: (1) provision of a source of fermentable nitrogen (*e.g.* urea) to ensure the maintenance of the nitrogen level in rumen fluid in the range of 80 to 150 mg/l (2) maintain the rumen nitrogen concentration at a steady level; by the continuous supply of a non-protein nitrogen source (3) supply of a rapidly fermentable carbohydrate source (*e.g.* molasses) to ensure a more efficient rumen environment (4) a source of protein (*e.g.* coconut poonac) and (5) supply of minerals as most of the basal feeds are considered deficient in minerals.

---

[1] *Opportunity cost is defined as the likely income from the best alternative opportunity foregone.*

*IV. Development of the feeding system*: Based on previous experiences in many tropical developing countries (Preston and Leng, 1987; Combellas, 1991; Habib *et al.*, 1991; Hendratno *et al.*, 1991; Leng *et al.*, 1991; Saadullah, 1991) a urea-molasses-multinutrient mixture (UMMM) was selected as the catalytic feed supplement. Molasses, although not available in the immediate locality, is available at three large sugar factories. Rice straw is available in abundance in all rural areas but only a small proportion is currently used as a source of animal feed and organic fertilizer. Therefore, initial trials were conducted at the SAREC/NARESA Buffalo Research Station, Narangalla, to develop a feeding system based on these materials. A urea-molasses-multinutrient mixture (UMMM) with the composition given in Table 1, was fed in the form of a paste/lick to four heifers and two lactating cows for 12 weeks. The UMMM lick was prepared daily by first mixing the rice bran and dicalcium phosphate using the layer method. The urea, molasses, magnesium sulphate, common salt and the micro-mineral mixture

**Table 1.** Composition of the Urea-Molasses-Multinutrient Mixture (UMMM).

| Ingredients | Weight (g) |
|---|---|
| Rice bran (g) | 700 |
| Molasses (g) | 300 |
| Urea (g) | 65 |
| Dicalcium phosphate (g) | 50 |
| Magnesium sulphate (g) | 25 |
| Common salt (g) | 25 |
| Copper sulphate (mg) | 150 |
| Zinc oxide (mg) | 200 |
| Cobalt sulphate (mg) | 3 |
| Sodium selenite (mg) | 3 |

($CuSO_4.5H_2O$, $ZnO$, $CoSO_4.7H_2O$ and $Na_2SeO_4.10H_2O$) were dissolved in a minimum quantity of water. The latter was then added to the former and mixed thoroughly. The final mixture was transferred to a bucket and compacted with a wooden rod. The UMMM was left in the buffalo shed for the cows to lick at their own free will. Generally, around 1 kg was consumed completely within 2-3 hours. Water and rice straw were available ***ad libitum*** in the shed, and 1-2 kg of green forage (grass or *Glyricidia*) was provided per animal per day. No concentrates were fed. Based on the results (Table 2), this diet was selected as the standard for the model SIMBUs to be established at Mahaweli System H.

**Table 2.** Straw intake of female buffaloes fed on Urea-Molasses-Multinutrient Mixture (UMMM) during the on-station pilot study.

| Week | Straw intake | | | | | |
|---|---|---|---|---|---|---|
| | kg/h/d | SD ± | % LW | SD ± | g/kg $W^{0.75}$ | SD ± |
| 1 | 3.68 | 0.37 | 1.13 | 0.19 | 48.47 | 6.99 |
| 2 | 5.64 | 0.22 | 1.73 | 0.22 | 73.91 | 7.23 |
| 3 | 6.02 | 0.24 | 1.79 | 0.13 | 78.50 | 7.31 |
| 4 | 6.64 | 0.65 | 2.02 | 0.39 | 86.24 | 14.89 |
| 5 | 6.39 | 0.27 | 1.94 | 0.32 | 82.33 | 9.00 |
| 6 | 7.04 | 0.77 | 2.20 | 0.46 | 92.41 | 17.61 |
| 7 | 7.39 | 0.44 | 2.27 | 0.36 | 98.60 | 9.87 |
| 8 | 7.49 | 0.50 | 2.47 | 0.16 | 96.12 | 6.77 |
| 9 | 7.86 | 0.72 | 2.34 | 0.18 | 100.08 | 7.44 |
| 10 | 7.71 | 0.71 | 2.31 | 0.15 | 99.85 | 7.94 |
| 11 | 7.85 | 0.82 | 2.39 | 0.22 | 102.42 | 9.50 |

*V. Establishment of first model SIMBU at an institutional farm*: Initially a prototype SIMBU was established on a 0.2 ha plot of land at the Nirawiya Livestock Farm of the Mahaweli Authority. A shed with essential features to house 3 cows and their follower calves was constructed with locally available, low-cost material. The land was fenced and a portion was cultivated with pasture and fodder legumes. Tree fodder varieties such as *Glyricidia* and *Leucaena* were grown on the perimeter fence and as hedge-rows. Inter-row spaces were used for cultivation of short term cash crops. Compost manure was prepared in pits behind the shed and used to fertilize the crops. Shed washings were directed to the fodder plots. During each harvesting season, rice straw was collected and stacked on wooden stands. Two Surti buffalo cows and one heifer were introduced to the unit and fed on grass, tree fodder, straw and UMMM. Since no wallowing was allowed, the animals were sprinkled with water four times per day at regular intervals during mid-day and afternoon, using a simple sprinkler device made with a perforated 12 mm PVC pipe fixed above the animals. This model unit was used for demonstrating the proposed system to the selected farmers and as a location for providing the necessary training. Planting materials of improved varieties of fodder were also supplied to farmers from this unit.

*VI. Establishment of model SIMBUs in village farms:* The nine small-holder farm families selected from Suddassanagama were provided with technical advice and material for construction of the model farm units in their homesteads using family labour. Each unit was supplied with two Surti buffalo cows and one heifer purchased from a state farm. The cows gave an average lactation yield of 1018±347 litres over a lactation length of 270.9±87.1 days at the farm of origin. Regular training workshops, field days and discussions were held with the farmers, to transfer knowledge and skills required to implement improved feeding, management and disease control practices, as well as to obtain feedback on the responses to such improved technologies.

*VII. Feeding trials*: The feeding system was tested and refined during four successive phases of study. Initially, the animals were fed for one month on a diet similar to that given at the farm of origin. The basal roughage consisted of cut grass, tree leaves and rice straw provided *ad libitum*. This was supplemented with 3 kg coconut poonac and 3 kg rice bran per day (Table 3, Diet 1). Subsequently, the concentrate supplement was replaced with other formulations shown in Table 3. These consisted of feeding 1 kg/day of UMMM as a lick for four months (Diet 2), 1 kg/day of UMMM enriched with 50 grams of good quality fish meal as a lick for six months (Diet 3) and the UMMM as a solid urea-molasses-multinutrient block (UMMB), for 12 months (Diet 4).

**Table 3.** Composition of test diets given during the four phases of on-farm trials.

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | Diet 1 | Diet 2 (UMMM*) | Diet 3 (UMMM) | Diet 4 (UMMB†) |
| Coconut poonac (g) | 3000 | — | — | — |
| Rice bran (g) | 3000 | 700 | 700 | 300 |
| Molasses (g) | — | 300 | 300 | 400 |
| Urea (g) | — | 65 | 65 | 120 |
| Dicalcium phosphate (g) | — | 50 | 50 | 20 |
| Magnesium sulphate (g) | — | 25 | 25 | — |
| Common salt (g) | — | 25 | 25 | 30 |
| Copper sulphate (mg) | — | 150 | 150 | — |
| Zinc oxide (mg) | — | 200 | 200 | — |
| Cobalt sulphate (mg) | — | 03 | 03 | — |
| Sodium selenite (mg) | — | 03 | 03 | — |
| Fish meal (g) | — | — | 50 | 30 |
| Cement (g) | — | — | — | 100 |

* Urea-molasses-multinutrient mixture; †Urea-molasses-multinutrient block.

The feeding systems are summarized in Table 4. The weight of each UMMB was 5 kg, which was generally consumed by each buffalo cow over a period of 5-6 days.

**Table 4.** Feeding systems adopted during the four phases of on-farm trials.

| Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| Rice straw | *ad lib.* | *ad lib.* | *ad lib.* | *ad lib.* |
| Green fodder (kg) | 1 - 2 | 1 - 2 | 1 - 2 | 1 - 2 |
| Rice bran (kg) | 3 | --- | --- | --- |
| Coconut poonac (kg) | 3 | --- | --- | --- |
| UMMM* (kg) | --- | 1 | --- | --- |
| UMMM with fish meal (kg) | --- | --- | 1 | --- |
| UMMB⁺ (kg) | --- | --- | --- | 0.7-1.0 |

* Urea-molasses-multinutrient mixture ⁺ Urea-molasses multinutrient Block

*VIII. Data recording:* Individual milk yields were recorded daily by the farmers. This was verified at fortnightly intervals by a technician, who also measured feed intake and chest girth. Records on reproductive events, health status, expenditure and income were also maintained.

## RESULTS AND DISCUSSION

### Rapid Rural Appraisal and Sample Survey

The RRA revealed that the primary income generating activity of the settler farmers in the Mahaweli System H was paddy cultivation. Although cultivation is usually possible during both Maha (North-east monsoon, September-December) and Yala (South-west monsoons, April-June) seasons, cultivation had been limited to only the Maha season for many years because of the lack of water. A few farmers resorted to vegetable cultivation during periods when rice cultivation was not possible. The income from both activities was seasonal and hardly sufficient to meet the family cash needs. In most instances, these settlers were compelled to borrow money from village lenders to meet their cash needs and often became entangled in a vicious agricultural debt cycle. A few settlers kept livestock, mainly cattle and/or buffaloes and poultry. The percentage of settlers keeping cattle and/or buffaloes at this location was low when compared to other areas of the country (Abeygunawardena *et al.*, 1994). It was observed that many farmers were keeping two herds; a buffalo herd for draught and a cattle herd for milk, based on the farmer perception that the buffalo was suitable mainly for draught and not for milk. This practice placed a heavy strain on the limited resources available in terms of land, feed and labour.

The sample survey covered a total of 100 livestock farmers, selected randomly from the Mahaweli System H area. They were interviewed using a structured questionnaire. The average holding was 0.97±0.12 ha of lowland and 0.22±0.32 ha of highland. The average family size was 4.8±1.0 heads and 95% of the chief householders were engaged in farming on a full time basis. The lowland was primarily used for paddy (rice) cultivation under irrigation, but when water was not available, vegetables were grown. The highland area was used as a home garden with a variety of multipurpose trees such as coconut, jak, banana, *etc.* Of the farmers included in the survey, 71% reared buffaloes and 47% also kept cattle. As shown in Figure 1a, the average herd size was 6.3±4.1 for buffaloes and 4.1±2.9 for cattle. The animals were grazed on communal grazing lands during the day and paddocked in the farmers' homestead during the night. The extent of utilization of common grazing grounds by farmers was; road sides (53%), scrub areas (35%), paddy fields after the harvest (68%) and irrigation canal banks (9%). In most locations animals were tethered to prevent them from trespassing into other farm properties. Only 1% of farmers used pasture and fodder resources from their own land. Buffaloes were used exclusively for draught by 95% of farmers, while 14% also used cattle for draught. Cattle were used mainly for milk (72%), while only a few buffalo

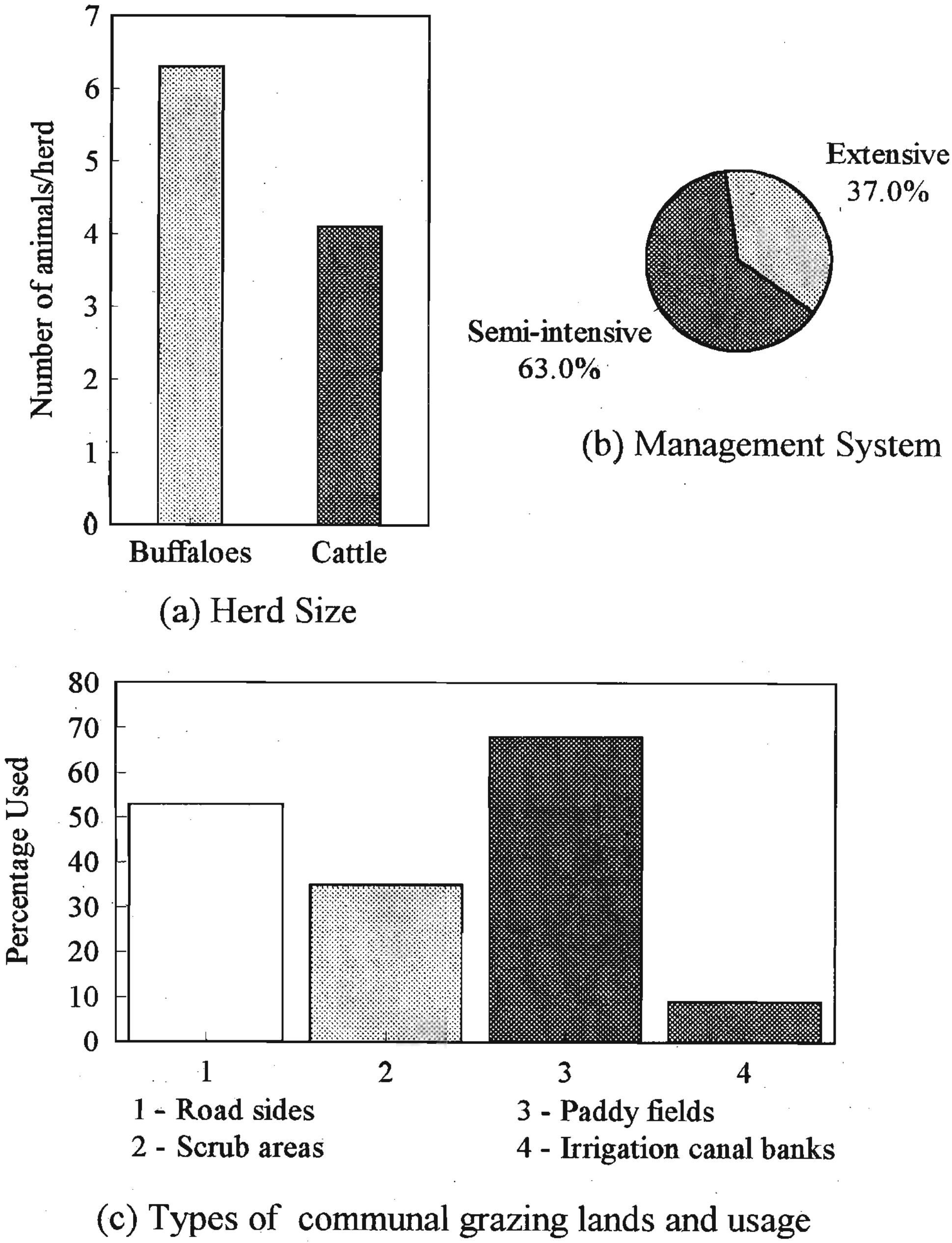



**Figure 1.** Average herd size, management systems and communal grazing areas of buffaloes and cattle.

owners (20%) milked their animals. The average milk yields for both buffaloes and cattle were 2.02 and 1.93 litres per animal, respectively. The average lactation length was 3.8±2.0 months for buffaloes and 5.7±2.3 months for cattle. The average daily milk production per farm was 3.28 litres from buffaloes and 4.05 litres from cattle. The contribution of farm power usage was 44.2% with animals, 30.5% from tractors and 23.3% from humans. Some farmers (23%) rented out buffaloes for draught to neighbouring farmers. In both buffalo and cattle farming, the major management constraints were the scarcity of grazing grounds (72%), restrictions on animal movements (17%) and trespassing (9%). Although crop residues were available in large quantities, only very few used these as animal feed (rice straw- 25%, maize stover- 17% and cowpea stover- 21%).

## On-Station Trials

The results of the on-station pilot study conducted at Narangalla Buffalo Research Farm are given in Table 2. Rice straw was provided *ad libitum* as the basal diet. The daily intake of straw increased gradually from around 4 kg to over 7 kg. This may have been due to the influence of the UMMM which increased the digestibility of the basal feed. Previous studies (Nolan and Leng, 1986) have shown that the limitations of rice straw feeding were (1) low rate of intake, (2) low total digestibility, (3) low propionate fermentation pattern in the rumen and (4) negligible content of both fermentable nitrogen and by-pass protein. There are a number of ways in which some of these limitations may be overcome, and of these the provision of urea with molasses proved to be the most convenient and acceptable to farmers (Preston and Leng, 1987). Many workers (Preston and Leng, 1987; Habib *et al.*, 1991) have shown that UMMM formulations improved the intake, especially of poor quality roughages . This improvement in the intake has been shown to be due to the maintenance of the rumen ammonia level at optimum levels (80 -200 mg N/l), and also at a constant level, which enhanced microbial cell wall synthesis and thereby increased the microbial population and activity (Preston and Leng, 1987). An additional benefit of the urea and molasses combination is that it is rich in rapidly fermentable carbohydrate and minerals which are readily available for microbial metabolism. Both these enhance the rumen microbial digestion of roughages which in turn increases the rate of passage of feed ingested resulting in an increase in feed intake (Preston and Leng,1987; Habib *et al.*, 1991). Roughage dry matter intake expressed as a percentage of body weight, increased over the experimental period from an initial value of 1.13±0.19% to 2.93±0.22% with a mean value of 2.0%. A similar trend in the roughage intake on the basis of metabolic body weight from 48.5±6.9 to 102.4±9.5 g/Kg $W^{0.75}$ was exhibited. This increase of 111% occurred within 11 weeks, and can be attributed to the effects of UMMM, which probably rendered a 'catalytic' effect rather than a 'supplementary' effect, as described above.

## On-Farm Trials

*Feeding and management system:* Animals were stall-fed on a basal diet consisting of natural green grass, tree fodder and rice straw provided *ad libitum*. The composition of the basal diet varied with the time of year; i.e. more green matter was fed during the wet season and more rice straw during the dry season. Animals were also given water *ad libitum*. As a means of alleviating heat stress, a few buckets of water were splashed on to the animals at regular intervals (2-4 hrs), during the warmest part of the day (11 am to 4 pm). Calves were kept separate from the cow in a calf shed and allowed to suckle for 30 minutes twice a day, following the morning and evening milking. This suckling management procedure has been shown to optimize both calf growth and postpartum reproduction as opposed to unrestricted suckling or early weaning (Abeygunawardena *et al.*, 1995b). No additional milk was given to the calves but hay and green grass were made available from a very early age. Calves were treated with pyrantel (250 mg per calf) at about 14 days of age, against *Toxocara vitulorum* which is considered to be the most prevalent and pathogenic gastrointestinal parasite (Roberts, 1989; Roberts, 1993). Animals were also vaccinated against foot and mouth disease, haemorrhagic septicaemia and black quarter disease, according to recommendations of the Department of Animal Production and Health.

*Composition of UMMM formulations:* The composition of the diets was modified during successive periods with a view to improving the quality of feed, while aiming to maintain the same level of productivity of the animal but at a lesser cost. As shown in Table 4, the conventional supplement (Diet 1) was replaced by UMMM. The composition of the UMMM was modified over time while monitoring the responses. In Diet 2, urea was provided at the rate 6.5% in the mixture. In Diet 3, the composition of the UMMM was essentially the same except that the mixture was enriched with 50 g of fish meal to provide bypass protein. When the UMMM was converted into a more convenient brick form (UMMB), the urea content was increased to 12% (Diet 4). Another change in Diet 4 was the ommision of minerals. This was done on the assumption that urea and molasses which are rich in minerals would be able to meet the requirements of the animals.

*Feed intake:* The data on daily feed intake and body weight are depicted in Figure 2. The feed intake on fresh weight basis was lower on Diet 4 than on Diets 2 and 3, which was due to the high content of straw in the roughage diet given with Diet 4 compared to that given during previous phases (Figure 2a). This resulted primarily from the increasing preference by farmers to use straw, as animals consumed it more readily than they had done earlier. When the intake was expressed on the DM basis (Figure 2b), the intake increased from 10.4 kg/day on Diet 3 to 11.4 kg/day on Diet 4. This increase is similar to that observed in the pilot study and also is in agreement with the work of many other workers (Preston and Leng, 1987; Habib *et al.*, 1991; Ranjhan, 1992). The increase in consumption of feed is attributed to the increased rate of passage (Preston and Leng, 1987).

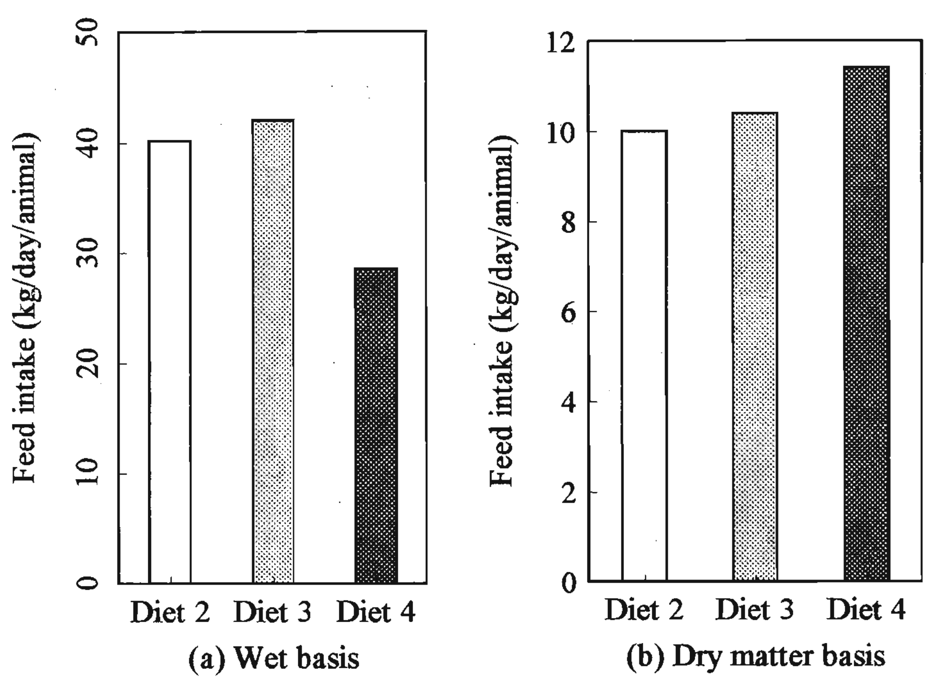
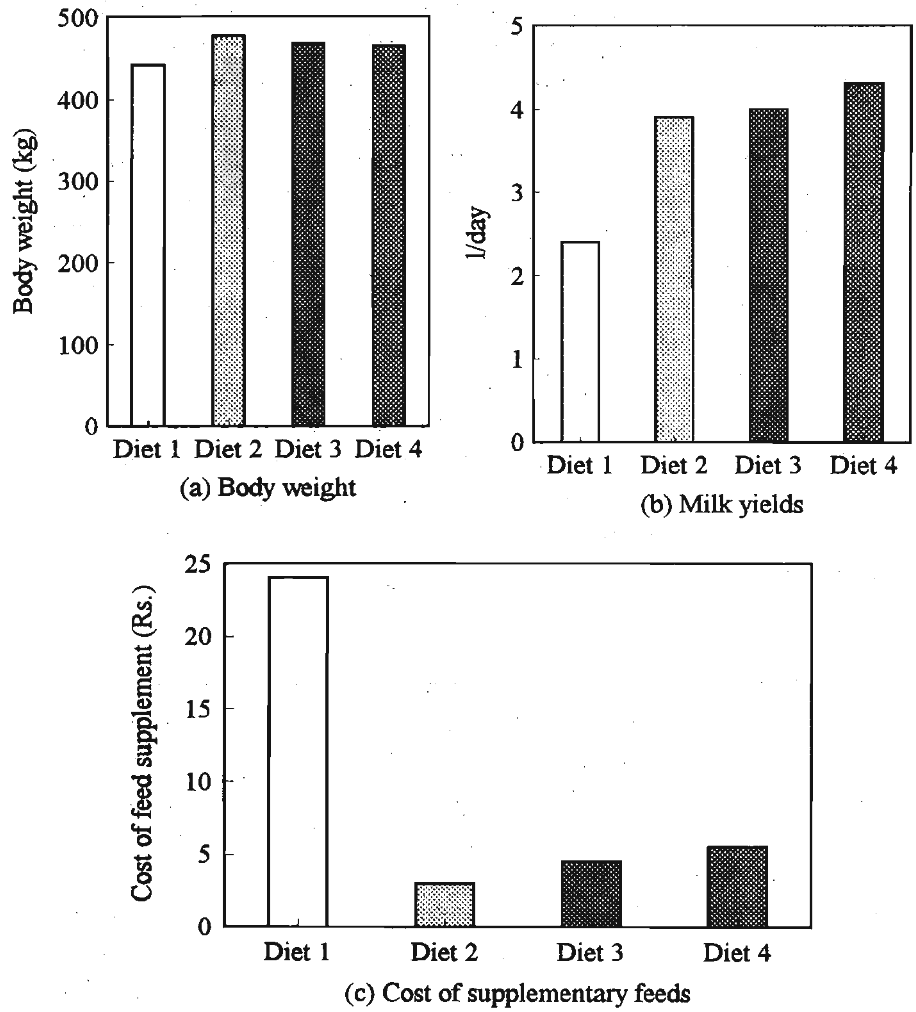



**Figure 2.** Feed intake of adult buffaloes in on-farm trials: (a) fresh weight basis and (b) dry matter basis.

*Body weight:* As shown in Figure 3a, there was an increase in body weight when the animals were fed UMMM as a lick, and this was maintained even when UMMM was fed as a brick. This increase was most likely due to the anabolic effect through an increase in feed intake. It is possible that the beneficial effects were brought about by the fermentable nitrogen provided as urea which maintained optimum levels of ammonia in the rumen resulting in an increase in rate and extent of digestion and an increase in microbial protein yield relative to volatile fatty acid production (Preston and Leng, 1987). The UMMM is also expected to provide other factors which enhances microbial cell synthesis such as glucose, amino acids, minerals (including S, K and P).

*Milk yield:* The milk yields ranged from 1.6 to 8.2 litres per animal per day (average 2.4±2.6 litres/day) before UMMM feeding (Figure 3b). The daily individual milk yields on Diets 2, 3 and 4 ranged between 1.4 to 9.9, 1.9 to 11.1 and 2.0 to 8.3 litres respectively, with mean values of 3.9±1.12, 4.0±2.32 and 4.3±1.40 liters per day, respectively, as shown in Figure 3b. Thus the animals were able to maintain or improve their milk yields when switched from traditional concentrate to UMMM supplementation, given either as a paste or as brick. The stimulatory effect of UMMM on milk yield of animals fed poor quality forages has been well demonstrated in many countries (Kunju, 1986; Preston and Leng, 1987). Preston and Leng (1987) demonstrated that feeding UMMM as a brick, resulted in an increase in both the feed intake and milk yield. They further demonstrated that animals consuming the UMMM provided as a brick had higher rumen ammonia concentrations and there was a significant relationship between the milk yield and rumen ammonia concentration.

*Reproduction:* The reproductive performance of animals after transfer to the SIMBUs was comparable to that observed in the farm of origin as well as in other state farms. The annual calving rate during the year 1994/95 was 62% and the calving interval was 584±80 days. The farmers were educated on oestrus detection and animals were hand mated following observed oestrus. If oestrus was not detected by 120 days postpartum, hormonal induction of oestrus was done using progesterone ear implants (Crestar, Intervet) for 10 days followed by timed artificial insemination or natural mating. This hormonal treatment was shown to be the most effective in inducing oestrus in postpartum anoestrous buffaloes (Abeygunawardena *et al.*, 1995c). The cost of this treatment was approximately Rs. 500 (US$10) and this was within affordable limits of the smallholder farmers. It was cost effective considering the increased milk yield obtainable during the life time of the cow.




| | | |
|---|---|---|
| Diet 1 | - | Coconut poonac (3 kg) + Rice bran (3 kg) |
| Diet 2 | - | UMMM paste (1 kg) |
| Diet 3 | - | UMMM paste with 50 g fish meal (1 kg) |
| Diet 4 | - | UMMB with 50 g of fish meal ( 1 kg) |

**Figure 3.** Body weights, milk yields and cost of feeding of adult buffaloes in the on-farm trials .

*Birth weight of calves and pre-pubertal growth rates:* The average birth weight of calves born on the SIMBUs was 30.42± 1.9 kg. This was slightly higher than the average of 27.1±4.0 kg recorded in state farms by Abeygunawardena *et al.* (1995a). The increase of 8.4% may be attributed to the beneficial effects of UMMM on maternal nutrition as shown by Lindsay *et al.* (1982). The pre-pubertal growth rate during the first 12 months (256±73 g/day) was similar to the mean of 250±130 g/day recorded for Surti buffaloes on state farms (Abeygunawardena *et al.*, 1995a). The growing animals, however were not given UMMM supplementation.

*Cost of feeding:* As shown in Figure 3c, the cost of feeding the UMMM either as a paste or a brick was considerably less than that of the standard diet given at the farm of origin. The reduction of cost from Diet 1 to Diet 2 was 87.5%, but the cost of Diet 3 was slightly higher that Diet 2 due to inclusion of fish meal. A further increase in cost occurred with conversion of UMMM paste into a brick form, but this was deemed acceptable given the convenience and greater acceptability of the brick by farmers.

## Costs, Benefits and Farmers' Perceptions

The approximate total cost of establishing the model SIMBUs was Rs. 45,000 (US$ 1=Rs. 55), including the cost of the shed and animals. The farm families provided labour for the construction of the sheds and for maintenance of the units. The enthusiasm and willingness of the farmers to participate in the project was very high. The costs borne by the farmers were mainly in terms of family labour for maintenance of the shed, collection of straw, provision of feed and care of the animals and assisting project personnel in keeping records of inputs and outputs. Direct financial costs were for farm implements and veterinary care. The opportunity cost of labour for other income generating activities appeared to be low.

The major benefit to the farmers was the regular cash flow from sale of milk. The average daily milk yield per household was 4.3±1.4 litres. At the current farm gate price of liquid milk this would yield Rs 51.70 per day. If converted to a value added product such as 'curd', the daily earnings would increase to approximately Rs 129.20 per day. Assuming that the cost of labour and energy for heating the milk (usually from firewood) is zero, the revenue can be considered as a net income of the household. This supplementary income, whether from liquid milk or from value added products, was considered sufficient to meet the daily cash needs thus obviating the need to borrow money from village lenders, a practice all too common in the region. It was also observed that during long spells of drought, these households were the only ones which had a regular cash income, as other agricultural activities such as rice or vegetable cultivation were not possible.

Other benefits observed were (a) use of dung as manure in both home garden plots and the paddy fields, (b) use of non-lactating dairy buffaloes for draught and (c) the increase in total assets owned by farmers through increase in herd size with time. The average herd size at the commencement of the trial was 4.2 and after 2.5 years it had increased to 8.1 animals. Given the current market prices of Rs. 10,000-16,000 for animals aged 2-3 years, this is a promising method of improving the asset base of small holder farmers. One important observation was that these model units made heavy demands on family labour and on leisure time of the farmers. In two units where family labour was limited, the performance was poor and the families have conceded their inability to cope with the management demands of the intensive system.

Overall, the project has clearly demonstrated the extent of potential resources available to rural small-holders (such as family labour, crop residues and tree fodder) which have very low or zero opportunity costs when no alternative uses are available. It has also shown the benefits of using buffaloes for milk production, which were hitherto considered purely as draught animals. Although, these farmers were very skeptical of new technology at the inception and hence reluctant to change their management practices, they have gradually learnt to accept the technologies delivered through this project, as positive results were forthcoming. In many areas of the dry zone, not only in irrigated new settlement areas, but also in 'purana' or traditional villages, extensive rearing of cattle and buffaloes has become difficult. Several studies have highlighted the need for intensification in order to sustain this valuable rural industry (Ozawa *et al.*, 1978; Abayawansa *et al.*, 1993; Abeygunawardena *et al.*, 1994). As shown in this study, intensification of buffalo and cattle farming with dairy type animals can be a viable option. The model SIMBUs developed through this study can be adapted to suit each local environment and the capital costs can be reduced by using low cost housing material.

This study also demonstrates the value of the farming systems research (FSR) approach in small-holder livestock technology generation and transfer, which is novel to livestock research in Sri Lanka. Using FSR, this study demonstrated a viable, cost effective solution to pressing problems faced by the resource poor smallholder peasant farmers. The FSR differs from the conventional transfer of technology approach (TOT) in many aspects (Ellis, 1992). Firstly, needs of farmers were identified through close interaction with them. Secondly, farmers became active partners in the experimentation and transfer processes. Thirdly, farmers became convinced of the value of new technologies by demonstration of positive responses, cost and benefits within their own land holdings. Fourthly, the main mode of dissemination of new innovations was from farmer to farmer with research and extension workers acting as facilitators. The TOT which was the popular model in 1970s and early 1980s proved disappointing when dealing with resource poor farmers operating under diverse ecological conditions with complex agronomic practices, poor markets and high risk climatic conditions (Chambers *et al.*, 1989; Ellis, 1992). FSR emerged subsequently and became popular due to the involvement of the farmer as an active player in the technology generation and transfer process, rather than a passive recipient. It also departs from the conventional model which implies a linear transfer mechanism, in that the emphasis is given to the constant feed-back between researchers and farmers, which ensures progressive refinement in the process of technology generation and transfer. In the present study, the farmers were involved from the inception, in constraint and goal identification, in actual experimentation and technology generation, and in the refinement and transfer processes.

In conclusion, this study demonstrates (1) the appropriateness and the applicability of new technology generated by conventional research (i.e. use of urea-molasses multinutrient formulations as feed supplements for ruminants; limited suckling and use of exogenous reproductive hormones for optimizing postpartum reproduction), (2) the feasibility of rearing buffaloes intensively on a low cost feeding system based on crop residues (rice straw) and agro-industrial byproducts (molasses), through the use of idle family labour, in an area where grazing land has dwindled and animal movements severely restricted, (3) value of intensive buffalo rearing as a regular income generating activity which would help farmers to avoid the vicious rural agricultural debt cycle and (4) the value of a farming systems approach to technology generation and transfer for improved livestock production by resource-poor peasants in rural Sri Lanka.

## Acknowledgements

This study was conducted with the financial assistance from the SAREC/NARESA Buffalo Research Programme. The cooperation of the National Livestock Development Board, Mahaweli Livestock Development Division and more importantly of the nine small-holder farmers at Sudassanagama in Mahaweli System H are gratefully acknowledged. We are indebted to the research assistants (U.B. Herath, P. Nadasiri and D.K. Dayananda) and to the technical assistants (Janaka Herath, Abeykoon Banda and Jayadasa Weeratunga, and Ramani Ruwanpathirana) who have worked with commitment and enthusiasm to make this exercise very fruitful. The constant encouragement and guidance of Dr. J.A.de S. Siriwardene, coordinator of the SAREC/NARESA Buffalo Information Dissemination Programme, is gratefully acknowledged.

## References


Abeygunawardena, H., Abayawansa, W.D., Rathnayake, D. and Jayatilake, M.W.A.P. (1994) Zebu cattle farming in Sri Lanka: Production Systems and Reproductive Characteristics. In: *Proceedings of the Final Research Coordination Meeting of an FAO/IAEA Co-ordinated Research Programme on "Strengthening Research on Animal Production and Disease Diagnosis in Asia through the Application of Immunoassay Techniques.*, IAEA-TECDOC-736, IAEA, Vienna, Austria, pp.37-51.

Abeygunawardena, H., Kuruwita, V.Y. and Perera, B.M.A.O. (1995a) Effects of different suckling regimes on postpartum fertility of buffalo cows and growth rates and mortality of buffalo calves. In: *These Proceedings* .

Abeygunawardena, H., Kuruwita, V.Y. and Perera, B.M.A.O (1995b) Effects of exogenous hormones on postpartum anoestrous buffaloes. In: *These Proceedings*.

Abeygunawardena, H., Abayawansa, W.D. and Perera, B.M.A.O. (1995c) Comparative study of reproductive and productive characteristics of indigenous swamp and exotic river type buffaloes in Sri Lanka. In: *These Proceedings*.

Abeygunawardena, H., Jayatilake, M.W.A.P. Abayawansa, W.D. and Ratnnayake, D. (1991) Opportunities and limitations of cattle farming in dry zone of Sri Lanka. *Sri Lanka vet. J.*, **38,** 26-27.

Abayawansa, W.D., Abeygunawardena, H. and Jayatilake, M.W.A.P. ( 1993) The traditional cattle farming system in the dry zone: A case study. *Trop. Agric. Res.*, **5,** 368-377.

Bastidae, P., Troconiz, J., Verdi, O. and Silva, O. (1984) Effect of restricted suckling on pregnancy and calf performance in Brahman cows. Theriogenology **21,** 289-293.

Chambers, R. (1983) *Rural Development - Putting the Last First.* Longman Group Ltd.: Essex, UK.

Chambers, R., Pacey, A. and Thrupp, L.A. (1989) *Farmer First - Farmer Innovations and Agricultural Research.* Intermediate Technology Publications: London, U.K.

Cockrill, W.R. (1974). *The Husbandry and Health of the Domestic Buffalo.* Food and Agricultural Organization: Rome, Italy.

Combellas, J. (1991) The importance of urea-molasses blocks and bypass protein in animal production: the situation in tropical Latin America. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*, pp. 115-131.

de Silva, L.N.A., Perera, B.M.A.O., . Tilakaratne, L. and Edqvist, L-Q. (1985) *Production Systems and Reproductive Performances of Indigenous Buffaloes in Sri Lanka.* Monograph, Swedish University of Agricultural Sciences: Uppsala, Sweden.

Ellis, F. (1992) *Agricultural Policies in Developing Countries.* Cambridge University Press: U.K.

Habib, G., Basit Ali Shah, S., Wahidullah, G. Jabbar and Ghufranullah. (1991) The importance of urea-molasses blocks and bypass protein in animal production: the situation in Pakistan. In: *Proceedings of Symposium on 'Isotope and Related Techniques in Animal Production and Health.* pp. 133-144.

Hendratno, C., Nolan, J.V. and Leng, R.A. (1991) The importance of urea-molasses multinutrient blocks for ruminant production in Indonesia. In: *Proceedings of Symposium on ' Isotope and Related Techniques in Animal Production and Health.* pp. 157-169.

Jayasooriya, M.C.N. (1993) Use of crop residues and agro-industrial byproducts in ruminant production systems in developing countries. *Animal Production in Developing Countries. Occasional Publication.* No. **16,** pp. 47-55.

Kunju, P.J.G. (1986) Urea molases block lick: a feed supplement for ruminants. In: *Rice Straw and Related Feeds in Ruminant Rations*. Eds. Ibrahim, M.N.M. and Schiere, J.B. Proceedings of International Workshop. Kandy, Sri Lanka. pp. 261-274.

Leng, R.A., Preston, T.R., Sansoucy, R. and Gorge Kunju, P.J. (1991) Multinutrient blocks as a strategic supplement for ruminants. *World Anim. Rev.* **67,** 11-19.

Lindsay, J. A., Mason, G.W.J. and Toleman., M.A. (1982) Supplementation of pregnant cows with protected proteins when fed tropical forage diets. In: *Proceedings of the Australian Society of Animal Production.* **14,** 67-78.

Nolan, J.V. and Leng, R.A. (1989) Manipulation of rumen to increase ruminant production. *Feeding Strategies for Improving Productivity of Ruminant Livestock in Developing Countries*, IAEA, Vienna. pp. 149-166.

Odde, K.G. (1990) A review of synchronization of oestrus in postpartum cattle. *J. Anim. Sci.* **68,** 817-830.

Owen, E. and Jayasooriya, M.C.N. (1989) Recent developments in chemical treatment of roughages and their relevance to animal production in developing countries. IAEA, Vienna. pp. 205-230.

Ozawa, S., Buvanendran ,V. and Gurusinghe, R. (1978) Survey of production characteristics, feeding and management practices of cattle in some regions of the dry zone of Sri Lanka. *Ceylon vet. J.* **26,** 10-13.

Perera, B.M.A.O., de Silva, L.N.A., Kuruwita, V.Y. and Karunaratne, A.M. (1987) Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at selected village locations in Sri Lanka. *Anim. Reprod. Sci.*, **14,** 115-127.

Preston, T.R. and Leng, R.A. (1987) *Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-tropics*. Penambul Books: Armidale, Australia.

Randel, R.D. (1981) Effect of once-daily suckling on postpartum interval and cow-calf performace of first calf Brahaman x Hereford heifers. *J. Anim. Sci.* **53,** 755-757.

Ranjhan, S.K. (1992) Carabao feeding and nutrition. In: *Carabao Production in the Philippines*. Eds. S.K. Ranjhan and P.S. Faylon. Field Document No. 13. Philippine Council for Agriculture, Forestry and Natural Resources Research and Development, Los Banos, Laguna, Philippines. pp. 131-162.

Richards, E.M. and Agalawatta, M. (1981) An inter-agroecological zone survey of cattle and buffalo management practices in Sri Lanka. *FAO Technical Report, CPC/SRL/30-SWE.*

Roberts, J.A. (1989) *Toxocara vitulorum:* treatment based on the duration of the infectivity of buffalo cows *(Bubalus bubalis)* for their calves. *J. vet. Pharmacol. Therap.* **12,** 5-13.

Roberts, J.A. (1993) *Toxocara vitulorum* in Ruminants. *Vet. Bull.* **63,** 545-568.

Saadullah, M. (1991) The importance of urea-molasses blocks and bypass protein in animal production: the situation in Bangladesh. In: *Proceedings of Symposium on ' Isotope and Related Techniques in Animal Production and Health.* pp. 145-156.

Starkey, P. (1989) Farming system research relating to animal power- An overview. In: *Proceedings of an International Research Symposium on 'Draught Animals in Rural Development'* Eds. D. Hoffman, J. Nari and R.J. Petheram, ACIAR Proceeding No. 27. pp. 82-93.

NA-143-95

# SUPPLY RESPONSE OF MILK AND MANAGEMENT EFFICIENCY OF CATTLE AND BUFFALO PRODUCTION IN SRI LANKA: A CROSS SECTION STUDY IN WET ZONE AND DRY ZONE DISTRICTS

C. Bogahawatte

*Department of Agricultural Economics, Faculty of Agriculture, University of Peradeniya, SRI LANKA*

**Abstract:** Milk accounts for approximately 3% of the daily per capita calorie consumption and 4.5% of the protein intake of an average Sri Lankan. Nearly 70% of the rural population is engaged in milk production. However, the domestic milk production from cattle and buffaloes is low and not sufficient to meet the local demand.

The main objective of this study was to determine the supply response of milk and management efficiency of cattle and buffalo farms in Kandy, Matara, Hambantota and Polonnaruwa districts in Sri Lanka. Kandy and Matara districts in the wet zone and Hambantota and Polonnaruwa districts in the dry zone were selected purposively. The sample consisted of 86 farmers from Kandy, 72 farmers from Matara, 75 farmers from Hambantota and 85 farmers from Polonnaruwa. Both cattle and buffaloes were reared in their farms. A field survey using a questionnaire was carried out between April 1991 and March 1992. The supply response model consists of milk productivity and herd size equations estimated by Ordinary Least Squares. The management efficiency of buffalo and cattle production was estimated by using the method of Timmer (1980) as modified by Russel and Young (1983).

There was considerable variation in the supply elasticities estimated for the districts. The supply elasticities for cow milk and buffalo milk in Kandy, Matara, Hambantota and Polonnaruwa were inelastic and low, indicating poor price responsiveness of the producers.

The result of technical efficiencies showed that the majority of the cattle and buffalo farms in the districts were not within 10% of their potential technical efficiencies. The variations of technical efficiencies between cattle and buffalo farms were relatively high within each district.

**Keywords:** Milk, supply response, management efficiency

## INTRODUCTION

The contribution of livestock to the national gross value of agriculture is estimated at 6 percent and milk production contributes only about 1.2 percent to the Gross Domestic Product (GDP) of Sri Lanka (Livestock Statistics, 1992). The value of the contribution of the livestock subsector to agriculture in the country is low (Economic and Social Statistics of Sri Lanka, 1993) as compared to other countries of South and South East Asia such as Pakistan (18 percent) and Philippines (30 percent). It is an important source of supplementary income for nearly 70 percent of rural agricultural peasants as 95 percent of the cattle and buffalo population in the country is owned by rural small-holders or by estate labour. In the peasant livestock holdings nearly 30-60 percent of the gross farm income is generated from livestock activities. Of the 10-11 million of farming population in the country nearly 3.5 million are directly involved in livestock (Sri Lanka Livestock Sector Policy Review, 1992). According to the population estimates in 1993, there are nearly 1.716 million cattle and 0.831 million buffaloes in the country. Of the total herd, 23 percent of cattle and 8.5 percent of buffalo are in production (Livestock Statistics, 1993). The annual per capita consumption of milk of 19.87 litres is much below the level of 41.6 litres recommended by the Medical Research Institute, to meet the nutrient requirements of an average Sri Lankan.

Like in other developing countries, Sri Lanka is also not self sufficient in milk, eventhough milk production is the principal element of the dairy sector. The daily consumption of milk is about 1.64 million litres/day . However the domestic production is estimated to be 0.864 million litres/day (Annual Report, Central Bank of Sri Lanka, 1993). During 1981-91, the value of milk imports increased from Rs 405 million to Rs 2796 million. In 1992 the value of milk imports was Rs 2943 million. The tariff on imported milk and milk products of 15 percent resulted in a price discrimination between the prices of domestic and imported milk and milk products.

The specific objectives of this study were to (i) determine the supply response of cattle and buffalo milk production in the wet zone districts of Kandy and Matara and dry zone districts of Polonnaruwa and Hambantota (ii) to determine the technical efficiency of cattle and buffalo farms in the above districts. Based on the results of the study, policies that are required for higher milk production in these districts are suggested. The estimates are based on a field survey done in these districts between 1991 and 1992.

## METHODOLOGY

### Data Collection

Kandy and Matara districts from the wet zone and Polonnaruwa and Hambantota districts from the dry zone were purposively selected for the study, to represent the major agroclimatic regions of the country. In each district the Divisional Secretaries (DS) divisions formed the primary basis of sampling. From each DS division, a random sample of cattle and buffalo farmers were selected for the field survey (Table 1). The selected farmers were interviewed using a questionnaire and information pertaining to their socio-economic characteristics, cattle and buffalo production and institutions were collected for the period 1991-92. In addition to the field survey, a participatory survey was also done with the assistance of field level livestock officers in the districts.

**Table 1.** Description of the socio economic field survey in the districts.

| District | Divisional Secretaries Divisions | No. of Farmers surveyed |
|---|---|---|
| Kandy | Gangaihala | 3 |
| | Thumpane | 6 |
| | Harispattuwa | 9 |
| | Meda Dumbara | 8 |
| | Udu Dumbara | 20 |
| | Minipe | 40 |
| | Total | **86** |
| Matara | Dikwella | 11 |
| | Kamburupitiya | 12 |
| | Matara | 31 |
| | Weligama | 15 |
| | Devinuwara | 3 |
| | Total | **72** |
| Polonnaruwa | Polonnaruwa | 8 |
| | Dimbulagala | 10 |
| | Tamankaduwa | 10 |
| | Hingurakgoda | 20 |
| | Lankapura | 36 |
| | Total | **84** |
| Hambantota | Suriyawewa | 4 |
| | Lunugamwehera | 8 |
| | Hambantota | 9 |
| | Weeravila | 18 |
| | Tissamaharamaya | 38 |
| | Ambalantota | 2 |
| | Total | **76** |

## Analytical Method

*Supply response of milk:* The total production of milk (litres/farm) is composed of the average milk production per cow (litres/cow) and herd size, i.e.

$$Si = Hi \cdot Yi \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where,

| | | |
|---|---|---|
| $Si$ | = | Supply of milk of i th farm (litres/farm); |
| $Hi$ | = | Herd size/farm; |
| $Yi$ | = | Average yield per cow (litres/farm). |

The supply elasticity for i th farm could be derived as follows.

$$dS = (S/H)\,dH + (S/Y).\,dY$$
$$dS/dP = (S/H)\,(dH/dP) + (S/Y)\,(dY/dP)$$
$$dS/dP.\,P/S = (S/H).(dH/dP).P/S + (S/Y).(dY/dP)\,.P/S$$

$$e_{s.p} = e_{h.p} + e_{y.p} \quad \ldots\ldots\ldots\ldots\ldots \quad (2).$$

where,

| | | |
|---|---|---|
| s.p | = | Total supply elasticity |
| h.p | = | Elasticity of herd |
| y.p | = | Elasticity of yield |

The yield and herd equations for the different districts were in linear or logarithmic forms and estimated by the method of Ordinary Least Squares. The estimated coefficients were used in computing the supply elasticities.

*Efficiency of cattle and buffalo management:* The economic efficiency of management could be used as an index to evaluate the performances of farms operating under different conditions. The differences in the farms may be caused by the pricing and technical efficiencies. Assume a profit function,

Maximizing $\prod = P.\,f(X1,X2,\ldots Xn;\,Z1,Z2\ldots Zm;\,e) - \sum Wi\,Xi \quad \ldots\ldots(3)$

where,

| | | |
|---|---|---|
| $\prod$ | = | profit earned by the farm in Rs ; |
| X1,X2,......Xn | = | variable inputs; |
| Z1,Z2,......Zm | = | fixed inputs; |

Wi (i=1.....n) = prices of the variable inputs;
e = residual term.

The first order condition of $\partial\prod/\partial X_i = Pf_i - W_i = 0$ states that the producer would be price efficient if marginal value product of using Xi equals its input price. The technical efficiency is measured against the most efficient production frontier that can be achieved under the existing technological condition of production in the farm. A simplified version of estimating the efficient production frontier as suggested by Russell and Young (1983) was based on earlier methods of Aigner and Chu (1968), Farrell (1975) and Timmer (1980). Here the production frontier was derived by using a linear production function. The intercept term of the estimated function was corrected by the addition of the highest positive residual which was generated in the estimated procedure. The technical efficiency (TE) is defined by,

$TE = Y_{j,a}/Y_j$ ..............................................(4)

or potential output of farm j, if it were on the frontier.

## RESULTS AND DISCUSSION

### Supply Response of Milk Production

The results of the yield and herd equations for cattle and buffalo in the districts are presented in Appendix Tables 1 and 2. These estimates were used for the calculations of the yield and herd size supply elasticities for cattle and buffaloes in the wet zone and dry zone districts (Table 2). In general, the yield and herd elasticity of * and ** : significant at 5 percent and 1 percent respectively for cattle and buffaloes were low and inelastic in the wet and dry zone districts. There was a high variation of elasticities between and within the wet zone and dry zone districts. Other than in Hambantota, the supply elasticities of milk were higher for cattle as compared to buffaloes in the districts.

**Table 2.** Supply response of milk production in the wet zone and dry zone districts.

| District | Cattle | | | Buffalo | | |
|---|---|---|---|---|---|---|
| | Yield elasticity | Herd elasticity | Supply elasticity | Yield elasticity | Herd elasticity | Supply elasticity |
| Kandy | 1.1979 | 0.1607 | 0.1925 | | | |
| Matara | 0.0007 | 0.0019 | 0 | 0.0016 | 0.0266 | 0 |
| Polonnaruwa | 0.0878 | 0.1632 | 0.0143 | 0.0057 | 0.2671 | 0.0015 |
| Hambantota | | 0.0987 | 0.0987 | | 0.5299 | 0.5299 |

In Kandy district the yield elasticity of milk was high and elastic as compared to herd size elasticity. For example, an increase of milk price by 10 percent would increase the average yield per cow by 1.2 percent as compared to 1.6 percent for herd size per farm. The relatively low response of herd size elasticity makes the total milk supply elasticity low and inelastic, indicating that dairy farmers were not price responsive in this district. In Kandy the yield and herd size elasticity of buffalo milk could not be estimated due to the poor nature of buffalo production data. However the yield and herd size elasticity for cow milk was higher in Kandy district as compared to the values for Matara district. The supply elasticity of buffalo milk for Matara district was almost zero.

In dry zone district of Hambantota, an increase of milk prices by 10 percent would increase the milk production by 0.9 percent as compared to 0.1 percent in Polonnaruwa district. In the Hambantota district the yield elasticity of milk could not be estimated and the herd size elasticity solely represents the supply elasticity of cow milk. Comparatively the supply elasticity of cow milk in this district was higher than that in Polonnaruwa. The low price responsiveness of the cattle and buffalo milk producers demonstrated in the results does not show much promise in terms of milk production through milk price increases. Milk price increases would have little value with simultaneous inflationary price effects on wages, feed prices, transport, equipment, medicines that would change the cost of milk production and milk marketing. Buffalo milk supply elasticity for Hambantota was relatively high as compared to that for Polonnaruwa and such a result may be related to the larger scale of buffalo production in Hambantota district.

## Efficiency of Milk Production

The technical efficiency of cattle and buffalo farms indicates the value of additional output of milk produced (litres/cow) on the most efficient production frontier. This value could be obtained from the pooled linear production function by adding the intercept to the largest of the estimated positive residual (Russel and Young, 1983). Table 3 shows the classification of farms by their technical efficiencies in Kandy district.

**Table 3.** Technical efficiency of cattle and buffalo farms in the Kandy District.

| Technical efficiencies | Number of farms | Percent of farms |
|---|---|---|
| < 0.10 | 77 | 89.5 |
| 0.50 - 0.59 | 1 | 1.2 |
| 0.60 - 0.75 | 2 | 2.3 |
| > 1.0 | 6 | 7.0 |

In the Kandy district, the technical efficiencies of the farms operated within <0.10-1.0. The results indicated that only 6 of the 86 cattle and buffalo farms (7 percent) in the district operated with technical efficiencies of greater than 1.0 (100 percent). Nearly 90 percent of the farms did not operate within 10 percent of their potential technical efficiencies. None of the inputs made any significant impact on the milk yield of cattle and buffaloes. Hence the pricing efficiencies could not be computed.

The technical efficiencies of cattle and buffalo farms in Matara district are shown in Table 4. In general, the technical efficiencies of buffalo farms were evenly distributed as compared to cattle farms. Only 1 of the 72 farms (1.3 percent) reported of technical efficiencies of >1.0 for cattle as compared to 12.5 percent for buffaloes. Nearly 21 percent of the buffaloes were with technical efficiencies between 0.61-0.70. For cattle, 8.4 percent of the farms reported technical efficiencies between 0.70-0.90 as compared to 14 percent for buffaloes. However 86.1 percent of the cattle farms were not within 10 percent of their technical efficiencies as compared to 25 percent for buffalo farms.

**Table 4**. Technical efficiency of cattle and buffalo farms in the Matara District ( Percent of farms).

| Technical efficiency | Cattle | Buffalo |
|---|---|---|
| < 0.10 | 86.1 | 25.0 |
| 0.11 - 0.20 | 2.8 | 4.3 |
| 0.21 - 0.30 | 1.4 | 2.8 |
| 0.31 - 0.40 | - | 1.4 |
| 0.61- 0.70 | - | 20.8 |
| 0.71 - 0.80 | 4.2 | 13.9 |
| 0.81 - 0.90 | 4.2 | 11.1 |
| 0.91 - 1.00 | - | 8.2 |
| > 1.00 | 1.3 | 12.5 |

In Hambantota district nearly 90 percent of the cattle farms were managed below 10 percent of their potential efficiencies in comparison to 41 percent for buffalo farms. Nearly 4 percent of cattle farms operated with technical efficiencies between 0.30-0.40. For buffaloes, 22.7 percent of farms were managed with a technical efficiency between 0.70-0.80 and 9.3 percent between 0.8-0.9 of technical efficiency. Only 2.7 percent of farms and 1.3 percent for buffaloes were managed with technical efficiencies of >1.0 (Table 5). The distributions were more even for buffalo farms as compared to cattle farms in the Hambantota district as in the Matara district. The pricing efficiencies were not computed for both cattle and buffaloes in the district as the inputs were not fitted in the yield equation.

**Table 5.** Technical efficiency of cattle and buffalo farms in Hambantota District (percent of farms reporting).

| Technical efficiency | Cattle | Buffalo |
|---|---|---|
| <0.10 | 89.3 | 41.3 |
| -0.1 | 4.0 | - |
| -0.09 | 1.3 | 2.7 |
| -0.09 | 2.7 | 9.3 |
| -0.09 | - | 12.0 |
| -0.09 | - | 22.7 |
| -0.09 | - | 9.3 |
| -0.09 | - | 1.3 |
| > 1.00 | 2.7 | 1.3 |

Nearly 73 percent of the cattle farms were not within 10 percent of the potential efficiencies as compared to 48 percent for buffalo farms in Polonnaruwa district (Table 6). Nearly 14 percent of the cattle farms were within 0.11-0.20 of their efficiency and only 6 percent over 100 percent of their efficiency in the district. As in the previous districts the technical efficiencies were more evenly distributed for buffalo farms within 0.31 -1.00 of efficiencies. Only 5 percent of buffalo farms were within >1.0 of their technical efficiencies (Table 6). In Polonnaruwa, the yield equations for cattle and buffalo did not show much significant impact of inputs used and hence the pricing efficiencies were not computed.

**Table 6.** Technical efficiency of cattle and buffalo farms (percent of farmers reporting).

| Technical efficiency | Cattle | Buffalo |
|---|---|---|
| <0.10 | 72.9 | 48.2 |
| 0.11 - 0.20 | 2.4 | 14.1 |
| 0.21 - 0.30 | - | 1.2 |
| 0.31 - 0.40 | 4.7 | - |
| 0.41 - 0.50 | - | 2.4 |
| 0.51 - 0.60 | - | 3.5 |
| 0.61 - 0.70 | 2.4 | 5.9 |
| 0.71 - 0.80 | 1.2 | 4.7 |
| 0.81 - 0.90 | 3.5 | 11.8 |
| 0.91-1.00 | 5.9 | 4.7 |
| >1.00 | 4.7 | 5.9 |

In summary, the technical efficiencies of cattle and buffalo farms were low with cattle farms performing worse than buffalo farms in the districts. However, the technical efficiencies were evenly distributed for buffalo farms as compared to cattle farms. In terms of policy implications the level of technical inputs used, management of inputs for production of cattle and buffalo farms requires to be further investigated.

## POLICY IMPLICATIONS

The results of the study indicated low supply elasticities for milk production and low technical efficiencies of cattle and buffalo production. This indicated the deficiencies in policy implications in terms of cattle and buffalo production.

The average herd size of cattle and buffaloes were low particularly in the wet zone districts. This may be related to the small farm size and poor feed availability for the farm animals. The majority of the buffaloes are reared for draught rather than for milk. Hence it is not surprising to expect a low average yield capacity of 1-3 litres/farm with a short lactation period of around 200 days. Poor calf management also contributed to the low production of the farms. Improved housing of animals particularly for buffaloes is a requirement. Because of these factors animals may be prone to several diseases. An improved livestock extension service may be required to develop the management ability of farmers. The breeding programmes for cattle and buffalo improvement need to be improved to serve the small farms. This may require training on correct detection of heat, availability of A.I or natural stud services and technical services. Poor feeding practices adopted by farmers may have contributed to low efficiencies of milk production. This may be attributed to high market prices of concentrates. Most of the compounded feeds are too expensive for farmers as most of the raw materials (maize, fish meal, meat meal) are imported. The poor quality of forages available in the communal grazing lands and the lack of improved pastures for stall feeding resulted in poor farm productivity. The use of low cost compounded feeds using local raw material may require further research. Increasing the producer price of milk will have little or no value in the face of escalating feed prices particularly that of concentrates. The producer price of milk is generally measured on the cost of production of milk in the farm and this may not be precise. This may or may not be better than parity pricing. The main question here is how the minimum price of milk should move over time as costs are subjected to much variation with the changes of input prices.

The use of any of these pricing mechanisms ignores the demand for milk. One of the major difficulties in setting up the price support mechanism is the absence of a mechanism to evaluate the low supply responses of milk producers distributed over time. Cost of production of milk affecting the minimum price is an alternative to price support schemes for milk. If the mandated price is lower than the market clearing price, the market will bid the price up. If the mandated price is above the market clearing price, surplus of milk would occur which could be removed by a consumer subsidy for milk. Alternatively, allowing the market price to float and making the direct payments to the farmers of the difference between that price and cost of milk may be possible. Credit availability with low interest payments for cattle and buffalo farms may be an important incentive for milk producers. Presently the rate of return of milk production may be lower than high rate of interest required for short and long term credit. The lowering of the interest rate requires reduction of budget deficit which may cause reduction of inflation.

## References

Aigner, D.Z. and Chu, S.F. (1968) On estimating the industry production function. *American Economic Review*, **58,** 826-839.

Annual Report (1993) Central Bank of Sri Lanka.

Economic and Social Statistics of Sri Lanka (1993) Central Bank of Sri Lanka.

Farrell, M.J. (1987) The measurement of productive efficiency, *Journal of Royal Statistical Society*, Series A (General), Part III, 120, 253-290.

Russell, N.P. and Young, T. (1983) Frontier production functions and the measurement of technical efficiencies. *Journal of Agric. Economics*. **34,** 139-149.

Timmer, C.P. (1980) On measuring technical efficiency. *Food Research Institute Studies in Agric. Economics, Trade and Development*, **9,** 99-171.

Livestock Statistics (1992) Ministry of Livestock Development and Rural Industries, Sri Lanka.

Sri Lanka Livestock Sector Policy Review (1992) Ministry of Livestock Development and Rural Industries, Sri Lanka.

## Definition of the Variables used in Appendix Tables 1 and 2

| | | |
|---|---|---|
| yld | = | milk yield of cattle/buffalo (litres/lactation/cow) |
| fs | = | family size |
| el | = | lactation length of European cattle (months) |
| il | = | lactation length of indigenous cattle |
| bl | = | lactation length of indigenous buffalo |
| ibl | = | lactation length of Indian buffalo |
| ibcl | = | lactation length of Indian crossbred buffalo |
| cbs | = | proportion of crossbred buffaloes/farm |
| fel | = | experience of livestock farming (years) |
| mgc | = | grazing hours/day for cattle in maha season |
| ygc | = | grazing hours/day for cattle in yala season |
| gh | = | average grazing hours for cattle |
| st | = | amount of straw fed to milking cows (kg/day) |
| pc | = | amount of forage fed to milking cows (kg/day) |
| pfc | = | price of concentrates (Rs/kg) |
| lc | = | labour cost for cattle (Rs) |
| cmp | = | price of fresh cow milk (Rs/litre) |
| bmp | = | price of fresh buffalo milk (Rs/litre) |
| nc | = | number of cattle per farm |
| nb | = | number of buffaloes per farm |
| ccm | = | chena cultivated per farm (ha) |
| llo | = | lowland cultivation per farm (ha) |
| tcmc | = | total cost of cattle production/farm (Rs) |
| bc | = | breeding cost of cattle/farm (Rs) |
| ctd | = | cost of total draught of buffaloes/farm (Rs) |
| mb | = | miscellaneous cost of buffaloes/farm (Rs) |
| eb | = | equipment cost of buffaloes/farm (Rs) |
| ib | = | number of indigenous milking buffaloes/farm |
| oib | = | number of other buffaloes/farm |
| pb | = | purchase price of buffaloes (Rs) |
| ppc | = | purchase price of cattle (Rs) |
| td | = | buffalo draught used/farm (animal hours) |

**Appendix Table 1.** Yield equations for cattle and buffaloes.

| District | Independent variables | R sq |
|---|---|---|
| Kandy | | 0.797 |
| Cattle | 21.041 +259.752** el - 1068.902** lc - 2.749 bc + 412.438** cmp + 1.132 pco + 4.119 pfc | |
| | (37.076) (226.464) (1.471) (51.953) (4.931) (34.006) | |
| Buffalo | Not Available | |
| Matara | | 0.589 |
| Cattle | - 4.849 + 0.198 cmp + 2.728 llo + 70.142** ll + 0.054* tcmc | |
| | (0.059) (0.802) (2.559) (0.0231) | |
| Buffalo | - 1.027 + 8.987 ygb + 15.646 llo + 16.299 ho + 5703.085** cbx + 64.377** bi + 0.059 pb + 0.807 bmp | 0.900 |
| | (12.299) (11.700) (22.599) (858.480) (18.143) (0.051) (5.317) | |
| Polonnaruwa | | 0.967 |
| Cattle | 47.833 + 0.159** pcx + 55.574 ll + 8.459* cmp + 1.223 sto + 0.927** bc - 5.575 gh | |
| | (0.278) (3.017) (3.166) (4.176) (0.057) (2.918) | |
| Buffalo | - 115.418 + 0.753 cmp + 12.735 ygb + 59.184** bi + 119.896** ibl + 96.034 **lbcl - 0.001 tcmb - 0.470 td | 0.958 |
| | (0.711) (11.89) (0.389) (20.118) (8.001) (0.187) (1.324) | |
| Hambantota | | 0.884 |
| Buffalo | 0.474 + 85.041** bl + 109.501** ibcl - 0.001 tcmc | |
| | (4.152) (19.599) (0.005) | |

Figures in parentheses are the standard errors

* and ** : significant at 5 percent and 1 percent, respectively

**Appendix Table 2.** Herd equations for cattle and buffaloes.

| District | Independent Variables | R sq |
|---|---|---|
| Kandy | | 0.172 |
| Cattle | 0.415 + 0042 fs + 0.439** cmp - 0.002 pc | |
| | (0.059) (0.101) (0.003) | |
| Buffalo | 1.174 + 0.230 ho + 0.103 llo + 0.851** ibo + 0.362** oibo - 0.044 nc | |
| | (0.154) (0.086) (0.277) (0.089) (0.090) | 0.323 |
| Matara | | 0.357 |
| Cattle | - 0.453 + 1.973** mgc + 0.019 cmp + 0.005* tcmc + 0.018 nb | |
| | (0.239) (0.369) (0.002) (0.019) | |
| Buffalo | 23.369 + 6.143 ho - 0.002** ctd + 0.057** mb - 0.000 pb + 1.254 bmp | |
| | (4.352) (0.001) (0.009) (0.010) (1.027) | 0.527 |
| Polonnaruwa | | 0.589 |
| Cattle | 0.210 + 4.210** sto + 0.010 pcx + 0.442** mgc + 0.286 cmp | |
| | (0.250) (0.014) (0.152) (0.189) | |
| Buffalo | - 2.739 - 0.105 nc + 1.142** sb + 0.784 ygb + 1.138** bmp + 0.011 cb + 0.048 td - 0.00004pb | |
| | (0.098) (0.314) (0.391) (0.234) (0.004) (0.042) (0.002) | 0.840 |
| Hambantota | | |
| Cattle | - 13.648 + 2.947 fs + 0.001 tinmy + 0.005 pnnic - 0.018 nb + 1.086 ygc + 5.928* cmp | 0.148 |
| | (2.055) (0.001) (0.008) (0.531) (0.927) (2.735) | |
| Buffalo | 13.032 + 3.569 llo + 0.001 pnnic + 0.103* mb + 8.470 bmp - 0.011* pb - 0.0009 | |
| | (2.958) (0.001) (0.037) (0.958) (0.004) (0.001) | 0.602 |

Figures in parentheses are the standard errors

* and ** : significant at 5 percent and 1 percent, respectively

NA - 143 - 111

# A FIELD SURVEY AND MICROBIOLOGICAL STUDIES ON RUHUNU CURD

K.K. Pathirana[1], C.P. Kodikara[2], D.K.M.P. Dassanayake[2] and S. Widanapathirana[2]

[1]*Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya*
[2]*Department of Microbiology, Faculty of Science, University of Kelaniya, Kelaniya*
*SRI LANKA*

**Abstract:** Ruhunu curd although acclaimed as a quality product from ancient times, has not been the subject of any authentic study. Out of 609 Grama Niladari (GN) divisions in the entire Hambantota district and the Divisional Secretaries (DS) division of Tanamalwila in Moneragala district, 511 GN divisions (84%) were covered in the study. All curd producers were identified by GN division and a house to house survey was done on a 5% sample to obtain basic information on curd production. Sixty samples of curd were subjected to microbiological studies. A total of 1,968 producers manufactured 19,015 pots of curd/day. The highest number of producers and the number of pots produced were from Ambalantota and Tissa DS divisions, respectively. The lowest in both the aforementioned categories were from Okewela DS division. Buffalo milk and the warm dry conditions of the area were largely responsible for the firm curd, since curdling was invariably poor on rainy days. Data on herd composition, health, breeding, feeding and management of herds owned by curd producers were also recorded. Microbiological study showed the presence of Coliforms in 31.25% and *E. coli* in 6.25% of the samples. Potentially pathogenic *Staphylococcus aureus* was present in 56.25% of the samples (10 -13 x$10^{10}$/g) indicating the need to improve the hygienic conditions. Yeasts and moulds were present in all samples with very high counts (1-5 x $10^5$/g) indicating a low keeping quality. *Aspergillus flavus/parasiticus* was present in 31.25% of the samples. Twelve Aspergillus isolates were producers of aflatoxin in artificial media. The pH of curd ranged from 3.09 to 6.22 whereas the maximum recommended is 4.5. Lactic starter culture organisms isolated were *Lactobacillus fermentum, Lactobacillus cellobiosus, Streptococcus lactis* and *Streptococcus lactis* sub species *diacetylactis*. Acidity, pH and extent of curdling of buffalo milk by individual isolates after 6-8 hrs of inoculation were satisfactory. The minimum inoculum required for satisfactory curdling was 0.5

**g of previously made curd or 34 x $10^6$ colony forming units. Indications are that by introduction of low cost, simple scientific procedures, the quality and hygienic standards of the curd could be improved.**

**Keywords**: Milk, curdling, fermentation, microbiology

## INTRODUCTION

Literature refers to the use of curd in Ruhuna as far back as in 167 B.C., during the time of King Dutugemunu. Curd is defined as the product obtained from buffalo or cow milk by lactic fermentation through the action of single or mixed strains of lactic acid bacteria as specified by Sri Lanka Standard 824 (1988).

Ruhuna extends over a large area of Sri Lanka, but Ruhunu curd is typically manufactured in and around Hambantota district. Ruhunu curd is now a popular milk product in Sri Lanka, but no authentic reports are available on its production process. Richards and Agalawatta (1981) in a survey of 9 districts but excluding Hambantota district, and de Silva *et al.* (1985) in a survey covering 16 districts including Hambantota district reported only on whether milk from herds surveyed were used for curd production or not and the pricing of curd. When compared to the very low population of milking buffaloes in many of the other districts, a higher percentage (95.5%) of farmers in Hambantota district milked buffalo cows (de Silva *et al.*, 1985), indicating the importance of buffaloes for curd production in the district. The important factors that determine the quality of curd are the level of microbial contamination and the quality of lactic starter culture used in fermentation. As the manufacture of curd is a cottage industry, no controlled fermentation using selected strains of lactic acid bacteria is possible.

The objectives of this study were to obtain baseline information on production of Ruhunu curd through a field survey and to study the microbiological aspects of curd production to assess the hygienic quality of Ruhunu curd and to isolate lactic starter culture organisms for future field application.

## MATERIALS AND METHODS

### The Survey

The area surveyed included the entire Hambantota district consisting of 576 Grama Niladari (GN) divisions in 11 Divisional Secretaries (DS) divisions and 33 GN divisions of the Tanamalwila DS division in Moneragala district bordering Hambantota district. Thus a total of 609 GN divisions in 12 DS divisions were included in the study (Table 1).

At the beginning, all curd producers were identified by GN divisions through respective Grama Niladaris using a simple questionnaire to obtain following information: the name and address of the producer, number of pots of curd produced, source of milk for curd manufacture (whether own milk, bought milk or a combination of the two) and the number of cattle or buffaloes owned. While a house to house survey was conducted to check on the information provided by Grama Niladaris, information provided by other producers not identified by Grama Niladaris were also included for completeness. The house to house survey covered 512 or 83.9% of the GN divisions. Based on the initial survey, a 5% sample of curd producers was selected for a comprehensive survey for which a questionnaire was pre-tested, re-formulated and used to obtain detailed information. Sixty samples of curd were subjected to the following microbiological studies. Ten grams of the each sample were diluted in 90 ml of 0.1% peptone water and serial tenfold dilutions were prepared according to Sri Lanka Standards (SLS) 824 (1988, Part 1).

**Table 1.** Area covered in the survey together with types of producers according to source of milk and the number of pots of curd produced.

| DS Division | GN Divisions | | | Producers of curd by source of milk | | | | | | | No. of pots produced | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Covered | % | Own (0)[1] No. | Own (0)[1] % | Bought (B)[2] No. | Bought (B)[2] % | O and B No. | O and B % | Total[3] | Per Producer per day | Total per day |
| Tissa | 44 | 38 | 86.4 | 75 | 62.0 | 43 | 35.5 | 3 | 2.5 | 121 | 29.07 | 3518 |
| Lunugamwehera | 36 | 29 | 80.4 | 44 | 63.8 | 24 | 34.8 | 1 | 1.4 | 69 | 33.83 | 2334 |
| Hambantota | 29 | 25 | 86.2 | 181 | 83.8 | 25 | 11.6 | 10 | 4.6 | 216 | 8.62 | 1861 |
| Suriyawewa | 19 | 15 | 78.9 | 86 | 96.6 | 3 | 3.4 | 0 | 0 | 89 | 9.07 | 807 |
| Ambalanthota | 58 | 54 | 93.1 | 360 | 96.8 | 7 | 1.9 | 5 | 1.3 | 372 | 7.33 | 2727 |
| Angunakola | 51 | 38 | 74.5 | 189 | 97.4 | 5 | 2.6 | 0 | 0 | 194 | 5.97 | 1159 |
| Thangalla | 72 | 68 | 94.4 | 241 | 98.0 | 1 | 0.4 | 4 | 1.6 | 246 | 6.53 | 1607 |
| Beliatta | 71 | 60 | 84.5 | 158 | 99.4 | 1 | 0.6 | 0 | 0 | 159 | 4.43 | 704 |
| Weeraketiya | 85 | 67 | 78.8 | 214 | 99.5 | 0 | 0.0 | 1 | 0.5 | 215 | 4.46 | 959 |
| Okewela | 27 | 27 | 100.0 | 51 | 100.0 | 0 | 0.0 | 0 | 0 | 51 | 2.37 | 121 |
| Katuwana | 84 | 69 | 82.1 | 129 | 98.5 | 2 | 1.5 | 0 | 0 | 131 | 6.08 | 796 |
| Thanamalwila | 33 | 22 | 67.0 | 95 | 90.5 | 8 | 7.6 | 2 | 1.9 | 105 | 23.0 | 2422 |
| Total | 609 | 512 | - | 1829 | | 119 | | 26 | | 1968 | | 19015 |
| Mean | 9 | - | 83.9 | 151.9 | 92.7 | 9.9 | 6.0 | 2.1 | 1.3 | 164.0 | 11.73 | 1585 |
| ±SD | - | - | 9.1 | 92.0 | 13.4 | 13.5 | 1.3 | 3.0 | 1.2 | 90.5 | 10.61 | 1005 |

[1] producers of curd with milk from their herds only [2] producers with only bought milk [3] producers with both sources

## Microbiological Studies

The total viable plate count was done according to Sri Lanka Standard Institute (SLSI) procedures 516:1 (1984). Dilutions from $10^{-1}$ to $10^{-12}$ were plated using the pour plate technique. Organisms were identified biochemically (Cowan, 1975). Dilutions of curd from $10^{-1}$ to $10^{-9}$ were plated using the SLSI pour plate technique for yeast and mould counts (SLS 516:2, 1984). Presence of coliforms and *E. coli* per gram of sample was detected using the triplicate tube method (SLS 516:3, 1984). *S. aureus* was identified according to SLS 516:6 (1984). Coagulase activity was tested following the procedures of Cowan (1975). The presence of *Aspergillus flavus/Aspergillus parasiticus* was determined by culturing the organism. Twelve isolates showing typical colony characteristics of *Aspergillus flavus/A. parasiticus* on agar were differentiated into the two species using colony colour and morphological characters in slide cultures. The isolates were grown in potato dextrose agar with zinc dust and $NaNO_3$ and tested for aflatoxin production at the end of 3 and 7 days. For identification of genera of moulds, the fungi were grown in slide cultures and the isolates were identified up to generic level using colony characteristics and morphology of the fungi. For isolation and identification of lactic starter culture organisms, dilutions of curd up to $10^{-6}$ were inoculated according to procedures of Harrigan and MeCance (1979) and de Man *et al.* (1960). The characteristic colonies were subcultured and identified as lactic streptococci and lactobacilli according to Starr (1981). Each of the starter culture isolates was inoculated into skimmed milk and the pH was measured 8 hourly.

# RESULTS

## Producers, Source of Milk and Pots of Curd Produced

Results are presented in Table 1. There were a total of 1968 producers, producing a total of 19,015 pots of curd/day. The highest number of producers and pots of curd were from Ambalantota and Tissa DS divisions, respectively. Most of the producers produced curd with milk from their own herds (92.7%), while the lowest number produced curd with milk from their own and bought milk (1.3%). The largest number of pots/producer/day was in Lunugamwehera DS division (33.83%).

Of all herds belonging to farmers involved in curd production, 44% had both cattle and buffaloes while 40% and 9% had only buffaloes and only cattle, respectively. Six percent of producers who did not own animals bought milk to manufacture curd.

## Composition and Reproduction/Breeding of Herds Owned by Curd Producers

The information is presented in Table 2. Buffalo herds were larger than cattle herds. Cows in milk, age at first calving and calving interval were all in favour of neat cattle indicating low production and reproduction efficiency of buffaloes compared to cattle.

**Table 2**. Composition, reproduction/breeding of cattle and buffalo herds.

| | Cattle | | Buffaloes | |
|---|---|---|---|---|
| | Range | Mean ±SD | Range | Mean ±SD |
| Total number/ herd | 2-150 | 16.8±42.3 | 6-281 | 42.6±49.0 |
| Heifer calves and heifers | 1-32 | 15.5±13.5 | 1-51 | 7.8±7.5 |
| Bull calves | 1-35 | 8.3±9.9 | 1-31 | 7.0±6.5 |
| Cows in milk | 1-62 | 15.5±8.9 | 1-53 | 15.3±9.4 |
| Dry cows | 1-54 | 9.4±6.3 | 1-42 | 11.5±8.2 |
| % cows in milk | - | 62.2±21.4 | - | 55.0±28.9 |
| Stud bulls | 1-4 | 1.6±0.9 | 1-8 | 1.9±1.9 |
| Age at first calving | 29-95 | 42.6±10.0 | 36-64 | 46.4±8.2 |
| Calving interval | 12-41 | 16.1±7.3 | 14-47 | 18.2±6.0 |

Just over 20% of the farmers were using AI, of whom a large majority (80.0%) consider it a successful method.

## Some Management Practices

Animals were brought to paddocks for the night by 85.7% of farmers while 14.3% allowed free grazing throughout the night. Hardly any of the farmers (4.1%) offered water or feed in the night paddocks. However, 89.8% of them believed that the animals receive sufficient drinking water.

In all herds, milking was done only once a day in the morning while the majority (93.9%) used only one hand for that purpose. In all cases, suckling was necessary at milking with the majority (83.7%) allowing it once, while 16.3% allowed it twice at each milking. Buffalo calves were allowed to suckle up to a more mature age than cattle. Milking time ranged from 5 am to 9 am with the majority of them (86.8%) milking between 7 am to 8 am. No concentrates were offered even at milking time. The total duration of milking ranged from 10 to 25 min. The average milk yield of cattle and buffaloes were 1.8±0.91 kg/d and 1.9±0.91 kg/d with maxima of 3.9 and 3.2 kg/head/d, respectively.

## Health Aspects of the Herds

Data on some health aspects are presented in Table 3. Buffaloes when compared with cattle were more susceptible to disease, particularly at a younger age, with a higher mortality and more frequent epidemics. A high percentage of farmers (93.8%) dewormed their calves using western drugs (82.6%) while the balance 17.4% used indigenous drugs. Most farmers (78.2%) wormed calves below one month of age. Vaccination of animals against epidemic diseases was practised by 69.4% of farmers.

## Manufacture of Curd

Milk was boiled within 3 hours after milking by 80.9% of farmers while the rest did so within the next hour. All use firewood as

**Table 3**. Data on some health aspects of the herds.

| | Cattle | Buffaloes |
|---|---|---|
| Mortality due to all diseases (% farms reporting) | | |
| Age up to 1 mo | Nil | 31.8 |
| 2-6 mo | 10.7 | 38.6 |
| >6 mo | 28.6 | 18.2 |
| No. mortality due to disease | 60.7 | 41.4 |
| Incidence of mortality due to epidemics (% of total reported) | 16.7 | 83.3 |
| Frequency of epidemics causing mortality less than every 3 year (% of total reported) | 12.8 | 87.2 |

fuel for boiling of milk with 81.8% producers using an aluminium container while the others (18.2%) used a clay pot for the purpose. Most of them (56.4%) transferred the boiled milk directly into pots, using a jug (34.5%) and 9.1% using a big spoon.

## Starter Culture and Curdling of Milk

Nearly all (98.2%) used previously prepared curd, 0.5-1.0 day old (74.5%) while the rest (1.8%) used yoghurt as the source of starter culture. The starter culture was mixed with unboiled water before adding, by 69.1% of the farmers. Many farmers (58.5%) determined the temperature at which to add the starter culture by touching the pot while 22.0% dipped a finger into milk for that purpose. Thermometers have not been used to determine the temperature at the time of inoculation. Traditionally, all the farmers used the period between 9 am to 4 pm for curdling of milk.

Minimum times required for satisfactory curdling after inoculation were 3, 3-5 and > 5 hrs as indicated by 11.2, 72.4 and 16.4% of the farmers, respectively. While all of them used clay pots for making curd, 96.4% did so in new pots while the rest re-used old pots as well, when available. Most of them washed (90.9%) and dried (85.5%) the pots before making curd.

## Other Practices Affecting the Quality and Hygienic Standards of Curd

Only 1.2% of the farmers, on an irregular basis, removed fat before making curd; which was always after boiling. The amount removed was subjective, without adopting any standard. The only additives used were water and sugar by 3.7% and 1.8% of the farmers, respectively. The reasons given for addition of water were, to prevent drying and "burning" of milk while boiling and to increase the amount of milk. Sugar was added with the notion that it promoted curdling. The main reasons for using buffalo milk as against cow's milk for curd making were: (a) tradition (44.2%), (b) greater consumer demand (34.6%) and (c) better quality (9.7%).

Farmers' perceptions on the important factor relating to quality was the firmness of curd. Dry and warm weather and adequate boiling received the highest recognition by farmers as factors contributing to firmness and flavour of curd. In fact, most of the farmers (98.2%) reported that rain even during a dry spell resulted in poor quality curd. Most of them (83.6%) were not aware of the role of the starter culture in curd making.

## Social and Economic Aspects

Most of the curd producers (83.7%) owned the milking herds. The "farm gate" price of fresh buffalo milk varied between Rs. 8.00 and Rs. 8.50 per litre depending on the unit price determined by the collectors, while Rs. 14.00 was paid by others. The "farm gate" price of curd calculated from basic data obtained from producers ranged from Rs. 15-30 (46.2%), Rs. 31-40 (38.5%) and over Rs. 40 (15.3%). While numerous factors affected the sale price of curd, the two main factors recognised were the quantity of milk produced (29.1%) and the season of the year (23.6%), the price being low during rainy seasons and high during festival seasons. Curd producers were generally unaware of the methods adopted by their close neighbours. Only 4.3% of them were aware of the procedures adopted by their neighbours.

## Hygienic Quality of Curd

Of the sixty samples of curd analysed, 19 (31%) were positive for coliforms and 4 (6%) contained *E. coli*. When the results were compared with the different procedures used in curd manufacture, it was revealed that 95% of the producers who did not wash the containers prior to their use in curd manufacture had their curd contaminated with coliforms. Coagulase positive *Staphylococcus aureus* was present in 34 (56%) of the samples analysed. The counts ranged from $10\text{-}13\times10^{10}$/g. As discussed earlier the practice of using the fingers in assessing the temperature of the milk and in mixing the starter culture with warm water before introduction of the culture may be the source of contamination. *Aspergillus flavus* or *parasiticus* was present in 19 (31%) of the samples tested and the colony forming units per gram ranged from 10-12. Twelve isolates of *Aspergillus flavus* and *parasiticus* were tested for aflatoxin production in artificial media. Aflatoxin $B_1$ and $B_2$ were detected in 3 isolates and $G_1$ and $G_2$ in 7 isolates. However, production of aflatoxin was not detected in curd when fungal isolates were inoculated to curd.

## Keeping Quality of Curd

The yeast and mould count of curd ranged from $1\text{-}5\times10^5$ per gram of curd. All samples contained contaminating yeasts and moulds. The contaminant fungi were of the genera *Penicillium, Mucor* and *Aspergillus*.

## Lactic Starter Culture Organisms

Twenty isolates of lactic starter culture organisms were identified using biochemical tests. The isolates were identified as *Lactobacillus fermentum*, *Lactobacillus cellobiosus*, *Streptococcus lactis* and *Streptococcus lactis* subspecies *diacetylactis* and *Streptococcus cremoris*. When the above isolates were tested for starter culture activity, 9 of the isolates were found to be of acceptable quality as starter cultures. The isolates were used singly and in combination in fermentation of buffalo milk and were found to be satisfactory in curd production.

# DISCUSSION

## Composition and Reproduction/Breeding of Herds Owned by Curd Producers

Mean herd size of 42.6 buffaloes reported in this study was somewhat comparable to 53.6 reported by de Silva *et al.* (1985) for buffalo herds in Hambantota district, which were higher than most of the mean herd sizes reported for cattle and buffaloes in other districts (Kumaratilake and Buvanendran, 1979; Richards and Agalawatte, 1981; Siriwardene *et al.*, 1982). In comparison with cattle, the percent buffaloes in milk, age at first calving and calving interval (Table 2) indicate a low reproductive/breeding efficiency in buffaloes.

The mean age at first calving of buffaloes in this study (46.4 mo) is comparable to the mean age (45.7 mo) for buffaloes from 16 districts reported by de Silva *et al.* (1985). The mean calving interval of 18.2 mo. was also similar. The percentage buffalo cows in milk (55%) in this study was within the range (49-63%) reported for buffalo cows by Richards and Agalawatte (1981).

## Management Practices and Milking

Only once a day milking has been reported earlier as well (Richards and Agalawatte, 1982; Siriwardene *et al.*, 1982). The absence of feeding of concentrates as found in this study is confirmed by the results in other reports (Kumaratilake and Buvanendran, 1979; Richards and Agalawatte, 1981; de Silva *et al.*, 1985).

## Health Aspects

Higher mortality in buffaloes during early life when compared with cattle is in agreement with the findings of Cockrill (1981). Only 69.4% of the farmers vaccinated their animals, whereas de Silva *et al.* (1985) reported that all the farmers had their animals vaccinated routinely.

## Producers, Source of Milk and Pots of Curd Produced

Since the survey covered 83.9% of the total GN divisions and identified 1968 curd producers surveyed, the total number of producers in the area may be estimated by projection to be around 2300-2400. The production of curd would then be approximately 22,600-22,700 pots of curd per day. Results indicate that a much smaller number of curd producers with bought milk (6.0%) produced a proportionately larger number of pots than those who produced curd with their own milk (92.7%), indicating a much larger scale of operation by the former category.

Correlation coefficients between the number of neat cattle or buffaloes owned and the number of pots of curd produced with own milk were 0.79 and 0.93, respectively indicating a greater relationship between buffalo milk production and curd making. While a very low percentage of farmers milked buffaloes in many other districts, 95.5% of buffalo farmers milked their cows in the Hambantota district (de Silva *et al.*, 1985), substantiating that a high percentage of buffaloes are being milked. This preference for buffalo milk in curd making is based on the belief that buffalo milk gives firmness, pure whiteness and flavour to the curd, confirmed by the greater consumer acceptance of curd produced from buffalo milk. The farmers' perceptions on important considerations for promoting firmness in curd were warm dry weather (100%), adequate boiling of milk (87.2%) and the use of clay pots (43.6%). They did not attach much importance to the starter culture in this respect. Their perceptions of the important factors which produce a good flavour were the use of buffalo milk (32.7%), clay pots (28.3%) and the type of feed offered to cows (14.5%).

## Manufacture of Curd

The observations made during the study indicate that procedures adopted in the handling of milk and curd making have an adverse effect on the quality and hygenic standards of curd. It is clear that production, quality and hygenic standards need to be substantially improved by encouraging farmers to adopt simple methods and technology, involving minimum interventions. The provision of drinking water in night paddocks and in addition some concentrates, at least at milking time would improve production. Since most of the farmers (83.7%) bring animals early in the afternoon to a night paddock, twice a day milking using both hands instead of one, will increase the yield. However, a practical means of preserving milk overnight has to be provided to promote milking in the evening. In fact, in the absence of an efficient marketing system for liquid milk, conversion of milk to curd has opened an avenue of marketing a value added product having a longer shelf life.

The use of a thermometer and/or gaining experience to assess the optimum temperature by touching the pot rather than dipping the finger in boiled milk, before adding the starter culture would help to minimize contamination. The introduction of hygienic washing and cleaning procedures during transfer of milk to pots and during the introduction of the starter culture could easily improve the quality of the product. The assessment of the farmers' understanding of the process of curd production indicate a very poor awareness of the importance and the role played by the starter culture.

However it is clear that experience and tradition have contributed to the development of practices suited to the warm and dry climate of the region for production of a firm curd, with a pure white thick fat layer. A low percentage of producers admitted using two adulterants only: water (3.7%) and sugar (1.8%).

## Social and Economic Aspects

Most of the production units provided employment for 2-3 persons. The estimated value added by marketing milk as curd, calculated from the basic data based on a maximum value of Rs. 12.00/l of fresh milk varied widely between groups. These values ranged from Rs. 6-16, 17-26 and over Rs. 26 per litre for 46.2, 38.5 and 15.3% respectively of the total number of pots sold. The absence of a well organized fresh milk collection network in this area may also have forced farmers to convert milk to curd. The importance of developing a practical curd production procedure, using some of the traditional methods of farmers, need to be recognized.

## Microbiological Studies

The hygienic quality of Ruhunu curd produced under village conditions was found to be poor as indicated by the presence of coliforms, *E. coli* and *Staphylococcus aureus* in a high percentage of samples analysed. The Sri Lanka Standard for curd (SLS1988:824, Part 1) specifies, that the product should be free of *E. coli*. The presence of *E. coli* indicates the potential for the presence diarrhoeal pathogens. Coagulase positive *Staphylococcus aureus* is a potential pathogen which is normally present in the nose and on the skin of a variable proportion of healthy people. The contributory factor for presence of *Staphylococcus aureus* in a large number of samples could be due to the practice of mixing starter culture with fingers and dipping them to test the temperature of boiled milk before adding the starter culture.

Yeast and moulds find conditions in fermented milk suitable for growth. They contribute to the alteration of its flavour and appearance and the formation of toxic products.

The isolates of *Aspergillus flavus* and *parasiticus* were found to be aflatoxin producers in artificial media but toxin production was not evident in curd at room temperature. Aflatoxin production has been reported in dairy products such as Cheddar cheese (Lie and Marth, 1967) brick cheese (Shih and Marth, 1972) and tilsit cheese (Frank, 1968). *Aspergillus flavus* has been detected in yoghurt (Jordano *et al.*, 1989) but the isolates have not been tested for aflatoxin production.

The high mould and yeast counts of samples as seen in the present study indicate that the product has a very poor keeping quality. Spoilage of fermented products is initiated by fungi and growth of spoilage flora commence as the pH of the fermented product gradually rises during mould growth. The Sri Lankan Standard suggested for yoghurt is < 1000 yeasts and < 1 mould per gram (SLS 824, part 2, 1989). The Sri Lanka Standard for curd does not refer to a limit for yeast and mould but excessive counts of moulds and yeasts ($10x10^4$ - $50x10^4$) as seen in the present study are indicative of poor manufacturing practices.

The isolates of lactic starter culture organisms found in Ruhunu curd were satisfactory in coagulating milk in eight hours to form a curd of acceptable quality. Inspite of some of the short comings in hygienic standards, Ruhunu curd is popularly consumed as a healthy food. There have been no complaints or adverse medical reports associated with consumption of curd.

## Acknowledgements

The financial assistance for this study provided by SAREC through NARESA is gratefully acknowledged. The authors are thankful to Divisional Secretaries, Grama Niladaris and the field veterinary staff for giving their fullest co-operation, Prof. M.U. de Silva for tracing the history of Ruhunu curd, Prof. D. Attapattu, Dr. S.S.E Ranawana and Dr. L.D.P. Dharmasena who through initial collaboration in various ways helped to carry out this study. Mr. A.T. Jayasinghe (Technical Assistant) worked extremely hard and did an excellent job in his capacity. Thanks are due to Mrs. K.P.R. Priyadharshani for technical assistance in microbiological studies. Finally we thank Miss. Sumithra Samarasinghe for typing the paper.

## References

Cowan, S.T. (1975) *Manual for the Identification of Medical Bacteria.* Cambridge University Press, Cambridge.

Cockrill, W.R. (1981) The Water Buffalo. A review. *British vet. J.* **137**, 8-15.

de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A medium for the cultivation of Lactobacilli. *Journal of Applied Bacteriology* **23**, 130-135.

de Silva, L.N.A., Perera, B.M.A.O., Tilakeratne, L. and Edquist, L.E. (1985) Production systems and reproductive performance of indigenous buffaloes in Sri Lanka. *Report 3*, Swedish University of Agricultural Sciences, Uppsala.

Franik, H.A. (1968) Diffusion of aflatoxins in food stuffs. *Journal Food Science* **33**, 98-100.

Harrigan, W.F. and MeCance, M.E., Eds (1979) *Laboratory Methods in Food and Dairy Microbiology.* Harcourt Brace Jovanovich, London.

Jordano, R., Jordal, M., Martinez, P. and Pozo, R. (1989) Aflatoxin - producing strains of *Aspergillus flavus* in yoghurt. *Journal of Food Production* **52**, 823-824.

Kumaratilake, W.L.J.S. and Buvanendran, V. (1979) A survey of production characteristics of indigenous buffaloes in Sri Lanka. *Ceylon vet. J.* **27**, 10-13.

Lie, J.L. and Marth, E.H. (1967) Formation of aflatoxin in Cheddar Cheese by *Aspergillus flavus* and *Aspergillus parasiticus*. *J. Dairy Science* **50**, 1708-1710.

Richards, E.M. and Agalawatte, M. (1981) An inter-agroecological zone survey of cattle and buffalo management practices in Sri Lanka. *Field Document 1*, CCP/SRL/30 (SWE), FAO, Rome.

Richards, E.M and Agalawatte, M. (1982) A sample survey analysis of buffalo management practices and herd profitability in the different agro-ecological zones of Sri Lanka. *Workshop on Water Buffalo Research in Sri Lanka*, 24-28 Nov. 1980. SAREC report R 3: pp. 31-43.

Shih, C.N. and Marth, E.H. (1972) Experimental production of aflatoxin on brick cheese. *J. Milk Food Technology* **35,** 585-587.

Siriwardene, J.A. de S., Wickremasuriya, U.G.J.S. and Balachandran, S.S. (1982) Management practices of buffalo in small farms. *Workshop on Water Buffalo Research in Sri Lanka*, 24-28 Nov.1980. SAREC report R 3: pp. 11-19.

Sri Lanka Standard (1984) 516:1

Sri Lanka Standard (1984) 516:2

Sri Lanka Standard (1984) 516:3

Sri Lanka Standard (1984) 516:6

Sri Lanka Standard (1988) 824, Part 1

Sri Lanka Standard (1988) 824, Part 11

Starr, M.P., Ed. (1981) *The Prokaryotes*. Volume 11 Springer, Verlag, Berlin.

# PRELIMINARY ANALYTICAL OBSERVATIONS ON PERSISTENCY OF MILK YIELD IN BUFFALO IN SRI LANKA

I.D. Silva [1], A. Dangolla[1] and K.F.S.T. Silva [2]

*[1]Department of Veterinary Clinical Studies, Faculty of Veterinary, Medicine and Animal Science,[2] Department of Animal Science, Faculty of Agriculture, University of Peradeniya SRI LANKA*

**Abstract:** The daily milk yield in buffalo cows had been observed to fluctuate substantially after day 75 of the lactation period when compared to cattle. This fluctuation may vary from hypogalactia to even a temporary cessation of lactation (agalactia). This communication examines the persistency of milk yield in the first lactation milk records of 23 Murrah, Surti and their crosses from 3 farms in the dry, wet and intermediate zones. All observations were made over the period between day 75 up to at least day 178 of the lactation curve. The longest lactation period included was 368 days. The median, minimum and maximum of 10 day moving averages of the milk yield were 3.4, 0.33 and 10.40 litres respectively. The median reduction in milk yield in any given month was 10% of the previous month's yield. However, the monthly milk yield increased by approximately 110% of that of the previous month in 14 cows, at least once during the observation period. The variation in milk yield, in relation to the average yield, for 10 day periods was examined by calculating the weighted milk production (WMP). The WMP was calculated by dividing (or weighting) the difference between the maximum and minimum daily yield by the average yield for that period. The WMP was less than 1.5 in 10 cows. However, in 9 cows the WMP increased, at least twice, to over 1.5 indicating a substantially large variation in the milk yield in these cows.

An interesting finding was that, at least once during the study period, 13 cows showed an increase of ≥10% of their peak yield of the entire lactation during a 15 day period. Such unusual patterns have not been reported in cattle. Future studies on subsequent lactations of these buffaloes may provide information on factors leading to such variations in daily milk yield.

**Keywords:** Persistency, buffalo, milk yield, lactation curve, epidemiological parameters.

## INTRODUCTION

Inhibition in the let down of milk in buffaloes causing economic loss had been reported in India and the condition has been termed "Palaserlu" and "Dokas" (Kesavamurthy and Kotayya, 1973; Bhaskarsingh *et al.*, 1976). This condition was thought to be due to either a digestive disturbance, hypocalcaemia, mastitis, oedema or venous engorgement of the udder. Incidence had been observed to be relatively higher during winter (Kotayya *et al.*, 1976). Veterinarians have claimed that the condition subsided following treatment with stomachic powders, mineral mixtures or intramammary calcium gluconate or antibiotics. However, some veterinarians have claimed that the condition subsided even without treatment.

A similar syndrome had been observed in large organized buffalo farms in the dry zone of Sri Lanka. In order to study the occurrence of this phenomenon, it was decided to characterise the lactation curve of buffaloes in Sri Lanka. This preliminary communication describes some epidemiological parameters of the lactation curve in buffaloes, including the persistency which refers to the level at which the milk production is maintained as lactation progresses (Ensminger, 1993).

## MATERIALS AND METHODS

*Animals and locations:* Past first lactation records of cows from three farms in three different agro-climatic zones in Sri Lanka were examined. The farms were located in the mid country wet zone, the dry zone and the low country intermediate zone. Cows with complete records (n=23) were identified and the data relating to these animals were analysed.

This study was based on information available from records between 1987 and 1993. All observations were made for the period between day 75 up to at least day 178 of the first lactation. The minimum and maximum number of days included in the calculations were 103 and 380, respectively.

*Statistical analyses:* The following four calculations were made on the defined period of the lactation curve. The moving averages of the milk yield for periods of 10 days was calculated for each animal. The persistency of the milk yield of every cow for each month was calculated as a percentage of previous month's yield. The weighted milk production (WMP) which is the variation in the milk yield in relation to the mean, was determined by calculating the difference between the maximum and minimum daily milk yield divided by the average yield for the 10 day periods. Aberrant increases represent sharp increases in the moving average noticed during 15 day periods. This increase was quantified as a proportion of the peak yield of the lactation of that particular cow.

## RESULTS

Figure 1 shows the daily milk yield of 2 cows. Figure 2 shows the 10 day moving averages of 23 cows from the three different large farms in Sri Lanka. This figure includes data relating to 2 cows that had zero yield at least for one day. The mean of the 10 day moving average was approximately 4.5 litres, up to the 150th day of the lactation.

Table 1 denotes the persistency with respect to 30 day periods in the first lactation begining from day 75. The majority of observations on persistency were between 81 and 100%. The persistency of one cow was as low as $\leq$ 20% in both 30 day periods between 105 to 164 days in lactation. There were 5 cows whose persistency was more than 100% during the last stages of the lactation.

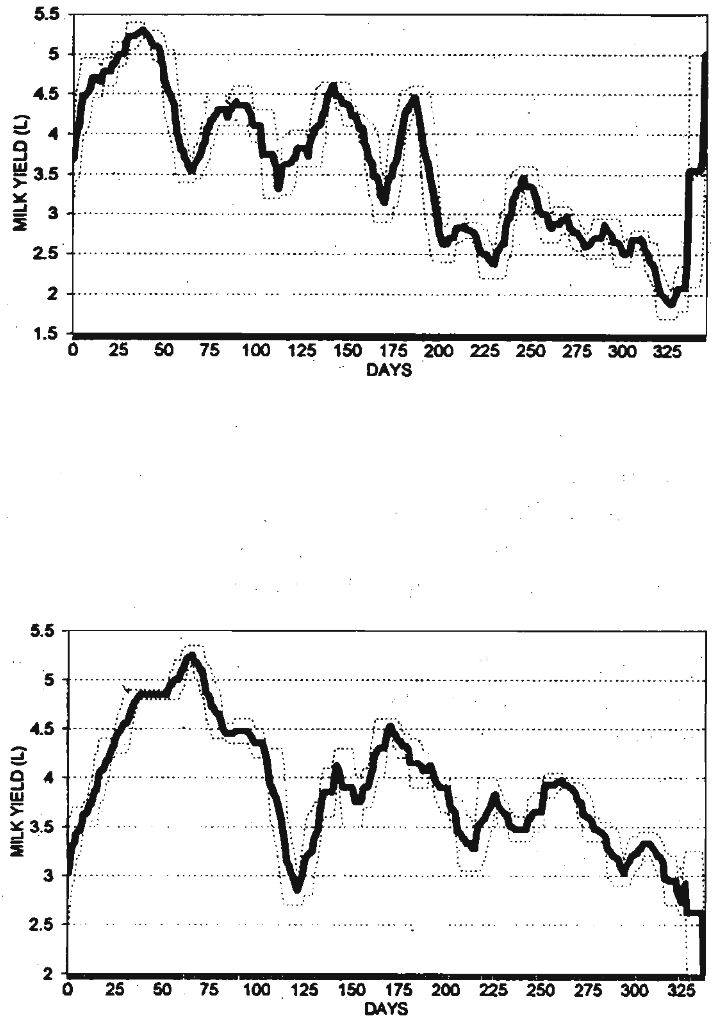



**Figure 1.** Daily milk yield of 2 cows during the first lactation.

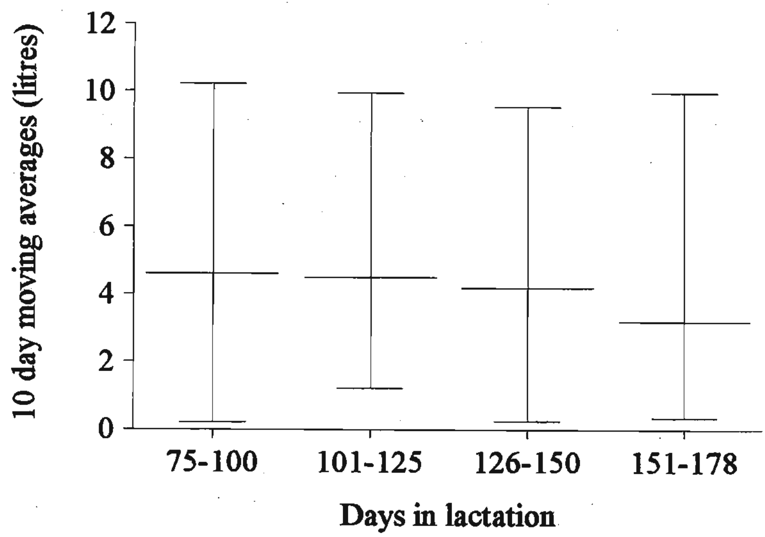



**Figure 2.** The mean, maximum and minimum of the 10 day moving averages of the milk yield in the first lactation of buffalo cows.

**Table 1.** Number of cows on their persistency of milk yield in relation to the periods during the first lactation.

| Persistency | 30 day periods from day 75 of lactation | | | | | |
|---|---|---|---|---|---|---|
| | 75-104 | 105-134 | 135-164 | 165-194 | 195-224 | ≥225 |
| 10-20% | | 1 | 1 | | | 11 |
| 21-40% | | | | | 1 | |
| 41-60% | | 2 | 1 | | 1 | 1 |
| 61-80% | 3 | 1 | | 4 | 1 | 15 |
| 81-100% | 11 | 12 | 8 | 8 | 10 | 18 |
| ≥101% | 9 | 7 | 13 | 8 | 4 | 11 |

Table 2 presents the frequency of cows in various WMP categories with respect to different time periods in the lactation. Majority of the WMP values were ≤ 1.5 irrespective of the period of lactation. However, an interesting observation was that a few WMP values were unusually high and were above 3.6.

**Table 2.** The frequency of cows in their WMP[1] and the length of the first lactation.

| WMP | 30 day periods from day 75 of lactation | | | | | |
|---|---|---|---|---|---|---|
| | 75-104 | 105-134 | 135-164 | 165-194 | 195-224 | ≥ 225 |
| ≤ 1.5 | 18 | 18 | 17 | 16 | 19 | 14 |
| 1.6 - 2.5 | 3 | 3 | 3 | 4 | 2 | 2 |
| 2.6 - 3.5 | 1 | 1 | | | | 2 |
| ≥ 3.6 | 1 | | 2 | 1 | | |

[1]Difference between the minimum and the maximum milk yield of 10 day periods as a proportion of the average for the same period.

The aberrant increases in the milk yield is tabulated in Table 3. The majority (14 out of 23) of the increases were ≤5% of their respective peak yield. Nevertheless, a substantial number of cows showed increases which were ≥20% of the peak yield.

**Table 3**. Number of cows with aberrant increases in the moving average, expressed as a percentage of the peak yield for that lactation, within ≤ 15 day periods.

| Aberrant increase as a percentage of peak yield | Days | | | | | |
|---|---|---|---|---|---|---|
| | 75-104 | 105-134 | 135-164 | 165-194 | 195-224 | ≥225 |
| ≤ 5% | 4 | 7 | 3 | 6 | 4 | 7 |
| 6% - 10% | 4 | 3 | 7 | 8 | 4 | 8 |
| 11% - 20% | 9 | 7 | 7 | 4 | 4 | 8 |
| ≥ 20% | 2 | 2 | 2 | 2 | 1 | 4 |

## DISCUSSION

The 10 day moving average was approximately 4 litres during the first lactation and the above average varied between 0 and 10 litres (Figure 1). It was interesting to note that there had been no yield (agalactia) in two cows on one or more days during a given 10 day period. However, those two cows had subsequently re-commenced milk secretion.

The persistency of the milk yield in the majority of buffaloes studied were similar to that of cattle (Ensminger, 1993). The obvious cause for the low values for persistency of 10-20% in a few buffaloes may be due to hypogalactia. This finding is largely in agreement with the observations made in India (Kesavamurthy and Kotayya, 1973; Bhaskarsingh *et al.*, 1976; Kottaya *et al.*, 1976). The elevation of persistency above 100% seen in some buffaloes could also be attributed to hypogalactia which had occurred in the previous month. However, >101% persistency further indicates that there should be other reasons contributing to an increase in the monthly yield, especially during the final days of lactation (Table 1).

Sometimes the above mentioned increase could be as high as ≥20% of the peak yield. Similar observations have not been characterised or quantified in dairy animals, to the best of our knowledge. Therefore, it would be interesting to further investigate these aberrant increases in milk yields in dairy animals, including buffaloes.

It is not surprising for most of the WMP values to be ≤ 1.5, because fluctuations in milk yield are relatively less when compared to the average milk yield. Nonetheless, it is apparent that in buffaloes, the variation in milk yield in relation to the average yield could be substantially large (Table 2). The above reported phenomenon of hypogalactia could also lead to considerably large values for WMP.

The buffaloes used for this study were from state owned farms where satisfactory milk and health records had been maintained. The records, do not provide any indication of factors which could be attributed to the reported degree of variations in milk yield.

## Acknowledgements

This study was supported in part by funds from Swedish Agency for Research Cooperation with Developing Countries (SAREC) awarded through the NARESA. We thank L.C.S. Alwis, S.S. Withanage, K.A.M.J. Silva and I.N.K. Irriyagolla for their assistance in data processing.

## References

Bhaskarsingh, K., Rao R.K. and Kotayya, K. (1976) Dysgalactia in buffaloes-Relationship with serum calcium, inorganic phosphorus and copper. *Indian vet. J.* **53,** 685-688.

Ensminger, M.E. (1993) Milk secretion and handling. *Dairy Cattle Science* Third Edition. Interstate Publishers Inc., Illinois.

Kesavamurthy, A. and Kotayya, K. (1973) Dysgalactia in buffaloes-A preliminary report. *Indian vet. J.* **50,** 558-561.

Kotayya, K., Baba, E.H. and Kesavamurthy, A. (1976) Dysgalactia in buffaloes - Studies on incidence and seasonality. *Indian vet. J.* **53,** 426-429.

# FACTORS AFFECTING CARCASS AND MEAT QUALITY OF INDIGENOUS BUFFALOES IN SRI LANKA

H. W. Cyril and A. Jayaweera

*Department of Animal Science, Faculty of Agriculture*
*University of Peradeniya*
*SRI LANKA*

**Abstract:** Two separate studies were conducted to study the factors affecting carcass quality and meat quality characters of indigenous buffaloes. In the first study, 60 randomly selected buffaloes (live weights ranging from 100 kg to 450 kg) were used, and in the second study 90 randomly selected buffaloes were used. Various factors (eg: dressing percentage, meat to bone ratio and organ percentage) affecting carcass quality of buffaloes were determined. Meat samples were analyzed for chemical composition (eg: protein, fat, ash and water content), colour and tenderness.

The results of the carcass quality study revealed that the dressing percentage and meat to bone ratio increased with increasing live weight. The dressing percentage increased (mean±SD) from 47.99% ± 0.14 to 54.35% ± 0.0 and meat to bone ratio increased from 2.39 ± 0.45 to 4.74 ± 0.0. There was no significant difference between the dressing percentage of males (51.66% ± 3.14) and females (51.05% ± 3.32). The meat to bone ratio was found to be higher in males (3.06 ± 0.81) than in females (2.70 ± 0.72). The percentage of organs was higher in females than in males. With increasing live weight, the percentage of fore-quarters increased from 51.74 ± 3.13 to 57.94 ± 0.0 while the percentage of hind quarters decreased from 47.90 ± 0.16 to 42.05 ± 0.0.

In the meat quality study, differences were observed in water, protein, ash and fat contents of the meat of young animals and adult animals. Respective mean values were 76.25% ± 1.12, 21.26% ± 1.56, 1.72% ± 1.03, 1.13% ± 0.06, for young animals and 75.26% ± 2.19, 21.77% ± 1.89, 2.87% ± 1.27, 1.19% ± 0.22, for adult animals. The shear value of the meat of young animals (6.63 kg ± 1.69), was found to be significantly lower than that of adult animals (8.79 kg ± 2.37). In most of the adult animals (57.89%), meat colour was in the range of 7 to 9 while it was in the range of 1 to 3 for most of the young animals (78.52%). Between male and female animals, there was a difference in the water and fat contents of meat. For male animals, mean values of water and fat contents were 75.51% ± 1.39 and 2.41% ± 1.08, respectively, and for female animals, the values were 75.26% ± 2.59 and 3.00% ± 1.44, respectively.

**Keywords:** Carcass quality, buffalo, meat consumption

## INTRODUCTION

The buffalo population in Sri Lanka was estimated at 825,000 heads in 1991 (Livestock Statistics, 1992), but had exhibited a decreasing trend over the last decade. Buffaloes are reared mainly for draught and milk purposes in Sri Lanka. Although there is a ban on the slaughter of buffaloes, they contribute to local beef production partially, through illicit sources.

The water buffalo offers promising features as an excellent source of meat. Since buffaloes have been bred and kept as draught animals for centuries, they have evolved as a meat animal exhibiting exceptional muscular development with some animals weighing 1000 kg or more. They show faster growth compared to some breeds of dairy cattle (Tilakaratne, 1980). If the buffalo is reared and fed for meat production, the meat will be tender, palatable and of high quality so that it would be attractive to the consumer (Oganojanovic, 1965). Hence, there is good potential for rearing buffaloes under suitable environmental conditions to increase the supply of meat and meat products.

Buffaloes are less prone to fat deposition and thus produce more lean meat than cattle. On carcass weight basis, the percentage of high quality cuts, first grade meat and total meat is higher in buffaloes than in cattle (Padda *et al.*, 1986). Since the present global consumer demand is for leaner meat, the buffalo has a vast market potential. Thus, buffalo meat could play more important role in the international meat market and the production of buffaloes solely for meat is now expanding in the other countries in Asia (Pathak *et al.*, 1987).

Maintaining healthy, well nourished and sturdy herds of buffaloes for high quality meat production is a challenge for developing countries like Sri Lanka. In Sri Lanka most of the buffalo meat was and still continues to be derived from old animals slaughtered at the end of their productive life as draught or dairy animals. As a result, much of the buffalo meat sold is of poor quality (Padda *et al.*, 1986), because buffaloes are generally reared under extensive systems of management and used mainly as a source of farm power.

Recently, there has been a greater interest in rearing buffaloes exclusively for meat production. However, the rate of improvement in carcass weight achieved in developing countries is low in comparison to the improvements achieved in developed countries. Pricing structures and meat technology concerning buffalo beef is also not attractive to the farmer (Padda *et al.*, 1986). In Sri Lanka, very little information is available on carcass characteristics and meat quality of indigenous buffaloes. This has been one of the drawbacks for buffalo production in the country. Hence, the potential of the buffalo as a meat animal has not been fully realized as yet.

This study was undertaken to examine the various factors affecting carcass quality and meat quality of indigenous buffaloes in Sri Lanka, with a view to assess the suitability of the buffalo as an alternate meat producer.

## MATERIALS AND METHODS

Two separate studies on factors affecting carcass characteristics and meat quality were conducted. The first experiment on factors affecting carcass characteristics was conducted using 60 randomly selected buffaloes, both male and female, slaughtered at an abattoir. Their live weights were recorded prior to slaughter. Data on carcass weight, organ weights, meat and bone weights were collected. Dressing percentage, meat to bone ratio, organ percentage, hind quarter and fore quarter percentages were calculated and tabulated in relation to sex and respective live weights.

The second study on factors affecting meat quality was conducted using 90 randomly selected local buffaloes. The meat samples were taken from the *Longissimus dorsi* muscle. Meat samples were directly used for determination of colour and tenderness on the first day after slaughter. Colour was assessed with Munsell colour chart and shear value was estimated as a measure for tenderness of meat.

For analysis of fat percentage and crude protein percentage, the Soxhlet method and Kjeldhal system were used respectively. Moisture percentage and ash percentage were also determined.

## RESULTS AND DISCUSSION

In the first study, the live weights of selected animals varied between 100 kg to 450 kg. The dressing percentage increased significantly from 47.99 % $\pm$ 0.14 to 54.35 % $\pm$ 0.0 with increasing live weights (Table 1). These results agree with the results reported by Tilakaratne (1980) and Cooper and Wills (1979). The increasing trend was highest in the live weight range of 100 kg to 200 kg. The dressing percentages of buffalo carcasses of 130 - 500 kg body weight have been found to be between 50 and 55% in different countries (Afifi *et al.*, 1974; Johnson and Charles, 1975; Tilakaratne *et al.*, 1980; Rosa *et al.*, 1980; and Pathak *et al.,* 1987).

The meat to bone ratio showed an increasing trend (Table 1), from 2.39 $\pm$ 0.45 to 4.74 $\pm$ 0.0, with the live weights increasing from 100 kg to 450 kg. The increase was highest (3.67 $\pm$ 0.27 to 4.74 $\pm$ 0.0), in the live weight range of 350 kg to 450 kg. According to Cooper and Wills (1979), meat to bone ratio increases with increasing live weight since the percentage of bone declines while percentage of lean increases with increasing live weight.

**Table 1.** Effect of live weight on dressing percentage and meat to bone ratio.

| Live weight (kg) | Dressing % (mean ± S.D) | Meat:Bone ratio (mean ± S.D) |
|---|---|---|
| 100 - 150 | 47.99 ± 0.14 | 2.39 ± 0.45 |
| 150 - 200 | 51.01 ± 3.47 | 2.75 ± 0.95 |
| 200 - 250 | 51.43 ± 3.33 | 2.44 ± 0.64 |
| 250 - 300 | 50.64 ± 2.98 | 2.67 ± 0.63 |
| 300 - 350 | 52.48 ± 1.82 | 3.39 ± 0.71 |
| 350 - 400 | 52.97 ± 3.64 | 3.67 ± 0.27 |
| 400 - 450 | 54.35 ± 0.00* | 4.74 ± 0.00 |

* SD is zero as measurement was made only on one animal.

According to this study, significant differences could not be observed in the dressing percentages among male and female animals (Table 2), which were 51.66 % ± 3.14 and 51.05 % ± 3.32 respectively. These results were comparable with earlier reports on nondescript and Murrah buffaloes (Dodsward, 1972; Tilakaratne, 1980).

**Table 2.** Effect of sex on dressing percentage and meat to bone ratio.

| Sex | Dressing % (mean ± S.D) | Meat:Bone ratio |
|---|---|---|
| Male | 51.66 ± 3.14 | 3.06 ± 0.81 |
| Female | 51.05 ± 3.32 | 2.70 ± 0.72 |

Among males and females, the meat to bone ratio showed a marked difference (Table 2). The meat to bone ratio was higher in males (3.06 ± 0.81), than in females (2.70 ± 0.72). In cattle according to Preston and Wills (1976) carcasses of steers yield a significantly higher proportion of edible meat to bone and less excess fat than those of heifers or cows. This phenomenon may be applicable to buffaloes too, and that may be the reason for the higher meat to bone ratio observed in male buffaloes than in females.

Organ percentage (Table 3) was higher in female buffaloes (36.68%) than in males (35.78%). Especially the liver, rumen and intestine accounted for a little higher percentage in females. Generally, females have higher amounts of fat deposits in many organs than males (Cockrill, 1967). This could be the reason for the observation of higher percentage of organs in females.

**Table 3.** Effect of sex on organ percentage (mean ± S. D.).

| Organ | Sex | |
|---|---|---|
| | Male | Female |
| Skin | 10.90±1.05 | 10.18±1.09 |
| Pluck | 2.81±0.30 | 2.79±0.33 |
| Liver | 1.40±0.17 | 1.65±0.36 |
| Spleen | 0.36±0.08 | 0.34±0.07 |
| Rumen | 13.15±1.11 | 13.59±2.79 |
| Intestine | 4.27±0.88 | 5.13±0.94 |
| Feet | 2.67±0.52 | 2.72±0.22 |
| Tail | 0.22±0.08 | 0.28±0.11 |

The percentage of hind-quarters and fore-quarters showed a marked difference with increasing live weight (Table 4). Fore-quarter percentage increased from 51.71% ± 3.13 to 57.94% ± 0.0 while the hind-quarter percentage decreased from 47.90% ± 0.16 to 42.05% ± 0.0 in the live weight range 100 kg to 450 kg.

Preston and Wills (1976) explained that body weight generally increases with age. It is expected that there will be a positive relationship between age and dressing percentage. Accordingly, fore-quarter percentage also increases with increasing dressing percentage. As stated by Abdullah *et al.* (1981), fattening increases with age and plane of

**Table 4.** Effect of live weight on fore-quarter and hind-quarter percentages .

| Live weight (kg) | Percentage (mean ± S. D.) | |
|---|---|---|
| | Fore-quarter | Hind-quarter |
| 100 - 150 | 51.71 ± 3.13 | 47.90 ± 0.16 |
| 150 - 200 | 51.66 ± 1.37 | 48.11 ± 1.50 |
| 200 - 250 | 54.24 ± 1.26 | 45.71 ± 1.29 |
| 250 - 300 | 55.11 ± 1.30 | 45.00 ± 1.30 |
| 300 - 350 | 57.14 ± 1.95 | 42.85 ± 1.95 |
| 350 - 400 | 58.02 ± 0.18 | 42.02 ± 0.27 |
| 400 - 450 | 57.94 ± 0.00 | 42.05 ± 0.00 |

nutrition, leading to heavier fore-quarters and relatively lighter hind-quarters.

According to the second study, a significant difference in meat composition could be observed in different age categories (young animals and adult animals). Protein, fat and ash contents (Table 5), increased with the age of the animals (protein from 21.26% ± 1.56 to 21.77% ± 1.89, fat from 1.72% ± 1.03 to 2.87% ± 1.27 and ash from 1.13% ± 0.06 to 1.19% ± 0.22). There was a significant reduction of the water content

**Table 5.** Effect of age on composition of buffalo meat.

| Component | Composition % (mean ± S.D) | |
|---|---|---|
| | Young animals (1-2 years) | Adult animals (2-10 years) |
| Water | 76.25 ± 1.12 | 75.26 ± 2.19 |
| Protein | 21.26 ± 1.56 | 21.78 ± 1.89 |
| Fat | 1.72 ± 1.03 | 2.87 ± 1.27 |
| Ash | 1.13 ± 0.06 | 1.19 ± 0.22 |

of meat with age. Reduction in the water content (76.25% ± 1.12 in young animals and 75.26% ± 2.19 in adult animals) may be due to increase of the fat content of meat with age. Comparable results have been reported by Moulton and Lewis (1940) and Anjaneyulu (1985). According to Lawrie (1961), irrespective of species, breed or sex, most of the composition parameters of muscle, other than water content increase with the age of the animal.

Differences were observed in the meat composition between male and female animals. The fat content of females (3% ± 1.44) was higher than that of males (2.41% ± 1.08), (Table 6). Meanwhile, the water content was little higher in males (75.51% ± 1.39), than in females (75.26% ± 2.59). This may be due to the low fat content in males compared to females. This agrees with the findings of Moulton and Lewis (1975). There were no significant differences in the protein and ash contents between males and females. These observations agree with the results reported by Agabeili *et al.* (1971).

**Table 6.** Effect of sex on composition of buffalo meat.

| Component | Percentage (mean ± S.D) | |
|---|---|---|
| | Male | Female |
| Water | 75.51 ± 1.39 | 75.26 ± 2.59 |
| Protein | 21.63 ± 2.10 | 21.60 ± 1.81 |
| Fat | 2.41 ± 1.08 | 3.00 ± 1.44 |
| Ash | 1.15 ± 0.12 | 1.21 ± 0.30 |

Increase in the shear value with the age (6.63 kg ± 1.69 in young animals to 8.89 kg ± 2.37 in adult animals) was found to be significant (Table 7). According to Cooper and Wills (1979) and Matassino *et al.* (1984), meat becomes less tender as the animal gets older. Lean meat muscle is made up of minute fibres bundled together with connective tissues. In young animals, the muscle fibres and bundles are small and that imparts tenderness to meat (Hill, 1988). The age of animals, beyond two years of age has a clear effect on tenderness which decreases due to lack of marbling and the increase in the connective tissue and size of muscular bundles (Regab *et al.,* 1966). Accordingly, shear values increase with age.

**Table 7.** Effect of age on tenderness of buffalo meat.

| Age of animal | Shear value (kg) (mean ± S.D) |
|---|---|
| Young (1-2 years) | 6.63 ± 1.69 |
| Adult (2-10 years) | 8.79 ± 2.37 |

The highest percentage of adult animals (58.89%) was in the darker colour range of 7 to 9 (Table 8), while the highest percentage of young animals (78.52%) was in the lighter colour range of 1 to 3. Hafez (1952) and Romans *et al.* (1985) explained that meat darkens as the animal ages and the Munsell test "Value" (i.e. - darkness) increased with maturity. According to Hill (1988), the colour of muscle deepens in older animals due to an increase of respiratory pigments such as haemoglobin and myoglobin.

**Table 8.** Effect of age on colour of buffalo meat.

| Colour range | Animal percentage | |
|---|---|---|
| | Young animals (1-2 years) *(n = 76)* | Adult animals (2-10 years) *(n = 14)* |
| 1 - 3 Lighter | 78.52 | 13.15 |
| 4 - 6 | 21.42 | 28.94 |
| 7 - 9 Darker | 0.00 | 57.89 |

Among carcass characteristics of indigenous buffaloes, dressing percentage has a positive relationship to the live weight. There is hardly any difference in dressing percentage of male and female buffaloes. Meat to bone ratio shows an increasing trend with increasing live weight. However, males have a higher meat to bone ratio than females. Organ percentage is higher in females than in males. Percentage of fore-quarters shows a positive relationship to the live weight while it is negative for hind-quarters.

In indigenous buffaloes, the composition of meat varies with age. Protein, fat and ash contents increase while the water content decreases with age. Tenderness of meat also declines with age. Colour of meat changes from a lighter range in young animals to a darker range in adult animals. Composition of meat is different in male and female animals in relation to water and fat contents. Females have higher fat content and lower water content compared to males.

## References

Abdullah, O.Y., Shahin, K.A. and Latif, M.G.A. (1981) Growth and development of water buffalo and Friesian cross-bred cattle with special reference to body and carcass composition. *J. Agric. Sci. (Camb)* **97**, 205-212.

Afifi, Y. A., Shain, M. A. and Omara, S. F. (1974) Meat production from buffalo male calves at different ages. *Agriculture Research Review. India.* **52**, 1-20.

Agabeili, A. A., Guseinov, I. A. and Serdyuk, S. (1971) A new buffalo breed - the Caucasian. *Zhivotnovodstovo, Mosk., Vol. 33 61-63. Anim. Breed. Abstr.* **40**, 25 (1972).

Anjaneyulu, A. S. R., Sengar, S.S., Lakshamanan, V. and Joshi, D. C. (1985) Meat quality of male calves maintained on different levels of protein. *Buffalo Bulletin* **4**, 45-47.

Charles, D.D. and Johnson, E.R. (1975) Live weight gain and carcass composition of buffalo (*Bubalus bubalis*) steer on four feeding regimes. *Australian J. Agric. Sci.* pp 327-344.

Cockrill, W. R. (1967) The water buffalo. *Scient. Am.* **217**, 118-125.

Cooper, M. McG. and Wills, M. B. (1979) *Profitable beef production, 3rd Ed.* Farming Press, Ipswich.

Dodsward, T .L. (1972) *Beef production,* Pergamon Press, Oxford.

Hafez, E.S.T. (1952) The buffalo - A review. *Ind. J. Vet. Sci. Anim. Husb.* **22,** 257-263.

Hill, D. (1988) *Cattle and Buffalo Meat Production in Tropics*. Longman, U.K.

Johnson, E.R. and Charles, D.D. (1975) Comparison of live weight gain and changes in carcass composition between buffalo (*Bubalus bubalis*) and *Bos taurus* steers. *Australian J. Agric. Sci.* **26,** 415-422.

Lawrie, R. A. (1961) Studies of muscles of animals. I. Difference of composition of beef *Longissimus dorsi* muscle determined by age and anatomical location. *J. Agric, Sci.* **56,** 249.

Livestock Statistics. (1992) *Occasional report, Dept. of Census and Statistics.* Colombo, Sri Lanka.

Matassino, D., Girolami, A., Ramunno, L. and Gambacorta, E. (1984) Comparison on meat quality between buffalo and bovine fed with hay and concentrates. *Animal Production* **3,** 111.

Moulton, C. R. and Lewis, W. L. (1940) *Meat through Microscope.* Univ. of Chicago, Illinois.

Oganojanovic, A. (1974) *Handling and Health of Domestic Buffalo.* (Ed. W. Ross Cockrill) 377, pp.

Padda, G. S., Sharma, B. D. and Sharma, N. (1986) Quality characteristics of buffalo beef. *Buffalo Bulletin* **5,** 27-29.

Pathak, N.N., Ranjhan, S.K. and Baruah, K.K. (1987) Growth responses, feed efficiency and carcass characteristics of Indian buffalo (*Bubalus bubalis*) calves fattened on different planes of nutrition. *Buffalo Bulletin* **6,** 77-81.

Preston, T. R. and Wills, M. B. (1976) *Intensive Beef Production.* Pergamon Press, Oxford.

Regab, M.T., Darwish, M.Y.H. and Malek, A.G.A. (1966) Meat production from Egyptian buffaloes. II Physical and chemical characteristics of buffalo meat. *J. Anim. Prod. U.A.R.* **6,** 51-62.

Romans, J.R., Jones, K. W., Costello. W. J., Carlson, C. W. and Ziegler, P.T. (1985) *The Meat We Eat.* 12th Ed. Interstate Printers Danville, Illinois.

Rosa, A., Creta, V. and Dzic, G. (1980) Results obtained in intensive fattening of young buffaloes. *Nutr. Abst. Rev., 1981,* **51,** 337, Abstr. 3011.

Tilakaratne, N., Matsukava, T., Buvanendran, V. and Thangarajah, P. (1978) Growth, feed conversion and carcass characteristics of cattle and buffaloes fed with grass and concentrates. *Ceylon vet. J.* **24,** 9-12.

Tilakaratne, N. (1980) Meat production from domestic buffaloes. In: *Proceeding of the Workshop on Water Buffalo Research in Sri Lanka. Nov 24-28, 1980.* Peradeniya, Sri Lanka.

NA- 143 - 149

## Session I - Production Systems and Uses

## Panel Discussion

**Q:** N. H. Shah - I would like to know how the small farmer having a land extent of only 2 acres is managing cattle for milk and buffaloes for draught. This would bring increased pressure on the system. In my opinion it would be more economical to keep a dual purpose animal, rather than two different species of animals. Have you made any comparisons on the returns by keeping one, two or three buffaloes on one acre of land? Have there been any economic benefit studies done?

**A:** H. Abeygunawardena - Most of the Mahaweli settlements are located in old villages which are supplied with water for agricultural purposes through an irrigation system, which has existed from ancient times. Farmers engaged in livestock rearing continue the traditional farming practice of keeping two herds and relying on communal grazing land. But this is going to be impossible in the years to come because the extent of communal grazing land available is diminishing. There has already been conflicts between crop and livestock farmers. They find it difficult to give up this practice because they will lose a portion of their regular income. Therefore it has become essential for them to continue to keep livestock. What we are trying to do is to discourage the management of two herds; one for milk and the other for draught. We encourage rearing of dual purpose animals that gives milk which could be converted to value added products such as curd and yogurt in order to provide sufficient income and thereby break the "agricultural debt" cycle, that farmers are generally subjected to.

**Q:** C. Devendra - In the Mahaweli area, did problem identification and definition actually focus on nutritional and feeding resources as the number one problem, and what were the other problems or priorities.

**A:** H. Abeygunawardena - During Phase I of the Project, we observed that buffalo farming was quite extensive and the most important problems identified were (a) low milk price (b) low production and (c) high cost of feed. Based on these observations we developed a system that would reduce the cost of production and solve problems created by land limitations.

**Q:** C. Devendra - What were the other problems beyond feeding and nutrition?

**A:** H. Abeygunawardena - Firstly, low production. Ninety five per cent of the buffalo population is indigenous and therefore upgrading is essential. Attempts have been made to upgrade the local animal but so far we have not achieved the desirable levels. Secondly, endemic diseases.

**Q:** D.K. Singh - What is the status of gastrointestinal worm infestation in these animals.

**A:** H. Abeygunawardena - We have not noticed any major problems related to gastrointestinal worms.

**Comment:** D.K. Singh - I raised this question specifically to share our experience, particularly in Gujarat in India. We have observed that if animals are dewormed there is a substantial increase in the milk production. We have also developed a deworming procedure by putting the drug into the urea molasses mineral block and the animal is allowed to lick the block for 5-6 day.

**A:** H. Abeygunawardena - Studies performed by a group of parasitologists in Sri Lanka have shown that *Toxocara vitulorum* is the most important parasite in buffalo calves. According to these studies calves treated at around two weeks of age with one dose of anthelminthic are free from the threat from ascariasis for the rest of their lives. We have adopted this treatment regime and do not see a need to incorporate an anthelminthic into the block.

**Q:** D. K. Singh - I don't contest your opinion that *T. vitulorum* in the most important nematode in the buffalo. I also agree that parasitism *per se* is not an important problem clinically, but if you remove subclinical parasitism, occasionally, the result is a definite increase in the milk production.

**Comment:** D.H.A. Subasinghe - I like to make a comment that nutrition actually was in fact identified as the most important problem as it influenced other factors such as reproduction and growth and even resistance to disease. Therefore emphasis was on providing a low cost nutritive feed to the animals.

**A:** H. Abeygunawardena - Another reason was that we have considerable information on the use of rice straw as a ruminant feed and on the value of the urea-molasses supplement as a catalytic feed, but that information has unfortunately not reached the farmer. In this project we are also exploring the possibilities of adopting these technologies at the small farmer level.

**Q:** S.K. Ranjhan - What is the price of buffalo milk in Sri Lanka?

**A:** C. Bogahawatte - Buffalo milk fetches around Rs.10-12 per litre when it is collected separately. Generally, it is mixed with cows milk and the farmers are paid on the monthly average fat percentage.

**Q:** S.K. Ranjhan - What is the price of cows milk?

**A:** C. Bogahawatte - The average price is around Rs 7.50. It varies from Rs. 3.00 to 7.50.

**Q:** I.D. Silva - You mentioned that the buffalo and cows milk go together to the collecting centre. May I ask you what was the source of data for the production values?

**A:** C. Bogahawatta - The data on production has been collected at the farm gate. The farmer indicated how much of cow's and buffalo milk was produced.

**Q:** C. Devendra - From your reference to technical efficiencies, do we infer that it involves both biological factors and policy issues.

**A:** C. Bogahawatte - Yes.

**Comment:** C. Devendra - You said in the conclusions that you are operating within 10 per cent of efficiency therefore, by inference there is 90% potential. That is an enormous figure.

**Q:** P. Bunyavejchewin - What is the cost of imported milk compared to the locally produced milk.

**A:** C. Bogahawatta - Generally the price of imported liquid milk is higher and varies between Rs 65 to Rs 75 per litre, while the price of local liquid milk is Rs 30 per litre.

**Q:** P. Bunyavejchewin - Is imported liquid milk very popular?

**A:** C. Bogahawatta - Yes, especially among the expatriates.

**Comment:** P. Bunyavejchewin - I would like to make two points. First on the work output of male and female buffaloes. In Thailand, we did on-farm monitoring and tried to compare male and female buffaloes in terms of work output. We found no significant gender difference in the work output. It must be emphasised that in Thailand more female buffaloes are used compared to males. Secondly, we are researching this aspect of breeding on F1 or F2 crossbreds; the results are yet to be published. Preliminary studies have shown that 49 chromosome males have a lot of abnormal sperms be it a F1 or F2.

**Comment:** N.U. Horadagoda - I have a comment regarding the incorporation of anthelminthics into the block. I feel that incorporating an anthelmintic especially a benzimidazole preparation into the block and feeding it over a length of time could induce the development of resistance by certain species of parasites.

**Comment:** D.K. Singh - With regard to worm burden in adult animals, I agree that it is not a major problem but if you deworm an animal there is an increase in milk yield of 800 ml to 1 litre per day. This also could be achieved by incorporating the drug into the block or it could be given in feed pellets. The latter is a very convenient method of administering the drug. The drug is not administered on a continuous basis. It is given once a year at a strategic time.

**Comment:** S.K. Ranjhan - I tend to partially agree with Dr. Singh. The use of anthelminthics in the block has been done in some of the countries. In Swaziland, we introduced the anthelminthic for one day and the animals consumed 400 to 450 gram per day. The animal received the medicated block only on one day. The use of the medicated block obviated the necessity to drench the animals. This is a particularly useful practice when a large number of animals are involved. This method of administration will certainly not bring about the resistance as mentioned earlier.

**Comment:** A. Dangolla - Regarding the adminstration of anthelminthic through the block, I am not too sure whether anyone has looked into the concentration of anthelminthic at the site of action where the worm is located. As far as it is known, there is no evidence of resistance to ascarids. I am not sure whether anyone has thought of the decay of the drug in blood and at the site of action.

**Q:** B.M.A.O. Perera - I want to discuss the milk pricing scheme, since quite a lot of interest was shown on milk pricing. There are several people here who I am sure could comment on the schemes presently operating in Sri Lanka. One is Mr. Walter Perera, who is the Manager of a large dairy cooperative. I would like to hear his comments on the subject.

**Comment:** W. Perera - As a cooperative supplying milk to Nestles and Milco and also manufacturing its own products, I would say that the present scheme in Sri Lanka is based on fat as well as on SNF. The standard that has been set is that, for milk with fat at 4% and SNF at 8%, farmers are entitled to receive Rs. 8.50 per litre for cow milk. In general there is no price differentiation given for buffalo milk but in our cooperative we are paying the farmers Rs. 15.00 per litre for buffalo milk and Rs. 10.50 for cows milk. We can afford to pay a higher price for milk since we are doing some processing of the milk .

**A:** C. Bogahawatta - In my studies I found that most of the farmers who deliver milk directly to milk collection centres were paid different prices for cow and buffalo milk, but where milk was purchased by a collector and transported in the same vehicle, farmers received an average price based on that month's average fat content.

**Comment:** W. Perera - As far as milk collection in the coconut triangle is concerned, we would collect even one litre and it is collected in separate containers, either as cow milk or buffalo milk.

**Q:** C. Devendra - While you submit that there is 10% efficiency currently you also submit that 90% opportunities exist. Considering biological and policy aspects, if we had to push resources for the future for dairy development which direction would you push that?

**A:** C. Bogahawatta - The scale of production is going to be a very important factor. One needs to increase the herd size assuming that one is going to increase the yield per cow with new technologies. The scale of production is our weakness. We are not going to expand our herd size in the wet zone because of the population growth and intense competition for land. Therefore it is more feasible in the dry zone, especially in the Mahaweli area where land is available and where much potential lies.

NA - 143 - 155

# BREEDING STRATEGIES FOR OPTIMUM UTILIZATION OF AVAILABLE RESOURCES IN RURAL BUFFALO PRODUCTION SYSTEMS

C. Chantalakhana and P. Bunyavejchewin

*Kasetsart University, Bangkok 10900, THAILAND*

**Abstract**: Buffalo raising is expected to continue to play an important role in rural farming especially in rainfed lowland and upland farming systems, as well as in some irrigated lowland farming systems. Breeding improvement of river and swamp buffaloes for rural farms has to take farming systems perspectives into consideration. Some breeding strategies for the improvement of buffaloes for draught, meat and draught, meat-milk-and-draught and for dairy purpose, are discussed. Some breeding schemes are proposed within the context of rural farming in the developing countries.

**Keywords:** Production systems, breeding, strategies

## BUFFALOES IN THE WORLD

There were approximately 148.9 million water buffaloes in the world in 1993, while there were only 90 million in 1961. Almost 96% (142.6 million) of them were found in Asia and the Pacific, while they were distributed in more than 50 countries around the world, from China and India to Brazil, from USA and Europe to Australia. The number of buffaloes by continent is shown in Table 1. Most buffaloes are raised by small farmers in developing countries.

The water buffaloes (*Bubalus bubalis*) can be classified into two breed types, the river and the swamp type.

A. River breeds consist of (a) Asian breeds such as those in India and Pakistan and (b) Mediterranean breeds such as those in Italy, Romania and Middle East.

B. Swamp type such as those in China and Southeast Asia (SEA).

**Table 1**. Number of buffaloes by Continent (Chantalakhana, 1991).

| Continents | | Decades | | |
|---|---|---|---|---|
| | | 1961/70 | 1971/80 (In millions) | 1981/90 |
| Asia | N | 88.04/107.00 | 108.39/120.31 | 122.73/136.27 |
| | GI[1] | 2.5 | 1.1 | 1.0 |
| Africa[2] | N | 1.05/2.01 | 2.06/2.35 | 2.37/2.55 |
| | GI | 3.5 | 1.8 | 0.9 |
| South | N | .063/.118 | .129/.495 | .542/1.234 |
| America[3] | GI | 7.7 | 17.7 | 9.7 |
| Europe | N | .528/.369 | .392/.437 | .440/.376 |
| | GI | -4.0 | 1.2 | -2.0 |
| World[4] | N | 90.52/109.97 | 111.43/123.95 | 126.43/140.86 |
| | GI | 2.5 | 1.1 | 1.1 |

1 GI = Growth Index, average annual percentage change.

2 Only for Egypt and Mauritius, some buffaloes are found also in Madagascar, Mozambique, S. Africa, Uganda, Zaire and Congo.

3 Only for Brazil, but some buffaloes are found also in Venezuela, Trinidad, Peru, Paraguay, Argentina, Ecuador, Colombia, Surinam, Honduras, Costa Rica, Bolivia and Uruguay.

4 Australia is not included, where about 200,000 buffaloes are found.

More than 70% of the total buffalo population in the world belong to the river type while almost 30% are of the swamp type.

During the past three decades the number of buffaloes in the world steadily and consistently increased by about 1 to 2% per year, except in some Eastern European countries, where buffalo numbers decreased during 1981-1990, as indicated in Table 1. The most obvious increase in the number of buffaloes took place in Brazil and some other Latin American countries.

In the year 2025 it is expected that there will be a human population of more than 8.2 billion in the world with about 3.3 billion people living in rural areas. Clearly, more meat and milk will be required for urban consumption, while rural farmers will continue to depend on buffaloes for draught power, manure, cash income as well as other non-cash values. The water buffalo will remain an important component contributing to sustainable farming systems and rural development in developing countries for many decades to come.

## BUFFALO PRODUCTION AND RURAL FARMING

In 1990, out of 136 million buffaloes in Asia more than 95% (129.5 million) were found in India (75 m), China (21.4 m), Pakistan (15 m) and Southeast Asian countries including Myanmar, Bangladesh and Sri Lanka. Most buffaloes in these countries are concentrated in the following crop/animal agro-ecosystems (Devendra, 1995): irrigated lowland farming systems, rainfed lowland farming systems and upland farming systems.

In India most milk production is obtained from dairy buffaloes while the use of buffalo for draught power is also important. In Pakistan and China buffaloes are used as draught animals as well as for meat and milk. In SEA the water buffaloes provide meat and draught power, while manure and other by-products such as hide, horn are also important.

### Buffalo Farming in Developing Countries

*South Asia*: Buffaloes in this region mostly belong to the river breeds. More than 67% of the world buffalo population can be found in Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka. Water buffaloes are a source of milk, meat and draught power i.e. a triple purpose animal. In India 25.5 million tons of milk are produced by buffaloes annually, while only 23 million tons are from cattle (Soni, 1991). In Pakistan, buffaloes produce three times as much milk (9.9 m tons) as do cattle (3.1 m tons).

According to Soni (1991), buffalo production in South Asia is mainly by small farmers who are engaged in traditional integrated small-farm systems in which crops are commonly a major component.

Farmers maintain buffaloes mainly because they depend on these animals to carry out agricultural operations. Most of the farmers are subsistence farmers with limited land area for cultivation. The number of livestock, especially bovine animals, kept per farmholding is rather low. Most of the farmers keep around one or two buffaloes for crop cultivation or for milk production. Meat and manure are generally considered as by-products.

In India and Pakistan, buffalo production systems can be generally classified into 3 types, i.e. (1) extensive, (2) semi-intensive, and (3) intensive (Soni, 1991). The extensive system of buffalo raising is commonly practiced by small farmers who keep one or two buffaloes and maintain them by grazing on common village land, roadside grass and on stubble. Natural grazing is supplemented with straw and other crop residues. The semi-intensive system of buffalo raising is generally associated with intensive systems of crop production. Buffaloes are raised mainly for milk production and are stall fed. Some farmers may raise 10 to 50 buffaloes. The intensive system is usually characterized by large-scale buffalo production units or colonies to provide milk for large city consumption. This kind of buffalo operation involves a relatively large number of dairy buffaloes, usually located near metropolitan areas.

*East and Southeast Asia:* According to Bunyavejchewin and Chantalakhana (1991), 95 to 99% of the buffalo production in China and most SEA countries can be classified as smallholder or small farm systems, in which buffalo raising is regarded as an integral part of a crop production system. Swamp buffaloes primarily are a source of farm power for tillage and transportation, and a source of manure for use as fertilizer for crops, while meat is a secondary product and milk is generally unimportant. The most common areas where buffaloes play prominent roles are in rainfed lowland or upland rice production.

Raising swamp buffalo for beef production is becoming more common in East and SEA. However, the number of commercial buffalo farms in most countries is still relatively few. Raising buffaloes for dual-purpose, i.e. for meat and milk, has also become an important area of buffalo development in certain countries such as the Philippines, Malaysia and Thailand.

## Rural Farmers and Their Buffaloes

Meaningful and useful breeding strategies have to be formulated with rural farming perspectives while keeping in mind future trends and changes in buffalo farming systems due to changing socio-economic conditions in the developing countries. Opinions of rural farmers who raise buffaloes on how they determine desirable traits, traditionally and economically, are important factors in the formulation of buffalo breeding strategies.

*Subsistence production:* Most buffalo production in India, Pakistan, and other South Asian countries, as well as in China and SEA can be characterized as subsistence production. Buffaloes are generally regarded as multi-purpose animals. Milk, meat, draught power, manure, and by-products such as hide, are important economic commodities, while priceless values of buffaloes are also significant, for example, serving as farm family assurance in case of crop failure due to natural hazards, being a source of long-term saving, fulfilling traditional and cultural needs, providing a source of social status and prestige and being a family companion especially for children. For rural farmers, buffaloes are generally used to satisfy multiple needs of the farm family :

(1) To produce milk, such as in India, Pakistan, Bangladesh; or to a certain extent in Sri Lanka, Nepal, China and the Philippines.

(2) To produce meat, such as in Pakistan, China and SEA countries.

(3) To provide draught power and manure for rural farming, which is commonly needed in almost every Asian country.

Breeding strategies should be formulated to serve the requirements of different farming systems and farmers' socio-economic conditions. This paper aims to discuss buffalo breeding strategies for some multi-purpose uses under rural farming conditions in order to make optimum utilization of locally available resources, such as feed and to promote sustainable farming systems.

## SOME BREEDING STRATEGIES

### Breeding Buffaloes for Draught Purpose

Recognizing that the working capacity of an animal is directly related to body size and weight, rural farmers generally select larger buffaloes for work. In many countries farmers prefer to use male animals for work due to its larger body size. However, during recent years more and more female buffaloes are used for work as well as for reproduction due to the shortage of draught animals.

The measurement of draught ability of an animal is rather complicated since it depends on age, sex, size, health and nutritional conditions, training, harness and working equipment, time of day, soil types and conditions, *etc*. Since the conditions under which the animals are put to use vary from one location to another, standard engineering or laboratory measurement might not be useful or meaningful to all farm situations.

Draught buffaloes are generally used for work during early morning hours, usually from 05:00 hr. until 10:00 or 11:00 hr. It has been well recognized that buffaloes are generally less heat tolerant than cattle due to fewer sweat glands in the skin. No breeding work has been done to improve heat tolerance in buffaloes. It is probably quicker and more effective to improve animal working efficiency by reducing heat stress through management or other body cooling methods.

Animal draught efficiency can also be significantly increased through the use of improved harness systems (Garner, 1957; Starkey 1989), or improved farm tools and implements (Starkey and Sims, 1989). However, most of these improved harness systems and tools have not been widely adopted by rural farmers due to various reasons. Many of these problems were due to a lack of farmer participation in the development of these tools and equipment. Other socio-economic factors and government policies, for instance, lack of credit for small farms, lack of farmer training, or low price for farm commodities, have had negative effects on the ability of rural farmers to adopt new technologies. Indeed, it will be very useful and helpful to find ways and means to extend existing improved farm tools and harness systems to farmers in rural areas. If this effort becomes successful, animal draught capacity will be much improved.

Breeding improvement of buffaloes for draught purpose through genetic selection within a population is expensive and slow as compared to improvement by other methods such as feeding and management, health care, and use of improved farm tools and harness systems. From breeding aspects, it appeared to be generally agreed that the draught ability of buffaloes could be improved as a secondary purpose within the improvement programs for meat or milk production (Chantalakhana, 1992; Gunawan and Vercoe, 1989) by simultaneously selecting for animal body weight and height.

Crossbreeding of swamp buffalo with Murrah and Nili-Ravi in order to improve draught ability as well as milk and meat has been conducted in some countries such as the Philippines and China. Some crossbreds were found to have lighter body weights than the swamp (local) buffalo at the same age, while the working ability of crossbreds having the same body weight and heat resistance were comparable to the local (Momongan *et al.*, 1989). It was reported that crossbred buffaloes in China were superior to local swamp buffalo in working ability, i.e. draught power, area ploughed per unit time, ploughing speed and duration of work. This could be attributed to the lighter body weight of the crossbreds (Xiao Yongzuo, 1989a).

*Castration of draught buffaloes:* In some Asian countries, male buffaloes are usually castrated by farmers at about 2-3 years of age before training for work. Castrated animals are believed to be more tame and not distracted by females while working. However, castration of larger males in effect has resulted in negative selection against superior breeding stock as evident by the decline in the average body size of buffalo in Thailand and some other SEA countries. Therefore, it is very important to provide superior buffalo bulls for breeding at village level. Artificial insemination of buffalo cows with semen from superior bulls has had a limited impact at farm level as far as draught buffaloes on small farms are concerned. This is due to various socio-economic, technical as well as institutional constraints facing village farmers.

## Breeding Buffaloes for Meat or Meat-and-Draught Purpose

Meat production from buffalo has provided an important part of total national beef supply in many Asian countries. In China and SEA countries, many buffaloes are sold for slaughter immediately after the planting season in order to obtain cash for purchase of fertilizer and other farm inputs. In some Asian countries, such as the Philippines, China, and Thailand, more and more farmers raise buffaloes for sale while buffalo draught power becomes a complementary source of farm power to the two-wheel tractor. As the beef demand rises with increasing number of people and their educational and income levels in Asian countries, it is anticipated that meat production from buffalo will become more economically important because cattle beef supply does not grow at a sufficient rate to meet the fast expanding demand. In a country like Thailand with approximately 60 million people, it was estimated that each year up to one million cattle and buffaloes were imported (smuggled) for slaughter from neighbouring countries, while according to official statistics only about half a million cattle and buffaloes were slaughtered in various municipal slaughter houses annually. It was estimated that illegal slaughtering in some areas could be up to 3 to 5 times the official records. Therefore, it is anticipated that buffalo raising for meat purposes at small farm level will increase in the future, while feeding and management will continue to rely on the use of low quality roughages such as rice straw, natural grasses and tree leaves. However, buffalo fattening or finishing operations utilizing supplementary energy feeds such as cassava chips or molasses could become profitable as beef demand rises. Hence breeding strategies to improve buffalo for meat or meat-and-draught can be quite useful for many Asian countries.

The traits related to meat production in buffaloes such as weights at different ages and growth rates are expected to be medium to highly heritable, with $h^2$ of 0.3 to 0.7 (Bhat, 1992; Chantalakhana, 1992). There has been no genetic study on buffalo carcass traits so far but judging from experiences in beef cattle breeding they can be expected to be highly heritable, which means that breeding improvement can be effective.

*Selection of swamp buffalo for beef and draught:* Superior male and female buffaloes can be selected based on their own performance such as weights at 6 or 8 months and at one year to 18 months of age and growth rates before and after weaning at 6 or 8 months of age. Body height at withers and conformation score can also be used as supplementary criteria, while some reproductive traits such as size of scrotum or udder should also be observed.

Performance testing of animals from known or pedigree parents selected from breeding herds after weaning needs to be conducted at a central location where buffaloes can be fed and managed under uniform and well-planned herd management. Since buffaloes will be used for breeding by village farmers after the test it is important to provide them a similar feeding and management regime while on the test. The testing period is relatively longer (up to 10 months or an year) if the animals are tested for growth performance on grass. During the test, buffaloes are allowed to graze in pasture during the day and return to the barn in the evening where rice straw or other crop residues are fed *ad libitum*. Mineral licks are also provided in the barn as well as clean drinking water. In the early morning hours before buffaloes are let out to graze, they are fed with feed supplements at about 2-3 kg per head. Feed supplements are composed of low priced and locally available ingredients such as cassava chips, leucaena leaf meal, *etc.* Feed supplements are provided to tested buffaloes in order to allow optimum genetic expression as far as growth rates and body weights and size are concerned. A selection and breeding scheme for buffaloes is shown in the following diagram (Figure 1).

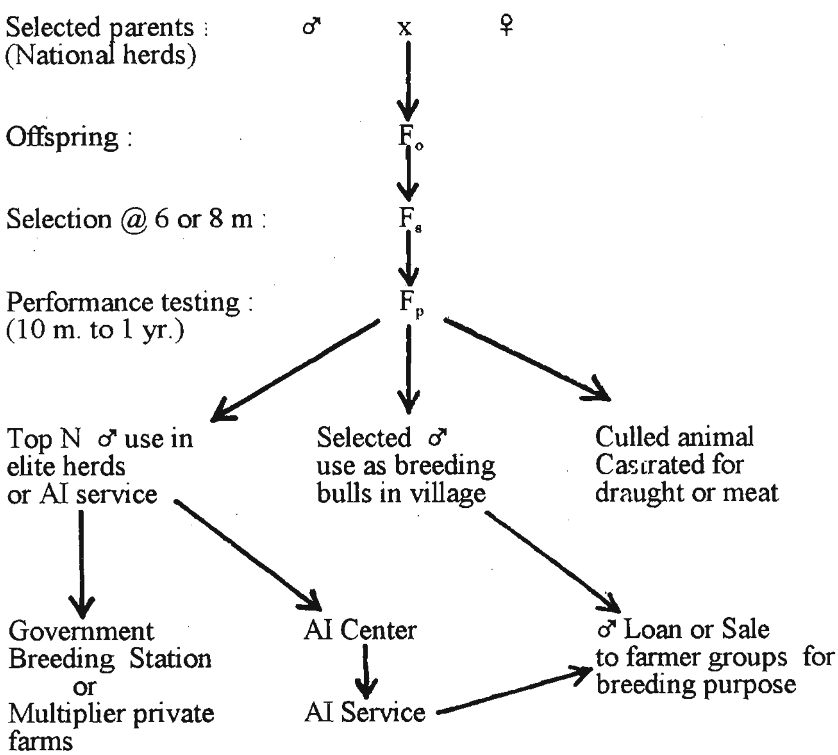



**Figure 1.** Selection and Breeding Scheme

An example of a buffalo selection scheme for meat and draught purpose in Thailand was reported by Konanta and Intaramongkol (1994) with satisfactory progress. This selection scheme implemented by the Thai government has been going on for more than 15 years. It started as a small scale project and is to be expanded in scale in the next five years (1996-2001) as the demand for meat and draught buffalo increases.

*Crossbreeding for beef and draught:* Crossbreeding of swamp buffalo with river breeds such as Murrah has been carried out in various countries in Asia in order to improve meat production and draught ability. $F_1$ crossbreds of swamp x Murrah are fertile and perform well under rural conditions. Their working ability is comparable to the swamp parent, while body size and growth rates in some cases equal or are slightly better as reported by Momongan *et al.* (1989), and shown in Tables 2 and 3.

**Table 2.** Least-square means (LSM in kg) for body weights of Philippine carabao and crossbreds.

| Age (months) | Philippine carabao | | Phil-Murrah $F_1$ | | Phil-Ravi $F_1$ | |
|---|---|---|---|---|---|---|
| | n | LSM | n | LSM | n | LSM |
| 0 | 102 | $26\pm1^a$ | 182 | $35\pm1^b$ | 38 | $35\pm1^b$ |
| 6 | 95 | $111\pm5^a$ | 172 | $127\pm6^b$ | 37 | $133\pm6^b$ |
| 12 | 85 | $160\pm6^a$ | 160 | $210\pm9^b$ | 31 | $215\pm9^b$ |
| 18 | 75 | $204\pm7^a$ | 125 | $281\pm15^b$ | 27 | $283\pm11^b$ |
| 24 | 58 | $239\pm9^a$ | 105 | $318\pm17^b$ | 24 | $332\pm19^b$ |
| 36 | 48 | $305\pm10^a$ | 98 | $462\pm24^b$ | 21 | $460\pm25^b$ |

Note: Significant difference ($p<0.01$) only between Philippine carabao and crossbreds.

One major concern relating to crossbreeding of the swamp buffalo ($2n = 48$) with river buffalo ($2n = 50$) is the unequal number of chromosomes, which result in unbalanced chromosome numbers ($2n = 49$) in the $F_1$ crossbreds. So far no breeding work in the field has been reported to indicate that the fertility of $F_1$ crossbreds is negatively affected by this cytogenetical phenomenon. However, Chavananikul (1994) reported that some recent investigations showed the occurrence of a meiotic impairment which affected the semen quality in the crossbred buffalo males carrying unbalanced karyotype of 49 chromosomes. No report on the effect of unbalanced chromosomes on the fertility of crossbred buffalo cows is available so far. $F_2$ and $F_3$ upgraded (backcross to river breed) buffaloes have been produced in different countries but the long-term ultimate breeding goal is often unclear while sufficient and good data are lacking to carry out comparisons of production and reproductive performance of crossbreds and their parental breeds.

**Table 3.** Work performance of Philippine carabao (PC) and Phil-Murrah $F_1$ (CB) steers.

| Parameters | Wet land condition | | Dry land condition | |
|---|---|---|---|---|
| | PC | CB | PC | CB |
| Depth of ploughing (cm) | 12.25 | 12.23 | 11.92 | 11.14 |
| Width of ploughing (cm) | 17.61 | 17.69 | 18.94 | 18.57 |
| Soil moisturé (%) | 42.96 | 43.74 | 22.92 | 22.55 |
| Hardness of soil (kg/ $cm^2$) | 1.66 | 1.66 | 1.91 | 1.92 |
| Ploughing velocity (m/sec) | 0.50 | 0.54 | 0.50 | 0.50 |
| Ploughing time (hour) for 2500 $m^2$ | 8.89 | 8.29 | 7.14 | 8.72 |
| Average draught force (kg) | 54.14 | 49.42 | 50.60 | 50.60 |
| Drawbar horsepower (PS) | 0.36 | 0.35 | 0.34 | 0.34 |

## Breeding Buffaloes for Meat-Milk-and -Draught

Crossbreeding between the swamp buffalo and the river breeds is often aimed at producing milk in addition to meat and draught in the crossbreds, as reported in China and the Philippines (Xiao Yongzuo, 1989a; Momongan *et al.*, 1989). In many developing countries, though UHT milk is available and can be kept without refrigeration, drinking milk is not always available to rural people, and furthermore milk is still expensive and not affordable by farmers. Crossbred buffaloes can produce 3 to 5 kg of milk per day which can be used for household consumption, where only 1 or 2 buffalo cows are kept. Hence, buffalo crossbreeding in this case is aimed at improving nutritional status and human health as well, especially for children and elders.

Only moderate levels of milk and meat production is expected from crossbred buffaloes since most dairy and beef characteristics in bovine animals are genetically negatively correlated. Under village conditions where feed resources and feed quality are commonly limited, milk production by an animal could mean that less nutritional supply is available for meat production. Similarly, to use crossbred buffaloes for farm work resulted in decrease of milk production by 12 to 17% (Xiao Yongzuo, 1989b). However, Zerbini *et al.* (1995) who worked on dairy cow traction in Ethiopia concluded that with appropriate feeding levels dairy cows could be used for draught purpose without any detrimental effects on fertility, except for extended calving intervals and a small reduction in milk (see Table 4). Further, feed supplementation to dairy cows used for work could results in a satisfactory level of milk production, but socio-economic considerations at small farm level may render feed supplementation of the multipurpose animals impractical and uneconomical.

Rural people in some countries are not natural milk drinkers and milk drinking could be problematic; marketing milk is also a complicated process. In such cases promoting milk production from buffalo might not be too useful. However, improved milk production of buffalo cows will definitely increase growth rate and body weight of the offspring at weaning age.

**Table 4**. Cumulative milk yield (kg) of $F_1$ crossbred cows over a period of two years.

| Treatment | 0-365d | 0-730d |
|---|---|---|
| Non working/non supplemented | 849 | 1,226 |
| Non working/supplemented | 1,792 | 3,186 |
| Working/non supplemented | 802 | 927 |
| Working/supplemented | 1,770 | 3,044 |
| Standard error | 152 | 219 |

Table 5 shows the levels of milk yield from different buffalo genotypes in China (Xiao Yongzuo, 1989b). It should be noted, however, that these buffaloes were raised on experimental farms and they were expected to be well managed for milk production.

**Table 5**. Milk yield of different buffalo breeds in China.

| Breed | Number of animals | Average lactation period (days) | Average lactation yield (kg) | Average daily milk yield (kg) | Highest daily milk yield (kg) |
|---|---|---|---|---|---|
| Local (Swamp) | 10 | 235 | 441±212 | 1.9 | 5.5 |
| MxL | 12 | 276 | 1096±261 | 3.7 | 9.5 |
| MxL($F_1$) | 60 | 270 | 1153±397 | 4.3 | 11.0 |
| Mx(MxL)($F_2$) | 12 | 291 | 1540±687 | 5.2 | 13.0 |
| Triple-Cross | 10 | 288 | 1981±470 | 6.9 | 5.3 |
| Murrah | 81 | 237 | 1573±524 | 6.6 | 7.0 |
| Nili-Ravi | 25 | 261 | 1873±690 | 7.2 | 9.9 |

## Breeding Buffaloes for Milk Production

The use of buffaloes as dairy animals by rural farmers has been well known in many countries such as India and Pakistan. River buffaloes of at least 15 breeds have been described by various authors (Mudgal and Sethi, 1989; Bhat, 1992). Some river breeds such as the Murrah and Nili-Ravi have been introduced to some other countries such as China and the Philippines to cross with local buffaloes in order to use the crossbreds as dairy or multi-purpose buffaloes. Different types of crosses such as that shown in the following breeding scheme (Figure 2) are commonly carried out in various countries, especially in SEA and China where only swamp buffaloes exist.

Mudgal and Sethi (1989) did a comprehensive review of performance for both meat and milk of different breeds of river buffaloes and some types of crossbreds between river and swamp buffaloes, mainly in China and the Philippines as further reported by Xiao Yongzuo (1989a and 1989b) and Momongan *et al.* (1989). Only the Murrah and the Nili-Ravi have been used more extensively so far in crossbreeding programs with the swamp buffalo in Asia. The milk yield and lactation period of dairy buffaloes of different genotypes appeared to be at satisfactory levels as shown in Table 6 (Xiao Yongzuo, 1989b).

(A) Local (Swamp) x Murrah (M)

↓

$F_1$ (50% M)

↙ ↓ ↘

| (B) Interse mating | (D) Triple cross | (C)upgrading (Backcross) |
|---|---|---|
| $F_1$ x $F_1$ | $F_1$ x Nili-Ravi | $F_1$ x Murrah |
| ↓ | ↓ | ↓ |
| $F_2$ (50% M) | $F_2$" (50% N 25%M) | $F_2$ (75% M) |
| ↓ | ↓ | ↓ |
| ? | ? | $F_3$ (87.5% M) |
| | | ↓ |
| | | ? |

**Figure 2**. Some Crossbreeding Schemes for Swamp x River Buffaloes.

**Table 6.** Milk production of different buffalo genotypes in China.

| Breed | Lactation sequence | Lactation period (d) | Lactation milk yield (kg) | Highest milk yield (kg) | Average milk yield (kg) |
|---|---|---|---|---|---|
| Triple | 1 | 311.5 | 2100.7 | 12.5 | 6.7 |
| Crossbred | 2 | 312.2 | 2574.2 | 15.7 | 8.2 |
| | 3 | 317.9 | 2704.9 | 17.0 | 8.5 |
| Nili-Ravi | 1 | 297.0 | 1500.0 | 7.4 | 5.0 |
| x Local | 2 | 351.8 | 2370.1 | 11.5 | 6.7 |
| | 3 | 324.3 | 2287.0 | 11.5 | 7.0 |
| Murrah | 1 | 269.6 | 711.9 | 16.7 | 6.3 |
| | 2 | 269.5 | 1921.8 | 17.5 | 7.1 |
| | 3 | 284.7 | 2073.6 | 14.8 | 7.2 |
| Nili-Ravi | 1 | 274.3 | 1825.4 | 19.9 | 6.6 |
| | 2 | 265.7 | 2087.2 | 19.9 | 7.8 |
| | 3 | 297.2 | 2096.5 | 18.7 | 7.5 |

Judging from existing reports, it is not possible to state clearly what breeds or genotypes are best suited for milk production. Variation due to feeding, management and environmental conditions apparently presented difficulty in genetic evaluation of various crosses conducted in different studies, while the numbers of animals in each study were usually not sufficiently large.

*Cattle vs. buffalo crossbreds for dairy production:* In countries where buffalo milk, which is exceptionally high in percent butterfat (7-8%), is not a traditional food for local people, there is a question whether cattle crossbreds such as Holstein crosses or buffaloes are more suitable for dairy production. In many Asian countries, Holstein crossbreds of 50% or higher can easily produce 15 kg milk per day or more, while buffaloes of river breeds or crosses usually average lower yields (6-8 kg). It appears that dairy farmers can obtain a higher income from raising crossbred dairy cattle than crossbred buffaloes in SEA countries, such as Thailand, where the benefit of high butterfat in buffalo milk cannot compensate for lower milk yields. However, crossbred buffaloes become more superior to crossbred cattle when they are considered as the multipurpose animals in integrated farming systems to utilize farm wastes and by-products, natural grasses and tree leaves, as well as marginal lands such as highway shoulders and scrub forest.

## CONCLUDING REMARKS

In rural areas especially under rainfed conditions, buffaloes have been incorporated as a vital component in sustainable agricultural production systems. They continue to provide renewable resources in terms of draught power and manure, while meat and milk are often secondary products in these systems. It is very evident in Thailand, that rice productivity during the last three or four decades has been sustained at a stable level, while chemical fertilizer use has been minimal. Thai farmers in rainfed areas have been using cattle and buffalo manure to maintain soil fertility. Manure is spread on to paddy fields in early rainy season and ploughed down before rice planting. Each adult buffalo can produce 4 to 6 metric tons of wet manure per year.

In most developing countries draught animal power remains an important source of energy input per unit of cultivated land area in spite of the increasing use of petroleum-based power in certain areas of some countries, wherever technically feasible and economically viable. Even today, water buffaloes provide 20-30% of the farm power in South China, Thailand, Indonesia, Malaysia, the Philippines and Indochina. Considering the socio-economic aspects of existing farming systems in these countries, which are not expected to change quickly or drastically, the mixed use of all these sources of power in suitable proportions will offer the highest benefit to the farmers, especially those engaged in rainfed agriculture.

In the last two decades, farm mechanization has increased at various rates in many Asian countries. This raises questions concerning the future of draught animal power. But it is clear that the benefits of mechanization on crop yield will be realized only when other production inputs such as fertilizer, insecticide and irrigation water become available and economical. It is therefore evident that under the prevailing rainfed and subsistence farming conditions in developing countries mechanization has not played a major role in sustainable crop production. For Asia, in general, in spite of the apparent increase in the use of farm machinery especially in irrigated areas, in most of the cultivated areas the use of draught animal power will remain relatively high. In many cases, the use of draught animal power can be strengthened so as to be complementary to the mechanized system.

Another important aspect of sustainable integrated farming systems practised by rural smallholders is the utilization of crop wastes and by-products, such as cereal straws, maize and sorghum stovers, pineapple waste, sweet potato vines and groundnut vines, by ruminant animals. Buffaloes are known to digest such poor-quality roughages more efficiently than cattle, and in developing countries small farmers commonly use most crop wastes and by-products to feed animals, especially buffaloes.

Buffalo raising as part of small farm agricultural systems has positive effects on social and economic sustainability and consequently on rural development. Buffaloes are usually looked after by the women, old folk and children, who are not employed otherwise. Old folk in the family feel that they are being useful and needed when tending buffaloes. One of the major reasons why rural people choose to remain at home during the dry season and not migrate to work in cities is because they have to look after their buffaloes or other livestock.

## References

Bhat, P.N. (1992) Genetics of river buffaloes. *Buffalo Production*, Chapter 2. Ed. N. M. Tulloh and J.H.G. Holmes. World Animal Science, C6. Elsevier Science Publishers, Amsterdam. pp 13-58.

Bunyavejchewin, P. and Chantalakhana, C. (1991) Buffalo Production in East and Southeast Asia: Problems and prospects. Papers presented at III World Buffalo Congress. In: *"Buffalo Production around the World : Problems and Prospects"*, Ed: C. Chantalakhana. Kasetsart University, Bangkok, Thailand. pp 30-45.

Chantalakhana, C. (1991) *Buffalo Production around the World: Problems and Prospects*. Proceedings of III World Bufflo Congress. Kasetsart University, Bangkok, Thailand. pp 80.

Chantalakhana, C. (1992) Genetics and breeding of swamp buffaloes. *Buffalo Production*. Chapter 4. Edited by N.M. Tulloh and J.H.G. Holmes. World Animal Science, C6. Elsevier Science Publishers, Amsterdam. pp 95-110.

Chavananikul, V. (1994) Cytogenetic aspects of crossbreeding for the improvement of buffalo. *Proceedings of the First Asian Buffalo Assoc. Congress*. Ed: P. Bunyavejchewin *et al.* Kasetsart University, Bangkok, Thailand. pp 153-159.

Devendra, C. (1995) Increasing research and development focus on the relevance of draught power from buffaloes. *Proceedings of Symposium on Improving Draught Capacity of the Multi-purpose Buffaloes in Small Farm Systems*, Edited by P. Bunyavejchewin *et al.*, Kasetsart University, Bangkok, Thailand. pp 3-12.

Garner, J.K. (1957) *Increasing the Work Efficiency of the Water Buffalo in Drawing the Plough and other Implements through the Use of Improved Harness*. Thailand United States Operations Mission. pp 11.

Gunawan, B. and Vercoe, J.E. (1989) Genetic improvement of draught animals. *Proceedings on Draught Animals in Rural Development,* Ed: P. Hoffmann *et al.* ACIAR Proceedings No. 27. pp 195-198.

Konanta, S. and Intaramongkol, J. (1994) Buffalo selection schemes in Thailand. *Proceedings of the First Asian Buffalo Assoc. Congress.* Ed: P. Bunyavejchewin *et al.* Kasetsart University, Bangkok, Thailand. pp 71-84.

Momongan, V.G., Parker, B.A., de los Santos, E.B. and Ranjhan, S.K. (1989) *Proceedings on Draught Animals in Rural Development,* Ed: D. Hoffmann *et al.* ACIAR Proceedings No. 27. pp 190-194.

Mudgal, V.D. and Sethi, R.K. (1989) Riverine breeds of buffaloes in Asia. *Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia.* Ed: M.K. Vidyadaran *et al.* Universiti Pertanian Malaysia, Malaysia. pp 27-44.

Soni, B.K. (1991) Buffalo production in South Asia: Its problems and propects. Papers presented at III World Buffalo Congress. In: " *Buffalo Production around the World: Problems and Prospects*", Ed: C. Chantalakhana. Kasetsart University, Bangkok, Thailand. pp 17-29.

Starkey, P. (1989) Harnessing for cattle and buffaloes: Options and research. *Proceedings on Draught Animals in Rural Development*, Ed: P. Hoffmann *et al.* ACIAR Proceedings No. 27. pp 280-292.

Starkey, P. and Sims, B. (1989) Animal-drawn implements : An overview of recent research and development. *Proceedings on Draught Animals in Rural Development*, Ed: D. Hoffmann *et al.* ACIAR Proceedings No. 27. pp 248-257.

Xiao Yongzuo. (1989a) Production performance of crossbred buffaloes in China. *Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia.* Ed: M.K. Vidyadaran *et al.* Universiti Pertanian Malaysia, Malaysia. pp 245-254.

Xiao Yongzuo. (1989b) The buffalo improvement programme in China. *Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia.* Ed: M.K. Vidyadaran *et al.* Universiti Pertanian Malaysia, Malaysia.

Zerbini, E., Wold, A.G. and Gemeda, T. (1995) Dairy cow traction: Effect of draught work on milk production and reproduction. *Proceeding of a Symposium on Improving Draught Capacity of the Multi-purpose Buffaloes in Small Farm Systems.* Ed: P. Bunyavejchewin *et al.*, Kasetsart University, Bangkok, Thailand. pp 170-189.

# PHYSIOLOGICAL RESPONSES OF LANKAN BUFFALOES TO STRESS AT WORK

A.A.J.Rajaratne[1] and S.S.E.Ranawana[2]

[1]*Department of Physiology, Faculty of Medicine, University of Peradeniya*
[2] *Veterinary Research Institute, Gannoruwa, Peradeniya*
*SRI LANKA.*

**Abstract**: A series of experiments was carried out to investigate the degree of stress that buffaloes undergo during different types of work in the field. Physiological parameters such as heart rate, respiration rate and rectal temperature were measured in Lankan buffaloes engaged in different types of work namely threshing paddy, ploughing muddy fields and ploughing dry lands before and at 30 minute intervals of working. Mean rectal temperature increased from 38.1°C to 39.2°C, mean respiration rate from 21 to 45 per minute and mean pulse rate from 63.3 to 84.5 per minute during a 1.5 h period of ploughing muddy fields. Mean rectal temperature increased from 37.4°C to 39.7°C, mean respiration rate from 20.3 to 75.5 per minute and mean pulse rate from 66.5 to 82 per minute when animals ploughed dry land for a period of 1.5 h. When the animals were used for threshing paddy for a period of 2 hrs, mean rectal temperature increased from 38.5°C to 40.5°C, mean respiration rate from 34.5 to 112 per minute and mean pulse rate from 68.9 to 107.3 per minute.

The results indicate that threshing paddy although not a very strenuous exercise, caused significantly higher stress in buffaloes compared to ploughing muddy fields. When water was poured as a method of alleviating stress in working buffaloes, there was a significant reduction in the pulse rate and respiration rate but there was no significant effect on rectal temperature. The results point to heat stress as the main stress factor in working buffaloes but the work load itself does not seem to cause a significant effect. Therefore, it is important to separate these two effects when studies are conducted on stress in buffaloes at work.

**Keywords**: Stress, work, respiration, rectal temperature, pulse

## INTRODUCTION

The use of animals as a draught power source has been recognized as extremely important and useful by people in all parts of the world. It is a conventional practice in Sri Lanka also to use animals for agricultural activities. The most common species of animals used for this purpose are buffaloes and cattle. Buffaloes are commonly used for ploughing, harrowing, levelling paddy fields and threshing paddy in Sri Lanka.

Efficiency of work performance is likely to be affected by several factors namely, species, duration and type of work and climatic conditions. Animals employed for draught in warm climates are often subject to stress from direct effects such as climate as well as indirect effects such as low quality feed (Devadattam and Mayura, 1978). Studies have been carried out in other countries to investigate the physiological changes occurring in cattle during work. However, similar studies in buffaloes are scarce. Therefore, the objective of this study was to monitor the physiological changes taking place during work in order to obtain maximum efficiency in working buffaloes.

## MATERIALS AND METHODS

As a tool for monitoring physiological changes, measurements of respiration rate, pulse rate and rectal temperature were made on working buffaloes, prior to the commencement of work and thereafter, at 30 minute intervals on different days. These measurements were made in buffaloes threshing paddy, ploughing muddy fields and also ploughing dry fields. Effect of pouring water was also studied when the animals were threshing paddy and ploughing dry land. Environmental measurements such as dry and wet bulb temperature were also made. The respiratory rate was measured first by observation of flank movements of animals for one minute without disturbing them. As pulse rate is a good indicator of the heart rate, pulse rate was measured by counting the pulse in the coccygeal artery for a period of one minute.

Rectal temperature was measured last in order to minimize the effect on other measurements. A nylon cord was tied to a clinical thermometer and the free end of the cord was tied to a snap-paper clip. By this device the thermometer could be clipped to the skin on the gluteal region. This prevented damage to the thermometer in case dung was voided during measurements.

Statistical analysis: Significance of differences between treatments as well as groups was tested by performing Student's t test.

## RESULTS

Table 1 presents means of physiological measurements made in buffaloes ploughing dry land. There was a gradual but significant ($p<0.02$) increase in rectal temperature and respiration rate as the work progressed. Although, there was a significant increase ($p<0.001$) in pulse rate at 30 minutes from the resting value, no significant increases were observed thereafter. There was a significant reduction in respiration rate, pulse rate and rectal temperature when water was poured on the animals. Although significant at probability level of 0.01, the drop observed in

**Table 1.** Mean rectal temperature, respiration rate and pulse rate in buffaloes ploughing dry land (n=4).

| Time (hrs) | Dry bulb Temp. ($^0C$) | Wet bulb Temp. ($^0C$) | Rectal Temp ($^0C$) | Respiration Rate ($min^{-1}$) | Pulse Rate ($min^{-1}$) |
|---|---|---|---|---|---|
| 0 | 25.0 | 20.6 | 37.4 | 20.3 | 66.5 |
| 0.5 | 25.6 | 21.7 | 38.2 | 32.0 | 79.0 |
| 1.0 | 27.2 | 22.8 | 38.4 | 51.5 | 79.0 |
| 1.5 | 28.3 | 23.3 | 39.7 | 75.5 | 82.0 |
| Pouring water | | | | | |
| 2.0 | 29.4 | 23.9 | 39.1 | 24.0 | 70.5 |

rectal temperature by pouring water was only 0.6°C. However, the respiration rate declined from 75.5 to 24 per minute and the reduction was very highly significant ($p<0.0001$).

Table 2 presents the means of the physiological measurements made in buffaloes ploughing muddy fields. There was a significant ($p<0.02$) increase in all the parameters when working, compared to the resting state. There was no significant increase in rectal temperature during working. However, a gradual but quantitatively small increase in respiration rate and pulse rate was observed during working.

**Table 2.** Mean rectal temperature, respiration rate and pulse rate in buffaloes ploughing muddy fields (n=4).

| Time (hrs) | Dry bulb Temp (°C) | Wet bulb Temp (°C) | Rectal Temp (°C) | Respiration Rate ($min^{-1}$) | Pulse Rate ($min^{-1}$) |
|---|---|---|---|---|---|
| 0 | 26.4 | 24.4 | 38.1 | 21.0 | 63.3 |
| 0.5 | 28.1 | 25.3 | 38.7 | 30.5 | 75.5 |
| 1.0 | 30.0 | 27.2 | 39.1 | 41.0 | 81.8 |
| 1.5 | 29.7 | 26.1 | 39.3 | 45.0 | 84.5 |

Table 3 presents the means of physiological measurements made in buffaloes threshing paddy. In general, there was a significant increase in rectal temperature, pulse rate and respiration rate as the work progressed.

**Table 3.** Mean rectal temperature, respiration rate and pulse rate in buffaloes threshing paddy (n=15).

| Time (hrs) | Dry bulb Temp (°C) | Wet bulb Temp (°C) | Rectal Temp (°C) | Respiration Rate ($min^{-1}$) | Pulse Rate ($min^{-1}$) |
|---|---|---|---|---|---|
| 0 | 29.0 | 24.5 | 38.5 | 34.5 | 68.9 |
| 0.5 | 29.9 | 24.2 | 39.2 | 66.3 | 76.2 |
| 1.0 | 29.4 | 24.1 | 39.6 | 97.1 | 82.8 |
| 1.5 | 28.7 | 24.2 | 40.1 | 103.3 | 96.9 |
| 2.0 | 28.9 | 23.3 | 40.5 | 112.0 | 107.3 |

However, the differences in rectal temperature and respiration rate between 1.5h and 2.0h were not significant. Pulse rate difference between 1.5h and 2.0h was statistically significant ($p<0.05$). There was a significant increase in all the parameters in this treatment compared to the other treatments.

Table 4 presents the effect of pouring water on working buffaloes. There was a significant reduction in respiration rate, pulse rate and skin temperature when water was poured on animals. However, there was no significant reduction in rectal temperature following this treatment.

**Table 4.** Effect of pouring water on mean rectal temperature, respiration rate and pulse rate in working buffaloes (n=8) at dry bulb temperature of 29.5 °C.

| | Rectal Temp. (°C) | Respiration Rate ($min^{-1}$) | Pulse Rate ($min^{-1}$) | Skin Temp. (°C) |
|---|---|---|---|---|
| Before | 39.9 | 105.6 | 95.5 | 41.4 |
| After | 39.6 | 48.6 | 82.6 | 36.9 |
| t value | 1.43 | 7.0 | 3.49 | 9.91 |
| Level of significance | NS | $p < 0.0001$ | $p < 0.004$ | $p < 0.0001$ |

## DISCUSSION

The resting values for rectal temperature, respiration rate and pulse rate in these buffaloes were comparable to those recorded by Rajaratne *et al.* (1983). Comparatively higher resting values seen in the buffaloes used for threshing paddy indicate the effect of high ambient temperature that prevailed at the time of measurement.

There is a direct relationship between the severity of exercise and the pulse rate, and animals are expected to have a pulse rate above 150 per minute when performing exercise at a sub-maximal work load.

Lower pulse rate seen in buffaloes used for ploughing muddy fields indicate that these animals were performing exercise at a rate well below the sub-maximal load. On the other hand, according to Johnson *et al.* (1963) a rapid heart rate and a higher cardiac output is indicative of thermal strain. Therefore, the increase in heart rate observed in these working buffaloes is a result of a combination of both work and thermal strain but the thermal strain was responsible for the major part of the effect.

Heat stress has been shown to increase respiratory activity in animals (Worstell and Brody, 1953). The contribution of respiratory evaporation to heat dissipation in cattle and buffaloes is much smaller than that of cutaneous evaporation. Indeed, it is well established that respiratory activity under conditions of heat stress is a measure of the inadequacy of the quantitatively more important cutaneous evaporation to maintain heat balance (Riek and Lee, 1948). High respiratory rate is an attempt to compensate for a poor heat balance brought about by some other causes (Bianca, 1962). On the other hand, exercise also increases pulmonary ventilation in order to provide a constant supply of oxygen required for metabolic activities. A moderate increase in respiration rate in the buffaloes ploughing muddy fields indicates that the severity of exercise is not sufficient to cause any excessive increase in respiration rate.

Bligh (1955) tested the validity of using rectal temperature as a measure of deep body temperature and concluded that the rectal temperature was 0.1-0.3°C lower than carotid blood temperature under conditions of thermo-neutrality and mild heat stress, whereas both temperature measurements were identical under severe heat stress. Rectal temperature is a fairly good indicator of the core temperature though there appears to be variations in different sites. There is a distinct diurnal variation in deep body temperature in cattle and buffaloes, the maximum being in early evening and the minimum in early morning ( Wrenn *et al.*, 1961; Rajaratne *et al.*, 1983). A rise in core temperature is often taken as a sign that the animal has failed to bring into action the proper adaptates to maintain heat balance (McDowell, 1966). Excessive heat in the body produces undesirable effects on a number of physiological processes, chiefly on metabolism by lowering the feed substrate to the cells and lowering calorigenic hormones in the body.

A change in any of these physiological processes will provide some indication of the animal's response to thermal stress. However, the animal's body temperature is the direct measure of its physiological response to heat stress. Other reactions have been generally considered supplementary, indicating either the way in which the animal attempts to keep its heat load in balance or some of the consequences of the failure to do so.

It is clear from the results that although threshing paddy is a light type of work, very high rectal temperature together with high respiration and pulse rate seen, indicate that the animals were severely stressed by performing this work. This increase is due to the thermal strain created by the insulation effect of a thick covering of straw over the ground. Therefore, extra care should be taken when buffaloes are used for threshing paddy. On the other hand, ploughing paddy fields caused a mild heat stress as indicated by comparatively lesser increases in the physiological parameters due to a constant loss of heat into water in the paddy fields. Wallowing is one of the main avenues of heat loss in buffaloes (Ranawana *et al.*, 1985). Although pouring water could be a useful method of alleviating heat stress, it was not sufficient to cause an appreciable reduction in the rectal temperature. The marked reduction observed in the respiration rate with the reduction in skin temperature, in the absence of any significant change in rectal temperature suggests that the respiratory centre situated in the medulla of the brain is influenced by the thermo-receptors in the skin.

The results of these experiments point to heat stress as the main factor responsible for physiological changes observed in working buffaloes, but the work load itself does not seem to cause a significant effect. Therefore, it is important to separate these two effects when studies are conducted on stress in buffaloes at work.

## Acknowledgements

We acknowledge the able technical assistance of Miss. S.Kulatunge, Mr. A.G.Jayasena and Mr. R.A.D.Nicolas in conducting these studies. The work was financially supported by the Swedish Agency for Research Cooperation with Developing Countries.

## References

Bianca, W. (1962) Tolerance to severe heat stress and the behaviour of respiratory minute volume in cattle. *Nature* London, **195,** 1208-1209.

Bligh, J. (1955) Comparison of rectal temperature and deep body temperature in the calf. *Nature* London, **176,** 402-403.

Devadattam, D.S.K. and Mayura, N.L. (1978) Draught ability of Haryana bullocks. *Indian J. Dairy Sci.* **31,** 120-127.

Johnson, J.E., Naelapaa, H. and Frye, J.B. (1963) Physiological responses of Holstein, Brown Swiss and Red Scindhi crossbred bulls exposed to high temperature and humidities. *J. Anim. Sci.* **22,** 432-436.

McDowell, R.E. (1966) The role of physiology in animal production for tropical and sub-tropical areas. *Anim. Prod.* **1,** 39-46.

Rajaratne, A.A.J., Ranawana, S.S.E. and Tilakaratne, N. (1983) Comparative tolerance of hot-humid climatic conditions by three species (*Bos taurus, Bos indicus* and *Bos bubalus bubalis*) of Bovidae. *Sri Lanka Vet. J.* **31,** 21-26.

Ranawana, S.S.E., Rajaratne, A.A.J. and Tilakaratne, N. (1985) Dissipation of body heat by buffaloes during wallowing. In: *Proceedings of the 3rd AAAP Congress*, May 6-10, Seoul, South Korea, pp 894-896.

Riek, R.F. and Lee, D.H.K. (1948) Reactions to hot atmospheric conditions of Jersery cows in milk. *J. Dairy Res.* **15,** 219-226.

Worstell, C.F. and Brody, S. (1953) Comparative physiological reactions of European and Indian cattle to changing temperature. *Research Bulletin 515.* Missouri Agricultural Experimentation Station.

Wrenn, T.R., Bitaman, J. and Syke, J.F. (1961) Diurnal patterns of body temperature. *J. Dairy Sci.* **44,** 2070-2080.

NA - 143 - 183

# EFFECTIVENESS OF DIFFERENT COOLING TREATMENTS IN ALLEVIATING HEAT LOAD IN WATER BUFFALO *(Bubalus bubalis)*: A SUITABLE COOLING METHOD

E.R.K. Perera and A.N.F. Perera

*Department of Animal Science, Faculty of Agriculture,*
*University of Peradeniya,*
*SRI LANKA*

**Abstract**: An experiment was conducted to determine a suitable duration and frequency of cooling treatment that would effectively alleviate the heat load in water buffaloes. Thermoregulatory responses of buffaloes to the application of the following eight different cooling treatments were compared with no cooling (control), using a minimum of 3 heifers/treatment. Covering with wet gunnies ($T_1$), six sprinkler applications ($T_2$-$T_7$) using different combinations of 30, 60, or 90 sec (unit) durations and 3 or 6 repeated sprinkling cycles, and 540 sec continuous sprinkling ($T_8$). Among the treatment groups, the heifers were balanced by age ($2.0 \pm 0.6$ yr) and body weight ($155.5 \pm 10.0$ kg). Data on skin temperature (ST), respiration rate (RR), heart rate (HR) and rectal temperature (RT) were obtained from all the animals at -10, 0, +15, +30, +45, and +60 min following application and withdrawal of each treatment. Data on ambient temperature and relative humidity were recorded at the same time intervals. Each treatment was repeated on 5 days. The temperature humidity index (THI) remained $\geq 81$ during all treatments. Skin temperature was reduced ($p \leq 0.05$) in response to all sprinkler treatments. Increasing the frequency and duration of sprinkling increased the effectiveness of cooling. Three repeated cycles of 30 or 60 sec sprinkling were not effective in lowering HR, RR or RT significantly. However, 6 repeated cycles of 60 and 90 sec sprinkling, and 540 sec continuous sprinkling decreased ($p \leq 0.05$) HR and RR. Rectal temperature was reduced ($p \leq 0.05$) by 6 repeated cycles of 90 sec sprinkling and 540 sec sprinkling only. Following the withdrawal of the cooling treatments, ST, RR, HR and RT increased over time at different rates to reach the pre-cooling values. Hourly application of either 6 repeated cycles of 90 sec sprinkling or 540 sec continuous sprinkling is recommended for effective dissipation of heat and reduction of the heat load in buffalo, at ambient conditions $\geq 81$ THI.

**Keywords:** Water buffalo, heat stress, cooling methods, thermoregulatory responses

## INTRODUCTION

The hot climatic conditions which prevail during the day time in buffalo rearing countries such as Sri Lanka, create stress in the buffalo, primarily due to the less efficient heat dissipation mechanisms. Provision of effective means of cooling to facilitate heat dissipation is required when buffalo rearing is practised under such conditions. Although wallowing is the natural and most efficient method of cooling for buffaloes, it limits buffalo rearing to the areas where wallowing facilities could be found. Besides, buffaloes cannot be allowed to wallow throughout the day time, as wallowing results in water pollution. Hence, the development of suitable alternative methods of cooling for heat stress alleviation in buffalo is essential. Limited available data suggest that covering the body trunk with wet cloth (Chikamune and Shimizu, 1985) and application of water on body surface (Minett, 1947) improved heat dissipation in the buffalo. Since water is a scarce resource, which cannot be wasted, determination of an appropriate duration and frequency of cooling system which would effectively cool the animal is vital. This study was conducted to determine the most suitable duration and frequency of cooling that would effectively facilitate heat dissipation and reduce the heat load in the water buffalo by evaluating the thermoregulatory responses to application and withdrawal of different cooling treatments.

## MATERIALS AND METHODS

The study was conducted in the Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka using indigenous buffalo heifers balanced by age (2.0 ± 0.6 yr) and body weight (155.5 ± 10.0 kg). After an adaptation period of 14 d, the following cooling treatments were applied at 1200 hr (for 5 d /treatment) using a minimum of 3 heifers/ treatment, and compared with no cooling (control):

$T_1$ = dorsal trunk covered by wet gunnies, $T_2$ - $T_7$ = combinations of 30, 60 or 90 sec (unit) sprinkling durations (USD), and 3 or 6 repeated sprinkler cycles (RSC) with 10 sec intervals between each, $T_8$ = 540 sec continuous sprinkling. A single nozzle hand sprinkler delivering 1500 ml/ min placed at 1.6 m height and 1.2 m distance from the heifers was used for sprinkling of water. Except for the cooling treatments, other management practices were identical for all the heifers which were housed together. Measurements on skin temperature (ST) at mid-flank and shoulder, respiratory frequency (RR), heart rate (HR) and rectal temperature (RT) were obtained from individual heifers at -10, 0, 15, 30, 45, and 60 min pre and post treatment. A thermistor probe attached to a tele-thermometer (Cole-Parmer model 747), visual counting of flank movements/min, and a stethoscope placed under the arm-pit were used to obtain ST and RT, and RR and HR, respectively. Ambient temperature (AT) and relative humidity (RH) inside the barn during application of the treatments were recorded using a hygro-thermograph (Cole-Parmer model SL 8368-50) placed at a height of 1.5 m inside the barn. Response to cooling (R) as estimated from the differences in physiological parameters before (-10 min) and immediately after application of treatments were subjected to analysis of variance procedures. Regression analysis was performed on the physiological responses to withdrawal of the treatments versus time, to determine the rate of change (C). Time taken to reach the pre-cooling physiological values was estimated by dividing R by C.

## RESULTS AND DISCUSSION

### Ambient Temperature and Relative Humidity

Ambient temperature within the animal housing complex during different treatment periods ranged between 26.0°C and 31.7°C with a mean value of 30.6°C. The differences in mean AT among different treatment periods were not significant ($p>0.05$). Similarly, RH ranged between 63% and 87% with a mean of 68%, and the differences between the mean RH of different treatment periods were not significant ($p>0.05$). Thus, the estimated temperature-humidity index (THI) based on the AT and RH that prevailed during different treatment periods were also not different ($p>0.05$), the mean value being $81\pm1.5$. These meteorological indices show that even the daytime indoor conditions were warm and exceeded the THI of 80 during the treatment periods.

## Physiological Responses to Cooling Treatments

The changes observed in different physiological parameters in response to application of different cooling treatments are given in Table 1. In the following discussion, the six different combinations of 30, 60 and 90 sec USD and 3 or 6 RSC will be designated as 30/3, 30/6, 60/3, 60/6, 90/3 and 90/6 respectively.

*Skin temperature:* Mean ST reduced significantly ($p\leq0.05$) in response to application of all the sprinkler treatments (Table 1). It was not possible to monitor ST accurately after covering the dorsal trunk with gunny bags, hence these measurements were excluded. A 10 sec interval was allowed between consecutive RSC in every treatment. This resulted in spending additional 20 sec and 50 sec time period between RSC in treatments including 3 and 6 RSC respectively. Hence the 0 min physiological measurements were obtained after a total of 110, 230, 200, 410, 290, and 590 sec, from the commencement of applications of 30/3, 30/6, 60/3, 60/6, 90/3 and 90/6 sprinkler treatments respectively. Hence, substantial reductions in ST just after the completion of sprinkler applications were anticipated. The observed significant reductions in ST in response to all the sprinkler treatments indicated the effectiveness of sprinkling in increasing heat dissipation from the body surface. However, whether the resulting body surface cooling were adequate to trigger a signal to the higher centers of thermoregulation, to lower the deep body temperature effectively would be revealed after evaluating the responses of the other physiological indicators which reflect the interior thermal status of the body.

**Table 1.** Responses of physiological parameters to application of cooling treatments.

| Treatments | Skin Temp. (°C) | Resp. Rate (per min.) | Heart Rate (per min.) | Rectal Temp. (°C) |
|---|---|---|---|---|
| T1 (G) | | 0.5 | 0.3 | 0.01 |
| T2 (30/3) | -2.0 * | -1.1 | -1.2 | -0.02 |
| T3 (30/6) | -2.1 * | -4.6 * | -4.5 * | -0.05 |
| T4 (60/3) | -2.0 * | -1.2 | -1.4 | -0.06 |
| T5 (60/6) | -3.1 * | -5.9 * | -3.4 * | -0.06 |
| T6 (90/3) | -4.1 * | -6.0 * | -2.7 | -0.04 |
| T7 (90/6) | -4.1 * | -7.7 * | -3.2 * | -0.16 * |
| T8 (540) | -5.0 * | -7.1 * | -4.4 * | -0.17 * |

* $p < 0.05$

*Respiration rate:* The mean RR reduced significantly ($p \leq 0.05$) in response to 30/6, 60/6, 90/3, 90/6 and 540 sec sprinkling treatments (Table 1). Despite the 14 d adaptation period allowed, the RR of heifers did not decrease, but increased insignificantly when covered with wet gunnies, possibly due to excitement. Respiration serves as an avenue for evaporatory heat loss in addition to its primary function of gas exchange. As the ambient temperature rises above the upper critical temperature, animals become increasingly dependent upon evaporation via sweat glands and respiratory tract to enhance heat loss. The dependency on evaporatory heat loss via the respiratory tract is greater in the buffalo because it has a relatively smaller population of sweat glands. If the heat load is reduced due to cooling, the demand for respiratory evaporation declines and as a result RR decreases. Significant reductions in RR in the buffalo in response to spray cooling have been reported (Chikamune and Shimizu, 1985; Chikamune *et al.*, 1986).

In this experiment, the response of RR to cooling increased in magnitude and became significant as the frequency and/or duration of sprinkling was increased. For instance, as the USD increased from 30 sec to 90 sec, even 3 RSC caused significant reductions in RR, while at lower USD (30 sec and 60 sec) only 6 RSC was effective in reducing RR. Similarly, despite having the same (180 sec) absolute total sprinkling duration (excluding the interval between RSC) in 30/6 and 60/3, RR was significantly reduced by 30/6 but not by 60/3 sprinkling (Table 1). The 30/6 treatment included 6 consecutive RSC, whereas the 60/3 sprinkling treatment which was non-effective included only 3 RSC.

*Heart rate:* The mean HR of heifers reduced ($p \leq 0.05$) in response to 30/6, 60/6, 90/6 and 540 sprinkling treatments. The other treatments were not effective (Table 1). Heart rate increases upon exposure to heat due to stimulation of peripheral vasodilation and augmentation of blood flow to the skin. Cooling facilitates heat dissipation and reduces the heat load in the animal, thereby lowering the demand for increased blood flow and HR. Reductions in HR of buffalo in response to water spraying have been reported previously (Chikamune and Shimizu, 1985). In this experiment, significant reductions in HR in response to 30/6, 60/6, 90/6 and 540 sprinkling suggested that these sprinkler treatments were more effective in facilitating heat dissipation and reducing the heat load in the animal.

*Rectal temperature:* The RT of the animals were reduced ($p \leq 0.05$) in response to 90/6 and 540 sec sprinkling, while the other cooling treatments were not able to lower the RT significantly (Table 1). Rectal temperature represents the deep body temperature, and is changed within limits reflecting the thermal status of the body. Effective cooling improves the efficiency of heat dissipation mechanisms and thereby reduces the heat load resulting in a lower RT. The results of this experiment indicate that, although every sprinkling treatment tested was able to facilitate heat loss from the body surface, only the 90/6 and 540 sec sprinkling treatments were capable in lowering the heat load of the animal effectively as evidenced by the significant reductions in all thermoregulatory indicators in response to application of these two cooling treatments.

## Physiological Responses to Withdrawal of the Treatments

In the subsequent sections, the discussion will be limited to explain the observed changes in physiological parameters following withdrawal of the most effective cooling treatments (90/6 and 540).

*Skin temperature:* Following withdrawal of the 90/6 treatment, the mean ST of heifers increased linearly ($p \leq 0.01$) from 31.4$^0$C (at 0 min) to 35.2$^0$ C (at 60 min). Correspondingly, the ST of heifers subjected to 540 sec continuous sprinkling also increased ($p \leq 0.01$) linearly from a mean value of 31.4$^0$ C at 0 min to assume a mean value of 35.6$^0$ C at 60 min following withdrawal of the treatment. The lowering of ST by sprinkler treatments created wider temperature gradients between the environment and skin (body surface), as well as between the core and the skin of the animal, both gradients favouring the flow of heat towards the skin. This would explain the observed increases in ST following the withdrawal of treatments.

Regression analysis of ST (°C) against time (min) after withdrawal of sprinkling revealed that the rate of increase in ST was not different (0.0627 °C *vs* 0.0696 °C per min) between the two treatments. Regression equations representing the change of ST following withdrawal of the 90/6 and 540 sprinkling treatments were $Y = 31.97 + 0.06 X$ ($r^2 = 0.89$) and $Y = 32.09 + 0.07 X$ ($r^2 = 0.87$), respectively.

*Respiration rate:* The RR of the heifers subjected to 90/6 sprinkling increased linearly ($p \leq 0.05$) from a mean value of 23.7 respirations/min (0 min) to 27.0 respirations/min (60 min). In contrast, the RR of heifers sprinkled for 540 sec continued to decline ($p \leq 0.05$) further from a mean value of 23.7 respirations/min at 0 min to a mean RR of 22.5 respirations/min at 30 min following withdrawal. Thereafter, the mean RR increased ($p \leq 0.05$) to reach 27.1 per min at 60 min (Figure 1). The sustained decrease in RR till 30 min following withdrawal of treatment reflected a long standing and effective reduction in heat load by treatment T8. Regression equations representing the above mentioned changes in RR (Figure 1) revealed that the RR of heifers subjected to 540 sec continuous sprinkling treatment increased at a similar rate from 30 min to that of the heifers exposed to treatment T7, but kept a lower RR than with treatment T7 throughout the 60 min monitoring period.

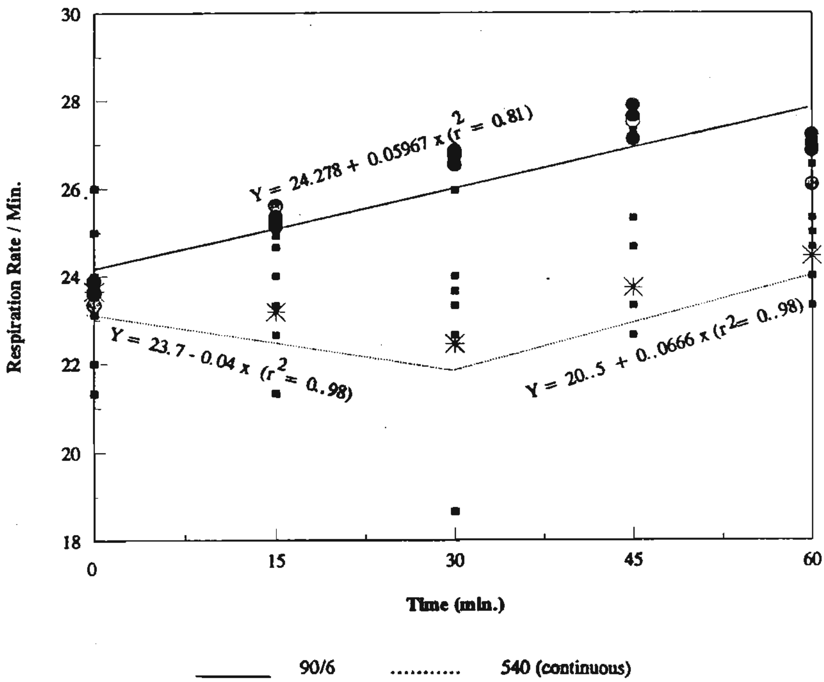



**Figure 1**. Change of respiration rate of heifers following withdrawal of sprinkling.

*Heart rate:* Following the withdrawal of 90/6 treatment, mean HR increased linearly ($p \leq 0.05$) from 58.9 beats/min at 0 min to 61.5 beats/min at 60 min. This change of HR over time (min) was represented by the regression equation $Y = 59.53 + 0.039 X$ ($r^2 = 0.75$). The mean HR of the heifers subjected to 540 sec continuous sprinkling ranged between 57.4 and 58.5 beats/min, and assumed a mean value of 58.1 beats/min during the 60 min monitoring period. Heart rate fluctuates in the direction of the changes in peripheral vasodilation and blood flow to the skin. The maintenance of a lower HR by the 540 sec sprinkler group through 60 min implied that this treatment was more effective than treatment T7.

*Rectal temperature:* The change of RT of the two treatment groups following the withdrawal of treatments is given in Figure 2. The mean RT of treatment T7 remained around 38.27 °C with a rate of increase of 0.00013 °C/min ($r^2$=0.50). In contrast, the RT of heifers subjected to 540 sec continuous sprinkling declined ($p \leq 0.05$) further till 30 min following withdrawal to reach 37.8 °C, and increased ($p \leq 0.05$) thereafter at a rate of 0.0057 °C/min ($r^2$= 0.99). The results suggested that 540 sec sprinkling was more effective than 90/6 treatment in maintaining a lower RT following the withdrawal of treatments.

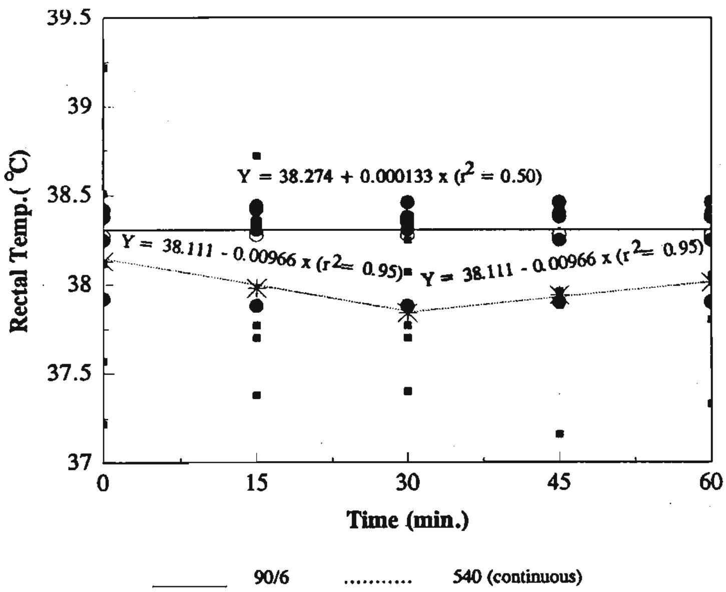



**Figure 2.** Change of rectal temperature of heifers following withdrawal of sprinkling.

*Time taken to return to pre-cooling values:* Based on the above observations on responses of physiological indicators to the application of the 90/6 or 540 sec sprinkler treatments, and the rates of change of these parameters following the withdrawal of the treatments, the approximate time taken by the four parameters to reach the pre-cooling values were estimated. In calculating these values, the rate of change to reach pre-cooling values was assumed to be linear. According to these calculated (predicted) values, the ST, RR , HR and RT of the 90/6 group of heifers would have reached the pre-cooling values in approximately 68, 128, 80 and 120 min respectively. The ST, RR, and RT of heifers subjected to 540 sec continuous sprinkling would have taken 73, 205, and 78 min to reach the pretreatment values. It was not possible to predict the time taken by the HR of this group to reach pre-cooling values because a representative regression line could not be derived due to the variation within the treatment. The results suggest that the physiological indicators did not reach their pre-sprinkling values after >60 min following the withdrawal of 90/6 or 540 sec sprinkler treatments. Hence, if any of these two treatments were re-applied after 60 min, it would prevent the thermal status of the animal from rising, by facilitating heat dissipation and lowering heat load ion buffaloes. Based on these results, hourly application of either 90/6 or 540 sec sprinkling is suitable to reduce the heat load in buffaloes at ambient conditions of ≥81 THI.

## SUMMARY AND CONCLUSIONS

The results indicate that all the tested sprinkling treatments were effective in improving heat dissipation from the body surface. Increasing duration and frequency of sprinkling improved the efficiency of cooling. Out of the 8 tested cooling methods, 90/6 and 540 sec continuous sprinkling were the most effective. These two treatments were able to maintain the physiological parameters at a lower level for ≥60 min following withdrawal of the treatments. Hourly application of any of these two sprinkler treatments when THI is ≥81 is suggested to facilitate heat dissipation and lowering the heat load in buffaloes and to prevent heat stress.

## Acknowledgements

The authors gratefully acknowledge the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for funding the research and the Natural Resources Energy and Science Authority of Sri Lanka (NARESA) for administration of funds.

## References

Chikamune, T., Kanai, Y., Lchikawa, T., Hamma, H. and Shimizu, H., (1986) Influence of solar radiation and effects of water spray on thermoregulatory responses and heat production in swamp buffaloes. *Jap. J. Trop. Agric.* **31**, 1-11.

Chikamune, T. and Shimizu, H. (1985) Covering of body trunks with white cloth effects on thermoregulatory responses. *Buffalo J.* **1**, 19-27.

Minett, F.C. (1947) Effects of artificial shower, natural rain and wallowing on body temperature of animals. *J. Anim. Sci.* **6**, 35-49.

NA-143-195

# PHYSIOLOGICAL RESPONSES OF LANKAN BUFFALOES TO DEHYDRATION

A.A.J. Rajaratne[1] and S.S.E. Ranawana[2]

[1]*Department of Physiology, Faculty of Medicine,*
*University of Peradeniya*
[2]*Veterinary Research Institute, Gannoruwa, Peradeniya*
*SRI LANKA*

**Abstract:** Buffaloes are widely known as shade and water loving animals. However, they also possess a remarkable ability to thrive under dry conditions. The main objective of this study was to investigate responses of buffaloes to dehydration.

Water balance was studied in four one year old female Sri Lankan buffaloes (mean body weight = 142.8 kg) by measuring water intake and water output. Total mean water intake, output and balance in the normal hydration was 21.8 l/day, 19.4 l/day and 2.32l/day respectively. Feed and urine water output was 5.34 l/day and 5.20 l/day respectively. The figure for water balance (2.32 l/day) represents water loss by cutaneous and respiratory evaporation.

When the animals were dehydrated by withholding water until they lost 7% of their body weights, the urine output was 595 ml/day. This value could be considered as the obligatory urine volume for Lankan buffaloes. Faecal water output during dehydration was 1.2 l/day.

These results show the ability of buffaloes to conserve water both by urinary and faecal routes as a response to dehydration.

**Keywoods** : Buffalo, water balance, dehydration

## Session II - Genotype and Environment

### Panel Discussion

**Q:** W. Perera - How do the cattle and buffalo banks function in Thailand?

**A:** C. Chantalakhana - The cattle and buffalo banks in Thailand were formed to help the poor rural farmers. Generally, during the cultivation period there is a big demand for buffaloes. Due to this heavy demand, the rates for hiring buffaloes are also high during this period, and affect the poor farmers. In order to reduce the burden during the cultivation period, the banks were formed with the help of NGOs. They are managed by the farmers and this helps the farmers to hire animals at a cheaper rate.

**Q:** M. G. Jeyaruban - How is the bull performance testing programme conducted in Thailand?

**A:** C. Chantalakhana - The bulls are selected at the government farms for draught traits. The top grade bulls are kept as farm replacements, and the second grade bulls are issued to farmers. In the meantime the best performers or "champion bulls" are purchased from the farmers and added to the top grade at the government farms. The programme is not very successful at the present time, mainly due to the availability of artificial insemination facilities and the preference by farmers for Murrah crosses over the local buffaloes.

**Q:** B.M.A.O. Perera - How are the breeding bulls distributed to farmers?

**A:** C. Chantalakhana - After the selection of elite bulls the second grade bulls are sold to farmers or given on loan. Majority of the buffaloes are sold to farmers on the condition that the animals should be used only for breeding purposes. The major problem encountered is that the farmers like to use these animals to breed their own cows and cows of their relatives, but not those of the neighbours.

**Q:** T.N. Jayatilake - What type of characters are looked for in the breeding programme?

**A:** C. Chantalakhana - Farmers want to have buffaloes mainly for draught purposes. They like an animal with black coat colour and large horns. As the appearance of the bull is not known with AI, the farmers are reluctant to use this method.

**Q:** T.N. Jayatilake - Is heat detection a major problem in buffaloes in Thailand?

**A:** C. Chantalakhana - No. As the herd size is about 2 to 3 cows, heat detection is not a major problem.

**Q:** T.N. Jayatilake - How is natural service carried out in Thailand?

**A:** C. Chantalakhana - The cows are brought to the bull breeding station for natural service.

**Comment:** V. Momongan - In the Philippines buffaloes are distributed over a vast land area and it is really difficult to artificially inseminate all the cows. In order to overcome this problem, about 100 breedable females are synchronized and artificially inseminated at the same time. In Indonesia the livestock technicians visit the villages at regular intervals. In order to facilitate the visits of the technicians, different coloured flags are used to indicate the requirements in a herd. A red flag is used to indicate a sick animal while a blue flag is used to indicate an animal in heat.

**Comment:** C. Devendra - In order to achieve better results in research and development regarding buffaloes, they should be looked at with a broader vision on an interdisciplinary approach. The systems approach should be adopted to include buffalo and crop production in the overall production system.

**Comment:** S.K. Ranjhan - Research and development in the buffalo sector should be undertaken with the concurrence of farmers. The farmers' views should always be considered in research, and the constraints in each particular region should be taken into consideration in transferring technologies to farmers.

**Q:** T.N. Jayatilake - What would be the effect of humidity as a stress factor in buffalo?

**A:** A.A.J. Rajaratne - If an animal is losing heat through evaporation loss, the animal will definitely be affected by high humidity. As buffaloes lose heat by wallowing, they appear to be less effected by the rise in humidity. But earlier studies have shown that humidity has an influence as a stress factor in buffalo because it hinders evaporative loss.

**Q:** T.N. Jayatilake - Farmers use buffaloes to thresh paddy in the night. Is this done in order to reduce stress in buffalo?

**A:** A.A.J. Rajaratne - We should consider the temperature humidity index with regard to buffaloes as the influence is regarded to be high. Buffalo has a dark coat colour and direct sunlight could be absorbed through the skin. The earlier studies at Undugoda, which were conducted during the rainy season, showed that buffaloes were stressed to a lesser extent when they were exposed to low temperature and high humidity than to high temperature and high humidity. Humidity plays a secondary role.

**Q:** B.M.A.O. Perera - Buffaloes are used in very muddy lands and their legs as well as lower body get wet during ploughing. Does this have any influence in reducing the stress in buffaloes?

**A:** A.A.J. Rajaratne - Yes. Even if buffaloes were to plough from morning till evening, they do not show stress. Heat stress is mainly due to accumulation of heat in the body rather than to work stress. Working in wet conditions has a definite influence in reducing stress in the buffalo. When buffaloes were ploughing in muddy land they were covered with mud, the rectal temperature increased by only 1°C and the respiration rate and heart rate remained at a low level. Therefore, the ploughing conditions where water is available reduces the stress factor in buffaloes.

**Q:** S.S.E. Ranawana - The buffalo is well known for its labile body temperature. Is there any close link between rectal temperature and respiration rate in buffalo during ploughing?

**A:** A.A.J. Rajaratne - Generally body temperature has an effect on the respiratory center in the brain. But in buffalo, when the water is poured over it, the respiratory rate decreases without a similar effect on the rectal temperature. This may be due to some thermo receptors in the skin, which effects the respiratory centre in the brain.

**Q:** C. Chantalakhana - We generally tend to look at the effects of temperature on stress. I am glad to note that both temperature and humidity have been considered in this study. The temperature humidity index is more meaningfull. The question that comes to mind is how much can you reduce the respiration rate and heart rate and what is the critical level at which the working ability of the animal is effected? At farm level, what kind of recommendation can be given because sprinkling would not be practical?

**A:** E.R.K. Perera - The temperature humidity index in this study was between 77 to 83. I feel that as animals are more comfortable working during the morning hours, we could try to reduce the levels to those found is the morning hours.

**Q:** S.S.E. Ranawana - If you want to see the effect of temperature on rectal temperature and other physiological parameters, the animals should be heat stressed. Would you say that the animals were heat stressed in your experiment, because the animals were under the shade and the temperature was in the range of 25 to 27°C ? This is a field situation.

**A:** E.R.K. Perera - In the previous experiment I found that the diurnal variation in skin temperature of the buffalo was more than the changes in cattle.

**Q:** S.S.E. Ranawana - Generally buffaloes are considered shade and water loving animals and therefore susceptible to water deprivation. But in dry areas in Australia buffaloes show good adaptability. Was the osmotic pressure measured in the urine of buffalo and if so what was the average osmotic pressure of buffalo urine?

**A:** A.A.J. Rajaratne - The osmotic pressure was not measured in this study. But the specific gravity was measured and the value for specific gravity was 1.05. This is the maximum value that any animal can achieve.

**Q:** S.S.E. Ranawana - Did you measure the moisture content of the buffalo faeces?

**A:** A.A.J. Rajaratne - The dry matter content of faeces had 50% moisture content. Despite that animals were eating and ruminating and did not show evidence of stress.

**Q:** K.K. Pathirana - What would be the effect of water intake on the feed intake and subsequent milk production in buffalo?

**A:** A.A.J. Rajaratne - Since this study was based on non-lactating animals it is difficult to extrapolate the result to a lactating cow. Restricting water to lactating animals might have some influence on the milk production. The lower water intake may lead to a more concentrated milk in buffalo.

**Q:** H. Abeygunawardena - Can the result from this research be used to find the minimum requirement of water to get the optimum production?

**A:** A.A.J. Rajaratne - It is very difficult to determine this from the present study. But future studies will be conducted to find this affect.

# NEW CONCEPTS AND STRATEGIES IN THE UTILIZATION OF FIBROUS CROP RESIDUES (FICR) AND AGRO-INDUSTRIAL BY PRODUCTS (AIBP) IN BUFFALO FEEDING

N. V. Thu

*Faculty of Animal Husbandry and Veterinary Medicine,*
*Cantho University, Cantho*
*VIETNAM*

**Abstract:** Under increasing human population pressure, the need for strategies for the efficient use and development of feeding systems based on fibrous residues and agro-industrial by products for buffaloes should be realized in Asia. China is a good example for learning the concepts of using FICR and AIBP to produce meat and save grain for human beings. The research and application under the field conditions of FICR and AIBP utilization for feeding ruminants is not only based on advances in knowledge on animal feeding and nutrition, but also on the benefits of re-cycling available local feed resources to the economy and the environment. Sustainable integrated farming systems practised by rural small-holders are based on the utilization of crop wastes and by products, such as cereal straws, maize stovers, sugarcane tops, bagasse, molasses, pineapple waste and groundnut vines by the ruminant animal. Buffaloes are known to utilize poor quality roughages more efficiently than cattle, and in developing countries small farmers commonly use crop wastes and byproducts to feed animals, especially buffaloes. Burning of straw and stubble or other agro-industrial by products is wasteful, creates pollution and sometimes causes serious accidents.

Buffalo raising as part of small farm agricultural systems has positive effects on social and economic sustainability, and consequently on rural development. One of the major reasons why rural people choose to remain in villages rather than go to work in cities is that they have to look after their buffaloes or other livestock.

**Keywords**: Fibrous crop residues, agro-industrial by products, buffalo, environment, social and economic sustainability.

## INTRODUCTION

In many developing tropical countries, ruminants including buffalo and cattle are fed on straw from cereal crops (mainly rice and wheat). As population pressure increases and the land area devoted to food crop production is extended, the use of crop residues and by products for animal feeding will increase. In some countries with specialized livestock production systems, straw is considered of such low feed value that it is often burnt. But in developing countries where livestock is integrated with cropping it is a valuable resource (Preston and Leng, 1987).

Draught animals appear to be able to work and to maintain body condition on a diet mainly of straw. In Vietnam, most often working buffaloes lose body weight during the working time because the main feed is the rice stubble in the fields and supplemented with rice straw at night. This results in reduced working capacity of the animals. In very extreme cases, farmers sell or slaughter their animals because of poor health (Thu and Hon, 1992). In Thailand, studies show that buffaloes are raised mainly on native feedstuffs and seasonal agricultural by products. Farmers do pay attention to their animals, especially the working ones as evidenced by the conservation of feed and forage and non-conventional feeds for use during times of scarcity. Variations of feed quality also exist throughout the year which can influence the performance of these animals (Wanapat, 1991).

Agro-industrial by products (AIBP) produced in many Asian countries have been used extensively. However, some of these resources have only limited application as a basal component in livestock feeds (eg: bagasse, palm pressed fibre, cocoa pods and rice hulls). The limitation of all these feed resources is the extremely low digestibility and, even when supplemented with essential nutrients, the intake is too low to support maintenance (Tables 1 and 2). Most of these residues arise from industrial processing and are therefore available in large quantities only at the factory site. The availability of the by products at the factory is an incentive towards finding ways of using them as ruminant feeds by 'relatively sophisticated' technologies to increase the digestibility such as briquetting, steam treatment and ammoniation.

**Table 1.** Some agro-industrial fibrous residues in Asian countries.

| Feedstuff | DM (%) | CP | CF | Ash |
|---|---|---|---|---|
| | | (% in dry matter) | | |
| Sugarcane bagasse | 92.7 | 2.5 | 39.7 | 4.5 |
| Banana stem/trunk | 5.3 | 0.2 | 1.5 | 1.1 |
| Cassava leaves | 100.0 | 23.2 | 21.9 | 7.8 |
| Corn cobs | 92.2 | 3.0 | 34.6 | 2.4 |
| Corn stover | 89.0 | 4.5 | 32.0 | 7.1 |
| Cotton seed hulls | 100.0 | 4.4 | 38.0 | 2.8 |
| Groundnut hulls | 100.0 | 6.2 | 45.7 | 11.1 |
| Groundnut straw | 100.0 | 8.1 | 40.6 | 8.7 |
| Rice hulls | 90.4 | 2.5 | 38.0 | 19.3 |
| Rice straw | 92.6 | 3.7 | 31.4 | 21.2 |
| Sorghum stover | 90.8 | 8.3 | 33.3 | 7.3 |
| Sugarcane tops silage | 24.8 | 1.7 | 7.7 | 2.2 |
| Pineapple pulp | 22.5 | 1.2 | 6.6 | 0.8 |
| Coconut leaf blades | 40.3 | 5.3 | 10.3 | - |

Sources: Gerpacio and Castillo, 1979; Devendra, 1979; Ranjhan, 1980; Castillo, 1983a.

## CONCEPTS OF USING FICR AND AIBP

Two events will influence, profoundly, the role of livestock in farming systems in developing countries in the next century. The first is the projected doubling of the world population, which will be most evident in the developing world. The second is the increasing aspirations in developing countries to acquire those amenities taken for granted in developed countries. Economic development will result in a massive increase in demand for energy. Yet the reserves of fossil fuel are likely to last less than 30 years ( Economist, 1994 quoted by Preston, 1995).

**Table 2.** Availability of some selected crop residues and agro-industrial by products in Asia and the Pacific.

| Crop | Residues and by products | Production ($10^6$ tonnes) | Production (% of world) |
|---|---|---|---|
| Rice | broken | 16.8 | 90.7 |
| | bran | 37.4 | 90.7 |
| | husk | 59.8 | 90.7 |
| | straw | 373.7 | 90.7 |
| Cassava | leaves | 5.9 | 38.0 |
| | waste | 28.0 | 38.0 |
| Cotton | seed meal | 8.0 | 43.8 |
| Groundnut | meal | 6.4 | 62.3 |
| Maize | germ meal | 15.0 | 19.4 |
| | stover | 64.0 | 19.4 |
| Sesame | cake | 0.8 | 67.3 |
| Sorghum | stover | 13.5 | 29.3 |
| Soya bean | soya bean meal | 7.3 | 10.8 |
| Sugarcane | bagasse | 48.6 | 41.2 |
| | green tops | 68.0 | 41.2 |
| | molasses | 12.6 | 41.2 |
| Wheat | bran | 14.6 | 30.3 |
| | stover | 145.7 | 10.03 |

Source: Wanapat, 1991

The justification for making better use of FICR and AIBP is supported by the following:

* There will be a competing demand for cereal grains - currently the basis of livestock production in the more developed countries. For example, in recent years China has increased the use of fibrous residues to produce beef in an effort to save grain for human consumption (Tingshuang *et al.*, 1995).

* Increasing importance to the production and utilization of renewable forms of energy, especially biomass. Natural

renewable resources will have to be used more efficiently, and the farming system will need to create more employment opportunities.

* Mechanization will be increasingly based on animal rather than mechanical power (Preston, 1995). Buffaloes in Asia are truly multi-purpose animals for work, meat, milk and manure (biogas and fertilizer) (Mathers *et al.*, 1985). They can adapt to the new situations.

* Observations under field conditions indicate that buffaloes thrive better than do cattle on coarse fodder. One special characteristic of feeding buffaloes on FICR and AIBP is that they are less discriminating in foraging and therefore consume a larger quantity of coarse fodder, not readily eaten by cattle (Bhattacharya, 1990). Langer *et al.*, 1968; (quoted by Bhattacharya, 1990) reported that when the ration was deficient in carbohydrate the rumen microbes in buffaloes could utilize dietary protein more efficiently.

## STRATEGIES FOR THE USE OF FICR AND AIBP FOR FEEDING BUFFALOES

The role of buffaloes and cattle must increasingly be a secondary scavenging one, to make use of byproducts and residues that might otherwise be wasted (Table 3). If the available resource is cereal straw, or cane tops or bagasse, it makes economic and ecological sense to offer twice the feed biomass that the animal can be expected to consume and utilize the less nutritious residue as fuel or as litter which will eventually be recycled into the soil and provide energy for N-fixing and methane-oxidising microorganisms, or burned directly as fuel.

**Table 3**. Potential supply of roughage (thousand tonnes) from some crop by products in Southern and South-east Asia.

| Crop by products | South-east Asia | Indian sub-continent | Total |
|---|---|---|---|
| Rice straw | 74162 | 114500 | 188662 |
| Maize, aerial | 7100 | 15500 | 22600 |
| Sorghum | 2142 | 33500 | 35042 |
| Cassava | 155 | n.a | - |
| Sugarcane bagasse | 8126 | 6264 | 14390 |
| Sugarcane tops | 10535 | 8600 | 19135 |
| Palm press fibre | 10535 | n.a | - |
| Peanut, aerial parts | 844 | n.a | |
| Pineapple process residue dehyd. | 48 | n.a | - |
| Pineapple process residue | 854 | n.a | - |
| Wheat and barley straw | - | 67000 | 67000 |

When fibre-rich crop residues and byproducts are the primary feed resource for ruminants, feeding strategies must be based on a clear understanding of the relative roles and nutritional needs of rumen microorganisms and of the host animal. The advances in ruminant nutrition that are most applicable to the animals managed by resource-poor farmers in tropical developing countries have until now been scattered through the scientific literature.

## GUIDELINES FOR OPTIMIZING UTILIZATION OF TROPICAL FEED RESOURCES

The proposed strategy considers the buffaloes and cattle as composed of two subsystems: the rumen and the animal.

## Feeding the Rumen Microbes

Ammonia is needed in the rumen to maximize digestibility and intake by the animals. There is also a need for macro and micro-minerals (P, S and Co), also some micro-nutrients (amino acids, peptides, etc.). It is necessary to optimize the ecosystem in the rumen for promoting rapid colonization and maximize the rate of intake of fermentable carbohydrate. The other approach is to treat crop residues with ammonia, or other alkalies or other treatments (steam or microbiology).

## Feeding the Animal

The aim is to increase the protein/energy (P/E) in the nutrients absorbed for metabolism by increasing the efficiency of rumen function, supplying bypass protein, controlling protozoans by using shrub foliages and reducing the external heat load. In the tropics there is a greater response by ruminants to supplementation strategies as compared with responses in temperate countries, and generally the requirements for protein (amino acids) per unit energy substrate will be greater for ruminants in the tropics than for those in temperate environments.

# FICR AND AIBP AS ANIMAL FEED

Although the meals made from leaves may have a high content of true protein in relation to crude protein, fibrous agro-industrial residues in general have low protein and high fibre as with rice and wheat straws, stovers of sorghum, millets and maize and sugarcane bagasse from sugarcane processing (Table 1). Many non-conventional feed resources have been identified and are now being utilized by the compounded feed industry (Castillo, 1983a; Acharya, 1993). Examples of these are agro-industrial by products, of which molasses is the most important. Other by products include pulp from citrus, pineapple and the sisal industries and rejected fruit and fruit wastes (Preston and Leng, 1987).

Over the period 1977 to 1990, 27 meetings were held on the use of fibrous crop residues in Asia, without any discernible impact at farmer level. There is a need to formulate and demonstrate rural-oriented strategies that will give priority to the on-farm use of local resources. Extending the information on fibrous crop residues to on-farm situations is considered in two phases:

- The information needed by farmers and their advisers,
- Mechanisms for delivering information to farmers

The benefits of addressing these phases more thoroughly will increase the current level of animal production and the socio-economic status of the rural poor (Devendra, 1993).

## Rice, Wheat, Corn and other Cereal Fibrous Residues

In Asia, especially in South-east Asia, there are abundant crop residues (Table 3) available for ruminant feeding. However, the intake and digestion of fibrous roughage by ruminants is limited by the rate of breakdown of feed particles, mainly by chewing, but also through micro fermentation in the rumen. Alternatives available to improve the intake and digestibility of fibrous roughage range from feeding at the proper stages and providing supplements (additional nitrogen, readily available carbohydrate and minerals) and to treat the roughage by physical, chemical or biological means (Na Phuket, 1993).

In the Philippines, chopping of rice straw or corn stover increased the voluntary intake of the roughages by buffaloes; soaking of the chopped feeds did not further increase intake (Doyle, 1982 quoted by Na Phuket, 1993). By using an alkali (such as sodium hydroxide, calcium hydroxide, ammonia, *etc.*) the bonds between hemicellulose and lignin and lignin and silica can be broken, removing the physical barrier to digestion (Davis *et al.*, 1983). Ammoniation is a preferred treatment because it provides both the alkali effect and nitrogen for microbial fermentation. It can be generated from urea or urine. Urea is easily transported and available in remote villages. According to information provided by the Ministry of Agriculture, the quantity of straw treated in China since 1985 has risen sharply. When used for beef production, 6 million tonnes of treated rice straw could produce over 300,000 tonnes of carcass meat (Dolberg and Finlayson, 1995).

Treatment of roughages has become very popular at the village level. Urea is however expensive to buy and farmers must determine how to obtain the best return from their investment. Nitrogen is a fundamental component of the farming system. It should not be wasted by practices such as the burning of dung, but should be recycled through crops, livestock, bio digesters and fish ponds.

The importance of urea in straw-based diets is demonstrated by an experiment in which rice straw was sprayed with 10% molasses alone or containing 1, 2 or 3% urea, sundried and then fed to female buffaloes the following day. The addition of molasses alone did not significantly affect intake or weight change but the addition of urea to molasses at various levels slightly increased daily feed intake and decreased weight loss in all animals (Suriyajuntratong *et al.*, 1974).

Concerning the biotechnological approaches to straw utilization by microbial systems, the biodegradation of lignin and ligno-cellulose by white rot fungi (actinomycete) has been studied. However, the results obtained so far show the need for further studies on genetic improvement of specific microbial strains, control of fermentation as well as understanding of the fundamental enzyme systems, before field application is possible (Neelakantan and Deodhar, 1993). Where basal diets of poor quality, such as rice straw or other crop residues were fed, buffaloes responded less to concentrate feeding than cattle. Thus, supplementation schemes should rely on supplying essential nutrients to roughage-based diets, preferably using roughage as supplements, such as legume crop residues or forage legumes produced on farms.

## Sugarcane By products

When complemented with rumen bypass supplements, sugar cane tops will support live weight gains in the order of 700 g/d in fattening cattle and 3000 litres lactational yield in milking animals (Ferreiro *et al.*, 1977 and Boodoo *et al.*, 1990a quoted by Preston and Murgueito, 1992). It is important to encourage selection, by offering the animal quantities of tops which exceed the expected intake by at least 50%. Under such circumstances, animals have been observed to select preferentially the growing point of the cane and to reject in large part, the leaf blade (Preston and Murgueito, 1992). Pregnant and lactating buffaloes and cattle used for work when fed with cane tops and grass, urea-molasses block and rice bran, sustained work and milk production (Thu, 1994). Cane tops can be ensiled with and without molasses and fed to cattle, sheep and goats (Castillo, 1983b). In Colombia, fresh sugar cane tops are usually chopped and then mixed with other forages to feed animals or is fed alone.

Carabaos (swamp buffaloes in the Philippines) seem to digest better than cattle the nutrients in the rations containing 60 per cent bagasse and 1 percent urea or 40% bagasse and 2 percent urea. Furthermore, carabaos had better nitrogen retention on both rations than cattle (Roxas *et al.*, 1969). In Brazil, where the integration of sugar and alcohol production results in large surpluses of bagasse, it is reported that more than 200,000 cattle are fed with steam hydrolysed bagasse (Preston and Murgueito, 1992). Alkaline hydrolysis of factory bagasse with sodium hydroxide is also an effective method of upgrading the bagasse (Martin *et al.*, 1977). The bagasse from artisan sugar production contains some 25% of soluble sugars in dry matter. This raises the total dry matter digestibility to almost 55% (Preston and Murgueito, 1992). Yuan *et al.* (1995) reported that when bagasse was treated with 5% of NaOH, 0.8 % of urea and 15% of molasses and then ensiled for one month, the digestibility in cattle was 21% higher for the treated ration than the untreated ones. The fermentation process of bagasse residue from sugar production changes the energy feed to protein feed. It has been claimed that the protein content of bagasse can be increased by 200% by fermentation and that the use of fermented bagasse residue to replace soybean meal as feed for dairy cattle can reduce feed cost by 40% and increase milk production by 5.2% (Jintong, 1995).

Molasses is a "concentrated" source of fermentable carbohydrate that is widely available in the tropics. It can be used as a basis of intensive cattle fattening and as a carrier for urea, minerals and other nutrients (multi-nutritional blocks) (Preston and Leng, 1987). Molasses can be mixed with urea and directly sprayed on rice straw for feeding cattle and buffaloes. The use of molasses-urea cake for feeding working buffaloes can improve working capacity and health (Thu *et al.*, 1995). Sansoucy (1995) reported that the various studies recently undertaken in different parts of the world concerning the manufacture and utilization of multinutrient blocks have led to real progress in the technology and its use.

## Other Agro-industrial By products

When banana fruits are graded for export, up to 25% may be rejected. They can be used for fattening cattle (Castillo, 1983b). Buffaloes and cattle also like to eat banana leaves and pseudo stems. In fruit canning plants, pineapple crowns, pulp and peel are the by products. In the Philippines, average daily weight gains of 0.38 and 0.48 kg were obtained when cattle were fed with pineapple pulp and pineapple leaf silage, respectively. In China, farmers obtain pineapple peel from the plants, then incubate under plastic covers to preserve it for gradual use. Banana peel is also a feed resource for cattle and buffaloes in Vietnam. Many fruit fibrous residues have yet to be tapped for their better utilization (for example, mango and water melon).

It is relevant to keep in view the amounts of FICR including AIBP and non-conventional feed resources (NCFR) that are generated in Asian countries. The total availability of these from field and tree crops is about 2098 x $10^6$ tonnes of dry matter, which increases at the rate of 2.9% per annum. The uses of AIBP and NCFR in Asia are accordant to their potential value and importance for individual species of animals. The bulk of the FICR is dry and of poor quality and only useful for low or medium levels of production and for draft animals (Devendra, 1993). In the context of "matching livestock systems to available resources" there is a need to promote closer cooperation between farmers and scientists. Important developments have been made recently in dual purpose systems for cattle production. However, in the case of buffalo production (especially, milk and meat), there is a need for more research to demonstrate the advantages of this species in using fibrous crop residues and by products.

Management of tropical forages and utilization of crop residues poses real problems, because of their low and rapid changes in quality. This directly effects animal productivity. For example, if corn stover is fed to animals directly at the time of harvest, the nutritive value of corn stover will not decline as much as when it is left in the field until the whole plot is harvested, before using it for grazing. Even though buffaloes have advantages over cattle, goat and sheep in the utilization of low quality feeds as FICR (Mc Dowell, 1985), for maximum production efficiency, feeding of forage alone is not enough (Eusebio, 1975 quoted by Na Phuket, 1993).

The availability of fibrous residues varies depending on the status of agricultural development. Countries with more advanced agriculture systems generally have more residues per hectare from different crops. Although many of the fibrous residues have been used in the past, there is a need to improve the efficiency of utilization. Other residues which have not yet been used and have potential as feed should be investigated. Buffalo production in Asian countries plays a very important role for work, meat, milk and manure, therefore there is a need for more research and development in utilization of FICR and AIBP for buffalo feeding, and thereby increase their usefulness, augment the income of villagers and minimize environmental pollution.

## References

Acharya, R.M. (1993) Livestock production systems, animal feeding and feed resources in India. In: *Feeding of Ruminants on Fibrous Crop Residues*, pp 5-7.

Bhattacharya, P. (1990) Buffalo. In: *An Introduction to Animal Husbandry in Tropics*. Co-published in New York, USA. pp 421-471.

Castillo, L.S. (1983a) Feeding value of crop residues of food crops grown in rice-based farming systems. In: *Asian Cropping 1983*. IRRI, Los Banos, Laguna, Philippines pp 385-406

Castillo, L.S. (1983b) Current utilization of fibrous residues in Asian countries. In: *The Utilization of Fibrous Agricultural Residues*. pp 33-49.

Davis, C.H., Saadullah, M., Dolberg, F. and Haque, M. (1983) Ammonia treatment of rice straw for cattle production in intensive agrarian agriculture. *Proc. Fourth Seminar on Maximum Livestock Production on Minimum Land,* Mymensingh, Bangladesh. pp 1-25

Devendra C. (1979) *Malaysian Feedstuffs*. Malaysian Agriculturre Research and Development Institute, Serdang, Selangor.

Devendra, C. (1993) Fibrous crop residues: strategy for the efficient use and development of feeding systems. In: *Feeding of Ruminants on Fibrous Crop Residues*. pp 8-20.

Dolberg, F. and Finlayson, P. (1995) Treated straw for beef production in China. *World Animal Review* 13-24.

Gerpacio, A.L. and Castillo, L.S. (1979) Nutrient composition of some Philippines feedstuffs. *Technical Bulletin* **21,** 117

Jintong, F. (1995) The use of fermented bagasse residues as feed for ruminants. In: *Proceedings of the Second International Conference on Increasing Animal Production with Local Resources*. Zhanjiang, China.

Mathers, J.C., Pearson, R.A., Sneddon, J.C., Matthewman, R.W. and Smith, A.J. (1985) The use of draught cows in agricultural systems with particular reference to their nutritional needs. In: *Milk Production in Developing Countries*. Ed. A.J. Smith. Edinburgh University Press. pp 476-496.

Martin, P.C., Cabello, A. and Elias, A. (1977) The use of fibrous sugarcane byproducts by ruminants. II. Effect of NaOH-pressure combination on the digestibility and chemical composition of bagasse and bagasse pith. *International Sugar Journal* **79,** 236.

Mc Dowell, R.E. (1985) Meeting constraints to livestock production systems in Asia. In: *Proceding of the Regional Workshop on Livestock Production Management*. Asian Development Bank, Manila.

Na Phuket, S.R. (1993) Swamp buffalo production systems. In: Buffalo Production. *World Animal Science* Volume C6, Elsevier, Amsterdam.

Neelakantan, S. and Deodhar, A.D. (1993) Biotechnological approaches of straw utilization by microbial systems for feed and industrial purposes. In: *Feeding of Ruminant on Fibrous Crop Residues*. Eds. K. Singh and J.B. Schiere. pp 248-257.

Preston, T.R. and Leng, R.A. (1987) Guideline for feeding systems & agro-industrial byproducts. In: *Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics*. PENAMBUL Books Ltd., Armidale NSW, Australia. pp 103-158.

Preston, T.R. (1995) Sugarcane for feed and fuel: recent developments. *World Animal Review* **82,** 84-89.

Preston, T.R. and Murgueito, E. (1992) Strategy for sustainable livestock production in the tropics. CONDRIT. Ltd, Cali, Colombia.

Ranjhan, S.K. (1980) *Animal Nutrition in Tropics and Sub-tropics*. Vikas Publishing House, New Delhi, India.

Roxas, D.B., Perez, C.B. (Jr) and Trinidad, E.E. (1969) The feeding value of sugarcane bagasse based rations for ruminants. I. Digestibility and nitrogen balance by cattle, carabaos and sheep. *Animal Sci.* **6,** 33-42.

Sansoucy, R. (1995) New developments in the manufacture and utilization of multinutrient blocks. *World Animal Review* **82,** 78-83.

Suriyajuntratong, W., Susksuchok, J., Pollarp, S., Sarapol, S. and Pison, U. (1974) The utilization of rice straw by water buffalo when supplemented with molasses and urea. *The Kesetsart Journal* **8,** 103.

Tingshuang, G., Zhenhai, Y. and Wei, K. (1995) A study on the strategy of feed development in China. In: *Proceedings of the Second International Conference on Increasing Animal Production with Local Resources*. Zhanjiang, China.

Thu, N.V. (1994) A study of the use of female cattle and buffalo in crushing sugarcane in Colombia. M.Sc. Thesis. Swedish University of Agricultural Sciences.

Thu, N.V., Dong, N.T.K., Hon, N.V., Quac, V.A. and Preston, T.R. (1995) Effect of Molasses-urea cake on performance of growing and working local buffaloes and cattle fed low nutritive value diet. In: *Proceedings of The Second International Conference on Increasing Animal Production with Local Resources*. Zhanjiang, China.

Thu, N.V. and Hon, N.V. (1992) Observations of the change of live weight and health of working buffaloes from the end of dry season to the beginning of raining season. *Selected Works of Scientific Research.* Faculty of Animal Husbandry and Vet. Med. University of Cantho. pp 82-91.

Wanapat, M. (1991) Better utilization of crop-residues and by products at village level to increase ruminant production in Thailand. In: *Proceeding of the International Workshop on Increasing Livestock Production by making better use of Local Feed Resources*, Vietnam.

Yuan, F.D., Lu, Y.L., Yun, M.M., Ling, Y.X., Yuan, P.Y., Ji, C.Z. and Bin L.S. (1995) The effect of alkaline treating sugarcane bagasse for increasing the nutrients digestibilities. In: *Proceedings of The Second International Conference on Increasing Animal Production with Local Resources*. Zhanjiang, China.

# DEVELOPMENT OF SYSTEMS OF SUPPLEMENTARY FEEDING FOR BUFFALOES IN SRI LANKA

S. Premaratne and A. Sivaram

*Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, SRI LANKA*

**Abstract:** Improving the productivity of the domestic buffalo is an urgent necessity for the Sri Lankan economy. Productivity of these animals mainly depend on the availability of quality feed throughout the year. Therefore, the objective of this study was to develop systems of supplementary feeding for buffaloes in Sri Lanka.

Experiments were conducted with four rumen fistulated male buffaloes to study the intake, digestibility and growth response of buffaloes fed rice (*Oryza sativa*) straw, supplemented with non-protein nitrogen and energy. Treatments consisted of 2% urea supplemented straw or plain straw with or without Jak (*Artocarpus heterophyllus*) seeds. Feed offered, refused and faeces excreted were measured and the dry matter intake and digestibility were calculated. Urine was collected to measure the nitrogen balance in animals. Animals were weighed before and after the experiment. Effect of these treatments on volatile fatty acid production, rumen ammonia and pH were also measured. Experiments were also conducted to study the effect of different fodders on dry matter intake of straw by milking buffaloes.

Dry matter intake of urea supplemented straw with jak seeds was higher compared to that with plain straw (102 *vs* 87 g/kg $w^{.75}$), whereas dry matter digestibility of plain straw with jak seeds was higher compared to plain straw (56% *vs* 48%). Inclusion of jak seeds improved the weight gain of buffaloes. Inclusion of fodders increased the straw dry matter intake by milking animals and the effect was prominent with glyricidia. In conclusion, it is evident that supplementation of straw based diets with urea and glyricidia improved the intake, digestibility and growth of buffaloes.

**Keywords**: Buffaloes, supplementary feeding, rice straw, fodder trees, urea feeding

## INTRODUCTION

The buffalo is an important source of draught power and to a limited extent a source of milk. Improving the productivity of these animals is an urgent need for the Sri Lankan economy. Unlike cattle, buffaloes are not widely distributed but are more concentrated in some regions of the country. In fact 70% of the buffalo population is in eight districts namely; Kurunegala, Anuradhapura, Hambantota, Batticoloa, Ampara, Trincomalee, Polonnaruwa and Kegalle. Productivity of these animals mainly depend on the availability of good quality feed throughout the year. Like in many other countries in the region, the rainfall distribution in Sri Lanka is not uniform throughout the year especially in the above mentioned districts. The pasture production curve usually follows the rainfall curve, resulting in a shortage of feed during the dry periods of the year.

Feed shortages during the dry periods can be overcome by use of crop-residues especially rice straw, non-conventional feedstuffs, agro-industrial by-products and tree fodder. The annual yield of rice straw is estimated at 3.0 million tons (Rajaguru, 1986) and the most effective way of utilizing rice straw is as a feed for ruminants. But straw alone can neither provide protein nor energy in sufficient amounts to meet the nutritional needs of the animal. Therefore, it is necessary to improve the nutritive value of straw either by pre-treatment or supplementation.

Addition of fodder leaves such as *Glyricidia maculata* (Glyricidia), *Leucaena leucocephala* (Ipil-Ipil) and *Tithonia diversifolia* (Wild sun-flower) could also improve the utilization of rice straw through improved protein nutrition and the supply of other nutrients to the rumen microbes. Addition of carbohydrates will also help to improve the energy nutrition of the rumen microbes. Therefore, the objectives of the present study were to develop systems of supplementary feeding for buffaloes in Sri Lanka.

## MATERIALS AND METHODS

*Experiment 1:* Four rumen fistulated male buffaloes, 2 years of age weighing 140-170 kg were used. They were tethered to rails in a well ventilated, partitioned building with free access to feed and water. During the experiment, they were housed in metabolism cages (1.95x0.9x1.9m) in order to record feed intake, faeces output and urine output.

The dietary treatments consisted of 2% urea supplemented straw (USS) or plain straw (PS) with or without Jak seeds (JS) and minerals. Dry matter intake of animals was 3% of their body weight, whereas the jak seed intake was limited to 5% of the total feed intake. Initially all the buffaloes were fed rice straw. The experimental diets were gradually introduced over a 10 day period. The experiment lasted 35 days which included a preliminary period of 20 days followed by a collection period of 15 days. During the collection period, feed offered, refused and faeces excreted were measured and the dry matter intake and digestibility were calculated.

Urine was collected to measure the nitrogen (AOAC, 1980) balance of the animals. Animals were weighed before and after the experiment. The effect of these treatments on volatile fatty acid production and rumen pH were also assessed. The four diets were fed to 4 animals in a Latin Square Design with 4 replicates.

*Experiment 2:* This experiment was carried out at the University Livestock Farm, Mawelawatte, Uda Peradeniya which is situated at an elevation of 1000 m above the mean sea level. The mean annual rainfall is about 2500mm.

Four male animals (12 - 18 months of age) weighing 60 -140 kg were used. During the day time, the buffaloes were allowed to graze in the field and in the night straw based diets were given (same as in Experiment 1). Intake of straw based diets during the night was measured for 30 days and nitrogen intake and weight gain were also measured. The design was a Latin Square with 4 animals.

*Experiment 3:* This experiment was also carried out at the University Livestock farm, Mawelawatte, Uda Peradeniya. Four milking buffaloes (weighing 379-482 kg) in different stages of lactation and pregnancy were used. Four experimental diets were used; (a) 2% urea sprinkled straw ad-libitum + minerals (b) 2% urea sprinkled straw ad-libitum + 5% Ipil-Ipil + minerals (c) 2% urea sprinkled straw ad-libitum + 5% wild sun-flower + minerals (d) 2% urea sprinkled straw ad-libitum + 5% glyricidia + minerals. The design was similar to that in the earlier experiments.

Animals were fed on one of the diets described earlier, during the night and allowed to graze in the field during the day time. Feed intake and nitrogen intake were measured for 30 days. Live weight gain/loss and milk production of animals were recorded. Data were statistically analyzed and the means were compared using Duncans Multiple Range Test (Steele and Torrie, 1960).

## RESULTS AND DISCUSSION

Table 1 presents the composition of feeds used in the study. Crude protein content of jak seeds was 14.7%, whereas the crude protein content of rice straw was 6.9%. These values are in agreement with the values reported by Ibrahim (1988). Table 2 presents the effect of supplementation on the dry matter intake, dry matter digestibility and nitrogen digestibility in buffaloes. Dry matter intake of plain straw was less ($p<0.05$) compared to all other treatments, whereas the highest ($p<0.05$) dry matter intake was observed with urea supplemented straw and jak seeds (Table 2). The higher intake of urea supplemented straw with jak seeds may have been due to increased palatability of this diet. Dry matter digestibility of plain straw was lowest (47.5%) compared to other treatments and the highest value was observed with the urea supplemented straw with jak seeds (55.6%). The higher dry matter digestibility in urea supplemented straw with jak seeds may be due to the better nutrient supply to rumen microbes with this diet. These results are in agreement with the work of Premaratne (1990). Nitrogen digestibility of the diets also followed the same trend. Nitrogen digestibility of plain straw was low but inclusion of urea or jak seeds improved the digestibility significantly. Also the addition of both urea and jak seeds improved the nitrogen digestibility significantly. This may be due to the balanced nutrient supply provided by these ingredients to the rumen microbes.

**Table 1.** Chemical composition of straw and supplementary feeds.

| Feed | Dry matter | Crude protein* | Fat* | Crude fibre* |
|---|---|---|---|---|
| Rice straw (%) | 93.15 | 6.9 | - | 33.6 |
| Ipil-Ipil (%) | 34.2 | 14.8 | 6.9 | 21.0 |
| Wild sun-flower (%) | 17.5 | 24.2 | 2.5 | 19.5 |
| Glyricidia (%) | 26.3 | 24.2 | 3.6 | 19.5 |
| Jak seed (%) | 94.92 | 14.71 | 3.0 | - |

* As percentage of dry matter

**Table 2.** Effect of urea and/or jak seed supplementation on the dry matter intake, digestibility and the nitrogen digestibility in buffaloes*.

| Item | Diet | | | |
|---|---|---|---|---|
| | PS | PS+JS | USS | USS+JS |
| DM Intake ($g/kgw^{0.75}/d$) | 87.8[a] | 91.4[ab] | 93.5[b] | 102[c] |
| DM digestibility (%) | 47.5[a] | 52.6[b] | 50.6[b] | 55.6[c] |
| N digestibility (%) | 32.7[a] | 41.1[b] | 40.5[b] | 53.1[c] |

* Mean of data from four buffaloes

Means within the same row having different superscripts differ significantly ($P<0.05$).

Table 3 presents the effect of fodder supplementation on the dry matter intake of milking buffaloes. According to Table 3, the total dry matter intake of urea supplemented straw with glyricidia was higher compared to urea supplemented straw alone, fed during the night. However, the inclusion of Ipil-ipil also increased the total intake of supplemented straw compared to supplementation with wild sun-flower. The lower intake of straw supplied with wild sun-flower may have been due to its poor palatability.

**Table 3**. Effect of tree fodders on the dry matter intake of straw in buffaloes.

| Diet | Dry matter Intake (g/kgw$^{0.75}$/d) |
|---|---|
| USS | 22.2 |
| USS + 5% IPIL | 25.4 |
| USS + 5% WSF | 23.3 |
| USS + 5% GLY | 26.7 |

In conclusion, it can be said that supplementation of straw based diets with urea and glyricidia improved the intake and digestibility of the feed.

## Acknowledgements

The authors gratefully acknowledge the financial support given by the SAREC/NARESA Buffalo Research Programme which made this study possible. They wish to thank Ms. Nilani Tissera, Mr. Daya Perera and the staff at Mawela Livestock Farm, University of Peradeniya for their valuable help during the study.

## References

Association of Official Analytical Chemists (1980) *Official Methods of Analysis*. (12 th Ed.) AOAC. Washington, D.C.

Ibrahim, M.N.M. (1988) *Feeding Tables for Ruminants in Sri Lanka.* Published by the Fibrous Feed Utilization Project, Kandy, Sri Lanka

Premaratne, S. (1990) Effect of non-protein nitrogen and fodder legumes on the intake, digestibility and growth parameters of buffaloes. In: *Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vieena, pp 53-61.

Rajaguru, A.S.B. (1986) Straw as ruminant feed in Sri Lanka. In: *"Rice Straw and Related Feeds in Ruminant Rations".* Eds. M.N.M Ibrahim and J.B Schiere. Straw Utilization Project, Publication No 02.

Steele, R.G.D. and Torrie, J.H. (1960) *Principles and Procedures of Statistics,* McGraw Hill, New York.

# COMPOSITION OF NATURAL HERBAGE AND IMPROVEMENT OF QUALITY OF SOME ALTERNATE FEED SOURCES FOR BUFFALO FEEDING

A.N.F. Perera and E.R.K. Perera

*Department of Animal Science, Faculty of Agriculture,*
*University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** The total buffalo population in Sri Lanka is 0.85 million, and more than 75% are reared under free grazing conditions in grazing lands on natural pastures or on other herbage. An experiment was conducted to evaluate the botanical composition and feeding value of natural forages. A series of experiments were conducted to evaluate the methods available to upgrade the feeding value of crop residues to feed buffaloes as a supplementary feed.

In natural forages, the highest grass component (72%) was observed in roadside forage and the highest legume component from scrub jungles (30%). Crude protein (CP) ranged from 8 - 16 % and *in vitro* dry matter digestibility (IVDMD) was 45 - 64 %. Rice straw was ensiled with biogas slurry liquid (BSL) up to 14 days and CP improved from 4 to 7 %, and IVDMD and *in vitro* organic matter digestibility (IVOMD) from 29 to 41 % and 25 to 39 % respectively. Different levels of urea treatment (0 - 6 % urea) and ensiling duration (0 - 21 days) of bagasse were evaluated. Urea treatment improved CP from 1% to 9 %, and IVDMD and IVOMD from 29 to 34 % and 28 to 33% respectively. Duration of ensiling had no effect. Treatment of palm press fibre (PPF) with urea (0 - 8 %) was studied. The IVDMD and IVOMD of PPF were improved from 22 to 32 % and 17 to 24 % respectively. Urea treated PPF had a higher CP content (33% *vs* 5 %) due to urea nitrogen. These results suggest the possibility of using different treatments on available crop residues to improve their utilization as supplementary feeds in buffalo production.

**Keywords:** Herbage, composition, alternate feeds, feed improvement, digestibility

# INTRODUCTION

The water buffalo (*Bubalus bubalis*) population in Sri Lanka has been estimated to be 0.85 million. They are mainly kept for draught purposes. Of the total population, 72 % is in the dry and dry intermediate zones, where year-round feed availability is limited. Generally buffaloes are not intensively managed, especially by the rural farmers due to their low income generation potential. However, the high price of buffalo milk and the increasing demand for buffalo curd has induced the rural buffalo farmer to extract as much milk as possible from their buffaloes.

One of the major factors that determines the efficiency of milk production is nutrition. Since a large proportion of buffaloes in Sri Lanka are in the dry and dry intermediate zones, they often face seasonal fluctuations in feed availability and quality. The natural feed sources are constantly subjected to seasonal fluctuations since their growth is mainly determined by the annual rainfall pattern. As a result, severe feed shortages are common, during inter-monsoonal dry spells and sometimes there is virtually nothing for animals to graze on. Therefore, alternate feed sources become vitally important to sustain, if not production, at least the condition of animals.

Rice straw is the most abundant crop residue available in Sri Lanka. It is available at the time when the dry spell begins and when natural forage availability is at a minimum. The total annual production of rice straw at present is 2.8 million metric tonnes. Of this, nearly 60 % is not used and is either allowed to decompose on the threshing grounds or burnt in the field. This enormous resource of valuable feed is not properly utilized as a ruminant feed due either to the lack of interest by the farmers or their belief that straw is low in feed quality. Therefore simple, economical and appropriate systems of treatment become important to upgrade this valuable feed resource.

Sugarcane by products are the second largest crop residue resource available as a potential feed for ruminant livestock feeding. Sugarcane green tops and bagasse are produced in large quantities during

8 months of the year. However, it is not utilized as an animal feed. Most of these by-products are allowed to decompose in the field (Khanal, *et al.*, 1995). Limitations with regard to their low digestibility and nutritive value can be overcome by pre-treatment (Devendra, 1992).

Though not much palm press fibre (PPF), is available in the country, the amount that is produced by the palm oil industry in the Southern region can be successfully utilized for livestock feeding with proper pre-treatment, as is done in countries like Malaysia, where large quantities are fed to ruminant livestock (Devendra, 1992).

Generally, all crop residues are low in nutritive value resulting in low intake and digestibility. By employing different pretreatment methods both the intake and digestibility can be improved making crop residues a suitable alternate feed resource for livestock feeding.

The objective of this study was to evaluate the natural forages and use of different pretreatments to improve the feeding value of some common crop residues in Sri Lanka.

## MATERIALS AND METHODS

*Botanical and chemical composition of natural forages:* Samples of natural forages were collected from waste lands, coconut lands, road sides, tank beds, fallow rice fields, scrub jungles and marshy lands in the wet zone, wet intermediate zone, dry intermediate zone and dry zone, in both wet and dry seasons. An iron quadrate measuring 0.5 x 0.5 m was used to collect random samples from each site for the determination of the botanical composition and feeding value. Samples from each site were placed in polythene bags and stored in an ice box to prevent any moisture losses. Botanical composition was determined by separating the fresh samples first as mono and dicot, thereafter as grasses and sedges and legumes and non-legumes. These were then dried separately at 60 $^0$C to determine the total dry matter. A second sample was dried in an oven at 60 $^0$C, until a uniform weight was obtained. Proximate and cell wall constituents and *in vitro* digestibility was determined. The composition of composite samples collected in both dry and wet seasons are given in Table 1.

**Table 1**. Botanical composition (% DM basis) of forages harvested from natural grazing lands.

| Location | Grasses | Sedges | Dicots | Legumes |
|---|---|---|---|---|
| Waste lands | 42 | 8 | 31 | 19 |
| Coconut lands | 58 | 7 | 20 | 15 |
| Road sides | 72 | 3 | 13 | 12 |
| Tank beds | 55 | 27 | 11 | 7 |
| Fallow rice fields | 28 | 53 | 12 | 6 |
| Scrub jungles | 12 | 2 | 46 | 30 |
| Marshy lands | 32 | 64 | 2 | 2 |

Each value is an average of 10 samples taken during two seasons.

*Rice straw ensiled with biogas slurry liquid (BSL):* Biogas slurry liquid was collected from an active Chinese type biogas unit, continually fed with dairy washings. Slurry was collected from the fermentation chamber, strained with a double layer of cloth and immediately transferred to a large vacuum flask for transportation. Fresh rice straw was mixed with strained BSL in the ratio of 1:1 and immediately packed into large polythene bags for ensiling. Separate samples were subjected to 0, 3, 6, 9, 12 and 15 day ensiling periods. Upon opening the treated bags, organoleptic characteristics, proximate and cell wall composition, ammonia nitrogen, total volatile fatty acids and *in vitro* digestibility were determined.

*Sugarcane bagasse treatment with urea*: Sugarcane bagasse was treated with 0, 2, 4 and 6 % urea solution (w/v) in the ratio of 1:1. After thoroughly mixing with the urea solution, the treated bagasse was stored in polythene bags for 0, 7, 14 or 21 days. At the end of the treatment periods the bags were opened and half of each sample was dried and ground to 2 mm particle size and the other half was stored in a freezer. Proximate and cell wall composition and digestibility were determined.

*Palm press fibre treatment with urea*: Palm press fibre was treated with 0, 4, 6, or 8 % urea solutions and allowed to react for 7 days in closed polythene bags. Urea treated samples were analyzed for proximate composition and detergent fibre. The rate of dry and organic matter disappearance of the components in the rumen of a cannulated buffalo was assessed for 24 hours.

Proximate analysis was done using standard methods (A.O.A.C., 1980) and detergent fibre according to Goering and Van Soest (1970). Rumen pH was measured electrometrically and total volatile fatty acids by steam distillation. *In vitro* digestibility (Tilley and Terry, 1963) was conducted using buffalo rumen fluid and *in sacco* degradation (Mehrez and Orskov, 1977) was determined using rumen cannulated buffaloes. All data were statistically analyzed using analysis of variance procedures (Snedcor and Cochran, 1967).

## RESULTS AND DISCUSSION

The major source of green feed for extensively reared buffaloes is the natural grazing lands (Wickramaratne, *et al*., 1993). The botanical composition of natural forages in these lands is given in Table 1. A high proportion of grass was observed in road side herbage samples (72%) and the lowest in herbage from scrub jungles (12%). Fallow rice fields were densely populated with sedges such as *Echinicloa spp., Cyprus spp.* and *Frimbristilis spp*. These monocot weeds are very common in rice fields and completely cover the field during fallow. They provide much of the bulk requirements of buffaloes grazing on fallow rice fields. Scrub jungles provide nitrogen rich feed due to the presence of leguminous forbes which are generally palatable (Perera, 1995). The highest content of DM (45 %) and lignin (24 %) were observed in fodder from scrub jungles (Table 2). This is mainly due to the presence of woody plant portions of dicots. Forages from all locations contained a CP content of less than 10 %, except the forages from tank bunds. This level of nitrogen is sufficient to maintain a rumen $NH_4$-N level to meet maintenance requirements. Road side grass exhibited the highest digestibility (643 g/kg). This is mainly due to the continuous removal of the mature vegetative portion of grass in road-side reservations, allowing regrowth which is tender (Perera *et al.*, 1993). The green biomass in natural grazing lands is subjected to severe fluctuations in both yield and quality.

**Table 2.** Chemical composition (DM basis) of forages harvested from natural grazing lands.

| Location | | Composition (%DM) | | | |
|---|---|---|---|---|---|
| | DM% | CP | ADF | ADL | IVDMD |
| Waste lands | 28.7 | 14.2 | 47.3 | 18.2 | 52.7 |
| Coconut lands | 32.2 | 12.1 | 40.7 | 12.3 | 47.3 |
| Road sides | 25.0 | 18.0 | 45.7 | 8.6 | 64.3 |
| Tank beds | 22.8 | 8.2 | 34.5 | 13.8 | 47.2 |
| Fallow rice fields | 22.6 | 16.4 | 48.7 | 13.6 | 52.6 |
| Scrub jungles | 45.3 | 14.7 | 54.3 | 24.1 | 45.3 |
| Marshy lands | 21.7 | 10.3 | 39.8 | 14.3 | 45.2 |

Composition of composite samples in both wet and dry seasons.

Rice straw, the largest crop residue resource available in Sri Lanka has been investigated in depth particularly with respect to upgrading the feeding value and utilization as a ruminant feed. Many chemical treatment methods have been suggested but the adoption of the procedures by farmers have not been very encouraging. Therefore, an environmentally friendly biological method was used in this study. The proximate composition, cell wall constituents and digestibility of rice straw ensiled with BSL is given in Table 3. Increasing the ensiling period

**Table 3**. Proximate composition, cell wall constituents, pH, total volatile fatty acids and digestibility of rice straw ensiled with biogas slurry liquid.

| | Ensiling duration (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Components (%). | 0 | 3 | 6 | 9 | 12 | 15 | SE ± |
| Dry matter | 44.7 | 43.6 | 42.7 | 42.3 | 41.9 | 41.6 | 0.36 |
| AI ash | 15.5 | 16.3 | 15.7 | 14.6 | 15.2 | 15.8 | 0.21 |
| Crude protein | $4.4^a$ | $6.2^b$ | $6.2^b$ | $7.2^b$ | $6.2^b$ | $6.4^b$ | 0.2 |
| Crude fibre | $26.4^a$ | $33.2^b$ | $33.0^b$ | $30.4^c$ | $30.4^c$ | $30.5^c$ | 0.29 |
| AD fibre | 55.5 | 54.6 | 55.4 | 54.7 | 51.4 | 51.5 | 0.61 |
| Cellulose | $27.4^a$ | $26.2^a$ | $23.7^b$ | $23.4^b$ | $20.5^c$ | $22.0^c$ | 0.65 |
| AD lignin | 12.2 | 14.9 | 16.4 | 16.5 | 14.5 | 13.6 | 0.7 |
| Ammonia - N | 0.19 | 0.21 | 0.24 | 0.23 | 0.23 | 0.23 | 0.01 |
| Non-ammonia-N | 0.08 | 0.07 | 0.08 | 0.09 | 0.08 | 0.08 | 0.01 |
| pH | 7.9 | 8 | 7.4 | 7.2 | 7 | 6.9 | 0.11 |
| Digestibility | | | | | | | |
| - Dry matter | $29.5^a$ | $30.3^a$ | $36.8^b$ | $36.3^b$ | $41.4^b$ | $41.1^b$ | 1.29 |
| - Org. Matter | $25.9^a$ | $26.2^a$ | $31.5^b$ | $32.5^b$ | $38.1^c$ | $39.1^c$ | 1.44 |
| Total VFA (µmol/100g DM) | $741^a$ | $942^b$ | $956^b$ | $944^b$ | $932^b$ | $940^b$ | 14 |

means followed by the same superscript within a row are not significantly different (P>0.05).

decreased the DM content, possibly due to breakdown of cell wall material (hemicellulose into pentoses) by the fermentative bacteria (Magno *et al.*, 1980). Increased level of CP observed with longer ensiling durations is a relative response resulting from a decrease in DM. Satisfactory fermentation was indicated by the significant increase (P<0.05) in TVFA. Both IVDMD and IVOMD were increased (P<0.05) by 13 units beyond 3 days of ensiling. This implies that BSL can be used as an alternative low pollution method to improve the utilization of rice straw as a ruminant feed. However, the acceptability by animals has yet to be investigated.

Urea treated bagasse exhibited a high CP content due to the addition of urea (P<0.05). However, the CP content of urea treated bagasse decreased in response to duration (P<0.05), perhaps due to the escape of free ammonia (Table 4). Both ADL and cellulose contents were reduced (P<0.05) with increase of the ensiling duration. The level of urea treatment improved (P<0.05) the IVDMD and IVOMD, but there was no response to treatment duration. This suggests that urea is too weak an agent to break down the lignocellulose bonding and therefore less effective in improving the nutritive of bagasse. A similar result may be

**Table 4.** Proximate and cell wall composition and digestibility of urea treated bagasse (%).

| | % Urea | | | | Duration (days) | | | |
|---|---|---|---|---|---|---|---|---|
| Components | 0 | 2 | 4 | 6 | 0 | 7 | 14 | 21 |
| Crude protein<br>T* D* | 1.2 | 8.3 | 12.5 | 19.3 | 11.2 | 10.4 | 10.2 | 9.5 |
| Ammonia - N<br>T* D* | 0.1 | 0.7 | 1.4 | 2 | 0.3 | 1.5 | 1.4 | 1.3 |
| AD Fibre | 25.3 | 24.9 | 24.8 | 24.7 | 24.9 | 24.7 | 23.4 | 22.8 |
| Cellulose<br>D* | 22.7 | 21.3 | 20.6 | 20.5 | 22.5 | 19.3 | 18.9 | 18.2 |
| AD Lignin<br>D* | 4.9 | 4.3 | 4.2 | 4.2 | 5.4 | 4.6 | 3.9 | 3.8 |
| AI Ash | 0.5 | 0.6 | 0.8 | 0.8 | 0.6 | 0.6 | 0.6 | 0.5 |
| Digestibility<br>- Dry matter | 29.5 | 30.6 | 33.9 | 34.1 | 31.5 | 31.3 | 31.7 | 32.9 |
| - Org. matter<br>T* | 28.2 | 29.4 | 31.6 | 32.6 | 29.9 | 30.0 | 29.6 | 30.5 |

T = Treatment (% Urea); D = Duration (days); * = $p < 0.05$;

obtained perhaps by merely supplementing urea at the time of feeding. Evidence is available to suggest that physicochemical methods such as steaming with chemical treatment or strong alkali treatment could improve its utilization (Devendra, 1979). However, more studies are needed to recommend suitable simple methods appropriate for local conditions.

Palm press fibre is a low quality roughage, but chemical treatment has been shown to ensure its potential as ruminant feed (Devendra, 1978). Treatment of PPF with urea up to 8% did not improve the levels of proximate components. But CP contents were elevated ($P<0.05$) due to the addition of urea-N. Urea treatment only reduced ADF ($P<0.05$), but there was no change between urea levels (Table 5).

**Table 5.** Effect of different levels of urea on proximate composition cell wall constituents, dry and organic matter degradation of palm press fibre (PPF).

| | Urea % | | | | |
|---|---|---|---|---|---|
| Component | 0 | 4 | 6 | 8 | SE± |
| Dry matter | 92.0 | 90.2 | 89.7 | 90.0 | 1.1 |
| Total ash | 7.4 | 6.9 | 6.8 | 7.1 | 0.6 |
| Acid insoluable ash | 1.8 | 1.7 | 2.1 | 2.1 | 0.6 |
| Crude protein | 4.6$^{a}$ | 24.6$^{b}$ | 29.1$^{c}$ | 32.8$^{c}$ | 2.4 |
| ADF | 54.0$^{a}$ | 46.2$^{b}$ | 48.9$^{b}$ | 46.3$^{b}$ | 1.8 |
| ADL | 41.0 | 37 | 38.8 | 37.3 | 2.1 |
| Cellulose | 22.1 | 18.9 | 20.3 | 19.7 | 1.7 |
| Disappearance in the rumen (at 48 h) | | | | | |
| - Dry matter | 22.5$^{a}$ | 27.3$^{b}$ | 27.8$^{b}$ | 31.9$^{b}$ | 3.1 |
| - Org. Matter | 17.2$^{a}$ | 17.8$^{a}$ | 17.9$^{a}$ | 24.1$^{b}$ | 2.9 |

means followed by the same superscript within a row are not significantly different ($P>0.05$).

Rumen incubation for 24 hours significantly improved IVDMD and IVOMD, but the level of improvement was not very promising. The low response to urea treatment by PPF is similar to that of bagasse due to the presence of high lignin. Therefore, stronger treatments may be needed to break the lignocellulose complex (Perera, 1992). Devendra (1978) suggested that alkali treatment did not improve the digestibility of CP in PPF due to the formation of soaps.

## Acknowledgments

The authors wish to extend their thanks to Miss. R. Ratnayake and H.G.D. Perera for their assistance in conducting the experiments. This study was conducted with financial assistance from the SAREC/ NARESA Buffalo Research Programme.

## References

A.O.A.C. (1980) *Official Methods of Analysis,* 12th Edition, Association of Official Analytical Chemists. Washington D.C.

Devendra, C. (1978) The utilization of feedingstuffs from oil palm. *Proc. Feedstuffs for Livestock in South East Asia.* Kuala Lumpur, Malaysia.

Devendra, C. (1979) The digestibility of chemically treated bagasse in molasses based diets for sheep and goats. *MARDI Res. Bull.* **1,** 103-115.

Devendra, C. (1992) Strategies for expanding utilization at the small farm level. In; *Non-conventional Feed Resources in Asia and the Pacific.* 4 Edition. FAO / UNDP.

Goering, H.K. and Van Soest, P.J. (1970) Forage Fibre Analysis. *U.S. Department of Agriculture Hand Book* No: 375. U.S. Government Printing Office, Washington D.C.

Khanal, R.C., Perera, A.N.F. and Perera, E.R.K. (1995) Ensiling characteristics of sugar cane tops and its potential as a ruminant feed. *Trop. Agric. Res.* 7: 177-185.

Magno, N.K., Carpenter, J.R., Nolan, J.C. and Campbell, C.M. (1986) Effect of addition of microbial inoculant, molasses and urea on silage quality of immature whole plant sugar cane. *J. Anim.* Sci. **63**, Suppl. **1,** 290.

Mehrez, A.Z. and Orskov, E.R. (1977) A study of artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci.* (Cambridge) **88**, 645-650.

Perera, A.N.F. (1992) Agricultural by-products as a feed resource for ruminants. *Proc. Eco Friendly Commercial Activities,* Organized by the Management for Development Foundation (MAD - Sri Lanka) and Norwegian Agency for Development Cooperation (NORAD), Colombo, Sri Lanka.

Perera, A.N.F. (1995) Potentials of livestock production in forestry systems. *Proc. Annual Forestry Symposium,* Forestry Unit, University of Sri Jayawardanepura, Nugegoda, Sri Lanka.

Perera, A.N.F., Wickramaratne, K.A. and Perera, E.R.K. (1993) Botanical composition and nutritive value of pastures in natural grazing lands of Ratnapura district. (Abst.). *Proc. 49th Annual Session of Sri Lanka Asso. Advancement of Science.*

Snedcor, G.W. and Cochran, W.G. (1967) *Statistical Methods.* 6th Edition, Iowa State University Press, Iowa.

Tilley, J.M. and Terry, R.A. (1963) A two stage technique for the *in vitro* digestion of forage crops. *Journal of British Grassland Society* **18,** 104 - 111.

Wickramaratne, K.A., Perera, A.N.F. and Perera, E.R.K. (1993) Botanical composition and nutritive value of pastures in natural grazing lands of Ratnapura district. (Abst). *Proc. 49 Annual Session of the Sri Lanka Assoc. Advancement of Science,* Colombo, Sri Lanka, December, 1992. pp. 56.

NA-143-239

# SERUM CONCENTRATIONS OF PROGESTERONE AND ITS PRECURSOR CHOLESTEROL IN BUFFALOES

R. Sivakanesan[1], J.G.S. Ranasinghe[1], C. Mariathasan[1] and H. Abeygunawardena[2]

[1]*Department of Biochemistry, Faculty of Medicine,*
[2]*Department of Veterinary Clinical Studies,*
*Faculty of Veterinary Medicine and Animal Sciences,*
*University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** The dependence of steroidogenesis in mammalian ovary on high density lipoprotein cholesterol (HDL-C) prompted a study on the relationship between serum progesterone and HDL-C concentration in buffaloes. All pregnant animals at the Narangalla buffalo farm were divided into two groups and one group was supplemented with urea treated straw. All the animals were allowed to graze freely. Blood samples were collected at fortnightly intervals and progesterone, total cholesterol (TC) and HDL-C concentrations were measured.

Pearson correlation analysis gave coefficient values of 0·083 (progesterone and TC all animals, n=124); -0·037 (progesterone and HDL-C all animals, n=128); 0·061 (progesterone and TC, control group, n=62); 0·112 (progesterone and TC, supplemented group, n=62); -0·007 (progesterone and HDL-C, control group, n=63); -0·082 (progesterone and HDL-C supplemented group, n=65) indicating very poor correlation. The TC concentration was lowest during the first two weeks postpartum in both control [1·22 ± 0·082 (SEM) mmol/l] and supplemented [1·42 ± 0·061 (SEM) mmol/l] groups and increased significantly up to 10 weeks (control 1·68 ± 0·094; supplemented 1·70 ± 0·089). Thereafter in the control group the levels fluctuated between 1·51 to 1·86 mmol/l whereas in the supplemented group it remained very close to 1·76 mmol/l. The wide fluctuations seen in the control group may be due to the availability of food. The TC concentration in the supplemented group did not differ significantly from that of the control group at any stage of the experiment. The HDL-C was lowest during the first two weeks after parturition; the values being 1·00 ± 0·068 (SEM) mmol/l and 1·10 ± 0·044 (SEM) mmol/l respectively for the control and supplemented groups. The HDL-C gradually increased over a period of 8 weeks after which it remained around 1·22 mmol/l in both groups. The differences between the control and supplemented groups were not significant at any stage during the experiment.

Five animals out of 18 in each group, control and supplemented, were cycling by 150 days and 133 days respectively. Supplementary feeding did not result in an appreciable improvement in the reproductive status and serum cholesterol. There was no correlation between either TC or HDL-C and serum progesterone concentrations, indicating the possibility that the progesterone synthesis is not influenced by the circulating levels of HDL-C and TC.

**Keywords:** Buffalo, progesterone, cholesterol, nutrition, ovarian activity

## INTRODUCTION

Cholesterol is the precursor for steroid hormone biosynthesis (Everett, 1947). Cholesterol in the ovary can be derived from two sources; cellular *denovo* synthesis from acetate or uptake of plasma lipoprotein cholesterol. The relative contribution of cholesterol synthesized *denovo vs* that derived from exogenous sources for steroidogenesis varies among species. The corpus luteum of the rabbit may utilize principally *denovo* synthesized cholesterol (Kovanen *et al.*, 1978), whereas rat (Anderson and Dietschy, 1978) bovine (Savion *et al.*, 1981) and porcine (Veldhuis and Gwynne, 1985) luteal tissues appear to depend on lipoprotein. The vast reduction of progesterone synthesis by ovarian cells when these cells are incubated in lipoprotein deficient media provides evidence for the major contribution of lipoprotein for progesterone synthesis (Tureck and Strauss, 1982).

There are species differences in uptake of lipoprotein by steroidogenic cells. Cultured luteal cells or luteinized granulosa cells of rat utilize high density lipoprotein cholesterol (HDL-C) for progesterone synthesis, even though low density lipoprotein (LDL) receptors are present (Anderson and Dietschy, 1978). In contrast, the rodent and swine ovarian tissues preferentially use LDL for steroidogenesis (Carr *et al.*, 1982).

Being herbivores, buffaloes depend on acetyl CoA formed during cellular metabolism for the synthesis of cholesterol. The dietary polysaccharides yield acetate as one of the volatile fatty acids during ruminal microbial fermentation. This is converted to acetyl CoA during cellular metabolism. Further, acetyl CoA is formed when cells oxidise glucose and fatty acids. It appears therefore that sufficient energy sources should be provided in the diet to enable the synthesis of cholesterol by the ruminant. Hence a connection such as dietary polysaccharides ---> acetate/acetyl CoA ---> cholesterol ---> lipoprotein cholesterol ---> progesterone might exist. This hypothesis needs to be tested and can be done if an assessment of some of the parameters in the above connection is carried out.

Therefore this study was conducted to determine the relationship between serum total cholesterol (TC) and HDL-C concentrations and serum progesterone in animals under the existing feeding pattern, namely grazing only, and in animals provided with a supplement in the form of urea treated straw.

## MATERIAL AND METHODS

The study was carried out from September, 1993 to July, 1994 at the SAREC/NARESA buffalo research station at Narangalla, Kuliyapitiya, located in the intermediate zone. The farm is about 238 acres in extent and is under coconut with natural and *Brachiaria miliformis* pasture. It receives an average rainfall of 2057±405 mm/annum occurring mostly during the north-east (October to January) and south-west (May to July) monsoons, but heavy precipitation is from the north-east monsoon. The average temperature is 30°C.

There are about 200 heads of indigenous buffalo (*Bubalus bubalis*) managed extensively under the coconut plantation. Usually animals are sent to pasture at around 7 a.m. and brought into night paddocks between 5.00 and 6.00 p.m. They are not provided with any supplementary feed. All animals were provided with wallowing facilities and calves are given anthelmintic at the age of 14 days. Routine vaccinations are done against Haemorrhagic Septicaemia and Foot and Mouth disease. Calves were allowed to suckle their dams *ad libitum* and cows were not milked. All the pregnant animals (n=35) in the farm were included in the study. The ages ranged from 4-8 years and almost all of them were multiparous animals.

Animals were divided at random into two groups (control and supplemented) at calving. The supplemented group was provided with 4 kg of urea supplemented straw (15 gm/kg of straw, sprayed 12 hr before the night feed) in addition to daytime grazing. The control group did not receive any supplementary night feed. Straw was withheld on the night previous to the day of blood collection to ensure that all animals had the same status.

Blood samples were collected from 8 months of pregnancy. Jugular blood (10 ml) was collected at 14 day intervals into vacutainers. One ml was transferred to oxalate fluoride bottles (30 parts of potassium oxalate and 1 part of sodium fluoride for 10 ml of blood) (Varley, 1980), soon after withdrawal for glucose, haemoglobin and packed cell volume measurements. Blood was transported to the laboratory within 2 hours of collection, centrifuged at 5000 rpm for 10 minutes and serum was separated. Aliquots were stored at -20°C until assay.

Total cholesterol and cholesterol in the HDL fraction were measured by the enzymatic method using Randox assay kits (Randox Laboratory Ltd, Diamond Road, Crumlin, Ireland). HDL fraction was obtained by phosphotungstate-$Mg^{2+}$ precipitation (Lopes-Virella, *et al.*, 1977). Plasma progesterone was measured by radioimmuno assay technique using antibody coated tubes and progesterone -$I^{125}$ as a tracer using the FAO/IAEA assay kits (Vienna). The standards were prepared at concentrations of 0.0, 0.1, 0.5, 2.0, 10.0 ng/ml.

## RESULTS

Postpartum cyclicity as evidenced by plasma progesterone elevations above 0.5 ng/ml (Perera, 1986) was observed around 85 days in both control and supplementary groups. Twelve animals out of 35 (35%) included in the study were cycling of which 5 animals belonged to the control group of 17 (30%) animals and 7 animals belonged to the supplemented group of 18 (39%) animals. However, a rise in serum progesterone above 0.5 ng/ml before 150 days postpartum, which is considered to be the average postpartum anoestrous period for buffaloes by Jainudeen, *et al.* ( 1982) was observed only in 3 animals in the control and 5 animals in the supplemented groups.

The number of anoestrous animals in the control and the supplemented groups during the entire experimental period of 240 days were 12 out of 17 (77%) and 11 out of 18 (62%), respectively.

### Total Cholesterol

The mean total cholesterol (Table 1) of the supplemented group was significantly higher ($p<0.05$) than the control group only at 0-2 weeks (1.42±0.06 mmol/l *vs* 1.22±0.08 mmol/l). Total cholesterol tended to increase from 8-10 weeks postpartum in both groups. It increased from 1.69±0.09 mmol/l to 1.87±0.1 mmol/l in the control and from 1.71±0.09 mmol/l to 1.78±0.05 mmol/l in the supplemented group.

### HDL Cholesterol

The HDL-C was lowest during the first two weeks after parturition, the mean values were 1.00±0.068 mmol/l and 1.10±0.044 mmol/l respectively, for the control and supplemented groups. The HDL-C gradually increased over a period of 8 weeks after which it remained at around 1.22 mmol/l (Table 2). The differences between the control and supplemented groups were not significant at any stage during the experiment.

**Table 1.** Serum total cholesterol concentrations (Mean±SD) in control and supplemented groups of buffaloes.

| Period (weeks) | Mean cholesterol concentration (mmol/l) | | | | p value |
|---|---|---|---|---|---|
| | Control | (n) | Supplemented | (n) | |
| 0-2 | 1.22 ± 0.08 | (15) | 1.42 ± 0.06 | (17) | 0.05* |
| 2-4 | 1.46 ± 0.07 | (15) | 1.44 ± 0.07 | (16) | 0.88 |
| 4-6 | 1.49 ± 0.10 | (15) | 1.64 ± 0.07 | (17) | 0.21 |
| 6-8 | 1.61 ± 0.09 | (15) | 1.63 ± 0.05 | (16) | 0.85 |
| 8-10 | 1.69 ± 0.09 | (15) | 1.71 ± 0.09 | (13) | 0.86 |
| 10-12 | 1.72 ± 0.08 | (14) | 1.70 ± 0.08 | (13) | 0.82 |
| 12-14 | 1.74 ± 0.09 | (14) | 1.76 ± 0.08 | (13) | 0.88 |
| 14-16 | 1.71 ± 0.13 | (13) | 1.80 ± 0.11 | (11) | 0.64 |
| 16-18 | 1.83 ± 0.1 | (13) | 1.63 ± 0.11 | (7) | 0.22 |
| 18-20 | 1.77 ± 0.24 | (7) | 1.78 ± 0.14 | (10) | 0.97 |
| 20-22 | 1.86 ± 0.09 | (9) | 1.72 ± 0.13 | (5) | 0.38 |
| 22-24 | 1.52 ± 0.09 | (3) | 1.78 ± 0.08 | (7) | 0.12 |
| 24-26 | 1.87 ± 0.1 | (10) | 1.78 ± 0.05 | (5) | 0.54 |

* Statistically significant

## Relationship between Progesterone and Total and HDL Cholesterol Concentrations

In order to determine whether the synthesis of progesterone is dependant on the circulating levels of lipoprotein cholesterol, the association between serum concentrations of progesterone and total cholesterol and cholesterol in the HDL fraction was ascertained by subjecting the data to a linear correlation analysis. Pearson correlation analysis of progesterone and cholesterol gave coefficient values of 0.083

**Table 2.** Serum high density lipoprotein cholesterol concentrations (Mean±SD) in control and supplemented groups of buffaloes.

| Period (weeks) | Mean HDL-C concentration (m.mol/l) | | p value |
|---|---|---|---|
| | Control (n) | Supplemented (n) | |
| 0-2 | 1.00 ± 0.07 (14) | 1.10 ± 0.04 (16) | 0.23 |
| 2-4 | 1.06 ± 0.08 (15) | 1.00 ± 0.06 (17) | 0.55 |
| 4-6 | 1.09 ± 0.07 (15) | 1.08 ± 0.05 (16) | 0.93 |
| 6-8 | 1.21 ± 0.07 (16) | 1.22 ± 0.03 (17) | 0.87 |
| 8-10 | 1.26 ± 0.07 (15) | 1.15 ± 0.05 (14) | 0.28 |
| 10-12 | 1.23 ± 0.06 (15) | 1.23 ± 0.05 (15) | 0.96 |
| 12-14 | 1.30 ± 0.08 (14) | 1.17 ± 0.05 (14) | 0.20 |
| 14-16 | 1.19 ± 0.09 (14) | 1.18 ± 0.04 (13) | 0.92 |
| 16-18 | 1.17 ± 0.11 (12) | 1.19 ± 0.07 (11) | 0.91 |
| 18-20 | 1.16 ± 0.05 (12) | 1.23 ± 0.04 (8) | 0.30 |
| 20-22 | 1.30 ± 0.07 (9) | 1.18 ± 0.08 (10) | 0.30 |
| 22-24 | 1.29 ± 0.06 (8) | 1.24 ± 0.06 (4) | 0.59 |

(progesterone and total cholesterol in all animals, n=124), -0.037; (progesterone and HDL cholesterol in all animals, n=128), 0.061; (progesterone and total cholesterol in the control group, n=62), 0.11; (progesterone and total cholesterol in supplemented group, n=62), -0.007; (progesterone and HDL cholesterol in the control group, n=63), -0.082; (progesterone and HDL cholesterol in the supplemented group, n=65) indicating very poor correlation.

## Body Weight

The mean pre-calving body weight (2 weeks before calving) of the control group (268±7.9 kg, n=17) was higher than that of the supplemented group (249± 6.0 kg, n=18), but the difference was not statistically significant. The mean body weight of the control group also was higher than that of the supplemented group during the entire experimental period (Table 3). Statistically significant difference was observed ($P<0.05$) between 12-20 weeks.

**Table 3**. Body weights (Mean±SD) of control and supplemented groups of buffaloes.

| Period (weeks) | Mean body weight (kg) | | | | p value |
|---|---|---|---|---|---|
| | Control | (n) | Supplemented | (n) | |
| 0-2 | 242 ± 7.0 | (18) | 229±20.0 | (17) | 0.138 |
| 2-4 | 232 ± 7.0 | (17) | 224±6.0 | (17) | 0.365 |
| 4-6 | 233 ± 7.4 | (17) | 220±5.5 | (17) | 0.142 |
| 6-8 | 231 ± 8.1 | (17) | 220±6.1 | (17) | 0.273 |
| 8-10 | 240 ± 9.0 | (15) | 217±6.7 | (14) | 0.054 |
| 10-12 | 232 ± 7.5 | (15) | 223±5.3 | (15) | 0.321 |
| 12-14 | 244 ± 8.8 | (13) | 220±7.7 | (14) | 0.043* |
| 14-16 | 245 ± 8.3 | (12) | 225±5.7 | (14) | 0.051* |
| 16-18 | 249 ± 7.5 | (11) | 219±7.2 | (13) | 0.009* |
| 18-20 | 241 ± 7.0 | (11) | 218±7.7 | (9) | 0.038* |
| 20-22 | 263 ± 14.3 | (8) | 226±8.2 | (10) | 0.031* |
| 22-24 | 251 ± 7.1 | (8) | 207±9.4 | (4) | 0.004* |
| 24-26 | 241 ± 1.1 | (3) | 228±7.3 | (6) | 0.470 |
| 26-28 | 261 ± 8.7 | (4) | 200±5.1 | (4) | 0.009 |
| 28-30 | 257 ± 26.6 | (3) | 243±8.8 | (6) | 0.525 |

* Statistically significant

## DISCUSSION

The low reproductive efficiency of local buffaloes still remains a major problem in buffalo farming. Field surveys have revealed that long periods of postpartum anoestrous is the primary cause for the extended calving interval (Perera, *et al.*, 1987). The genetic and environmental factors, play major roles in determining the reproduction capacity.

Among the environmental factors, rainfall is considered to be very important because it directly effects the availability of forages (Sindappa and Patil, 1979). In traditional dry zone farming systems buffalo cows are usually maintained on natural herbage and the quantity and quality of the forages are dependent on the seasonal rainfall (Mohan, 1990).

Limited studies have been carried out on the effect of diet on progesterone levels. Progesterone concentration in peripheral blood has been reported to decrease with poor nutrition (Hill, *et al.*, 1970) while Donaldson, *et al.* (1970) found the opposite results. On the other hand, Aspar, *et al.* (1975) revealed that in conditions of undernutrition it remained constant. In the present study, more animals in the supplemented group were found to be cycling compared to the unsupplemented group, indicating that feed supplementation had a marginal improvement on the reproductive status.

The total cholesterol concentration in Lankan buffaloes is slightly lower than the values reported by Jadhav, *et al.* (1977). The total cholesterol concentration increased after 8 weeks of calving irrespective of the group in the present study. However, Jadhav, *et al.* (1977) observed that it increased from the second week onwards in Gir cows. This difference may be due to species variation or other management factors. Increase in HDL-C during early postpartum period is in conformity with the observation of Puppione (1978). The increased HDL concentration during early lactation is an adaptation to the increase of apo C as a byproduct of VLDL catabolism by Lecithin Cholesteryl Acyl Transferase action.

Even though there is convincing evidence that steroid formation depends on the cholesterol supplied by circulating lipoprotein in many species of animals, the present study did not reveal any relationship between cholesterol and progesterone levels in serum. The total cholesterol concentrations and HDL-C in animals receiving urea supplemented straw were not statistically different to that of the unsupplemnted animals. Diane, *et al.* (1992) reported that supplementation of fat to dairy cattle rations stimulate lipoprotein cholesterol export by intestine to the major sites of *denovo* cholesterol synthesis in the ruminant, which can be utilized for steroidogenesis. Further, Pate and Condon (1982) observed that cows fed supplementary fat had higher progesterone concentrations either due to increased production or decreased clearance. In view of these observations, studies need to be carried out in local buffaloes to investigate the effect of fat on serum progesterone and lipoprotein concentrations and its impact on fertility.

Supplementary feeding with urea supplemented straw did not result in an appreciable improvement in reproductive status and serum cholesterol. There was no correlation between either TC or HDL-C and serum progesterone concentrations indicating the possibility that progesterone synthesis is not influenced by the circulating levels of HDL-C and TC. Further studies are required to elucidate the ovarian follicular lipoprotein cholesterol uptake mechanisms to understand ovarian steroidogenesis.

## References

Anderson, J.M. and Dietschy, J.M. (1978) Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, testis of the rat. *J. Biol. Chem.* **253,** 9024-9030.

Aspar, J.D., Aspros, J.E., Hixon, R.R. and Hansel, W. (1975) Effect of restricted feed intake on the sensitivity of the bovine corpus luteum to LH in vitro. *J. Anim. Sci.* **41,** 1120.

Carr, B.R., Macdonald, P.C. and Simpson, R.R.. (1982) The role of lipoproteins in the regulation of progesterone secretion by the corpus luteum. *Fertil. Steril.* **33,** 303.

Diane, J., Carroll, Ric, Grummer, R. and Murray, K. (1992) Stimulation of luteal cells progesterone production by lipoprotein from cows fed control or fat supplementary diet. *J. Dairy Sci.* **65,** 2205-2215.

Donaldson, L.E., Bassettand, J.M. and Thorburn, G.D. (1970) Peripheral plasma progesterone concentration of cows during puberty, oestrous cycle, pregnancy and lactation and the effect of under-nutrition or exogenous oxytocin on progesterone concentration. *J. Endocr.* **48,** 599.

Everett, J.W. (1947) Hormonal factors responsible for deposition of cholesterol in the C.L. of the rat. *Endocrinology* **42,** 364-374.

Hill, J.R., Lamond, D.M., Henricks, D. M., Dicky, J.F. and Niswender, G.D. (1970) The effect of under-nutrition on ovarian function and fertility in beef heifers. *Biol. Reprod.* **2,** 78.

Jadhav, S.S., Velhanker, D.P. and Deshpande, B.R. (1977) Variation in the Serum cholestrol levels in Gir cows during pregnancy, parturition and puerperium. *Indian vet. J.* **54,** (7) 536-544.

Jainudeen, M.R., Bongso, T.A., and Tan, H.S. (1982) Postpartum ovarian activity and uterine involution in the suckled swamp buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.* **5,** 181-190.

Kovanen, P.T. Goldstein, J.L. and Brown, M.S. (1978) High levels of 3-hydroxy 3-methyl glutaryl coenzyme A reductase activity and cholesterol synthesis in the ovary of the pregnant rabbits. *J. Biol. Chem.* **253,** 5126

Lopes-Virella M.F., Stone, P ., Ellis, S. and Colwell, J.D.A. (1977) Cholesterol determination in high density lipoproteins separated by three different methods. *Clin. Chem.* **23,** 882-884.

Mohan, V. (1990) Clinical and endocrinological studies on postpartum ovarian activity in Lanka buffaloes (*Bubalus bubalis*). Ph.D.,Thesis, University of Peradeniya.

Pate, J.L. and Condon, W.A. (1982) Effect of serum and lipoprotein on steroidogenesis in cultured bovine luteal cells. *Mol. Cell Endocr.* **28,** 571-580.

Perera, B.M.A.O. (1986) Use of RIA methods for studies on the reproduction of buffaloes. Nuclear and related techniques in animal production and health. In: *Proceedings of an International Symposium on the Use of Nuclear Techniques in Studies of Animal Production and Health in Different Environments*. Joint FAO/IAEA Division, Vienna. 17-21

Perera, B.M.A.O., De Silva, L.N.A., Kuruwita,V.Y. and Karunaratna, A.M. (1987) Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka. *Anim. Reprod. Sci.* **14,** 115-127.

Puppione, D.L. (1978) Implication of unique features of blood lipid transport in the lactating cow. *J. Dairy Sci.* **61,** 651.

Savion, N., Laherty, R., Lui, G.N. and Gospodarowicz, D. (1981) Modulation of LDL metabolism in bovine granulosa cells as a function of their steroidogenic activity. *J. Biol. Chem.* **256,** 12817- 12823.

Sindappa, T.N. and Patil, S.H. (1979) Seasonality of breeding and calving of buffaloes. *Indian vet. J.* **56,** 122-124.

Spicer, L.J.K., Sejrsen, A.A., Tucker, A. and Huber, J.T. (1984) Secretion of LH and FSH from over feeding dairy heifers. *J. Dairy Sci.* **67,** 1993-2000.

Tureck, R.W. and Strauss, J.F. (1982) Progesterone synthesis by luteinized human granulosa cells in culture: the role of denovo sterol synthesis and lipoprotein carried sterol. *J. Clin. Endocr.Metab.* **54,** 367.

Varley, H. (1980) *Practical Clinical Biochemistry* vol. 1, 5th edition. p 72.

Veldhuis, J.D. and Gwynne, J.T. (1985) Properties of LDL binding by cultured swine granulosa cells. *Endocrinology* **117,** 1067.

NA-143-251

# CHANGES IN GROWTH, RUMEN CHARACTERISTICS AND BLOOD METABOLITES OF INDIGENOUS BUFFALO HEIFERS IN RESPONSE TO SUPPLEMENTARY FEEDING OF UREA-TREATED *VERSUS* UNTREATED RICE STRAW

E.R.K. Perera and A.N.F. Perera

*Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, SRI LANKA*

**Abstract:** An experiment was conducted to examine the changes in feed intake, rumen parameters, blood metabolites and body weight gain of indigenous buffalo heifers in response to supplementary feeding of urea-treated *versus* untreated rice straw. Twelve indigenous buffalo heifers were allotted to two groups (n=6/group) balanced by body weight (89.0 ± 8.9 kg) and age (12.0 ± 0.5 months). During day time, all the heifers were offered "Guinea A" grass (*Panicum maximum*) *ad libitum*. At night, 4% urea-treated straw and untreated straw were offered to the treatment group and the control group, respectively. The group feed intakes of Guinea grass and straw were recorded daily. Representative samples of Guinea grass, straw and refusals were obtained periodically, for proximate analysis. Individual body weights were recorded monthly. Rumen samples were obtained every 4$^{th}$ week to determine rumen pH and rumen ammonia-N ($NH_3$-N). Blood urea nitrogen (BUN) and beta-hydroxybutyrate (BHB) were determined on the blood samples obtained on the same day. Urea treatment improved ($p \leq 0.01$) crude protein content of rice straw. The heifers on the treatment group had greater intake of straw dry matter ($p \leq 0.01$), total dry matter ($p \leq 0.01$), dietary nitrogen ($p \leq 0.01$) and dietary energy ($p \leq 0.01$) compared to the control group. Rumen ammonia content was higher ($p \leq 0.05$) in the treatment group animals. Rumen pH, circulating BHB and BUN were not different between the groups. But trends in rumen $NH_3$-N and BUN of the two groups suggested better utilization of available dietary protein by the treatment group, which resulted in higher ($p \leq 0.05$) body weight gain compared to the control group. The results suggest that supplementary feeding of urea-treated straw instead of untreated straw improved feed intake, protein nutrition, nitrogen utilization and growth performance in local buffalo heifers.

**Keywords:** Supplementary feeding, urea-treated straw, water buffalo, blood metabolites, body weight gain

## INTRODUCTION

The majority of the water buffaloes in Sri Lanka are subjected to poor and fluctuating planes of nutrition during the course of the year, as they are reared under natural grazing management with no supplementary feeding. The level of nutrition is a major determinant of the performance of ruminants (Grass *et al.*, 1982). Improvement of protein nutrition may accelerate the growth performance in buffaloes, and the changes in nutritional status would be reflected in rumen characteristics and circulating metabolites (Lee *et al.*, 1978). Urea treatment of rice straw has been shown to improve protein supply to the animal (Masaaki *et al.*, 1992; Saadullah *et al.*, 1981). Traditionally, untreated rice straw is offered as a dry season feed to buffaloes in Sri Lanka. Urea treatment is a simple processing method, which can be adopted by the rural farmers. Hence, in this experiment urea treatment of rice straw was chosen as a suitable method of improving protein nutrition for buffaloes with the objective of examining the responses in feed intake, rumen parameters, blood metabolites and body weight changes of indigenous buffalo heifers to supplementary feeding of urea treated rice straw instead of untreated straw.

## MATERIALS AND METHODS

The study was conducted at the Department of Animal Science, Faculty of Agriculture, University of Peradeniya using twelve (12) indigenous buffalo heifers. The animals were divided into two groups (n=6/group) balanced by age (12.0 ± 0.52 m) and body weight (89.0 ± 8.9 kg).

Medium quality Guinea A grass (*Panicum maximum*) was offered *ad libitum* to all the heifers from 0800 hr to 1600 hr. At 1600 hr, one group (treatment) was offered urea treated rice straw (4% urea treated straw ensiled for 7 d), while the other group (control) was offered untreated rice straw. Except for this difference all the other management practices were identical for both groups throughout the 40 wk experimental period. Group feed intakes of grass (G) and straw were recorded daily. Representative samples of G, urea treated straw (UTS), untreated straw (S) and refusals were collected biweekly to determine the dry matter (DM), organic matter (OM), and nitrogen content of the feeds and refusals, using the standard analytical methods (A.O.A.C., 1980). Rumen liquor samples were obtained from individual animals 3 hr post-feeding using a mouth tube, every 8[th] wk for the determination of rumen ammonia (Markham, 1942) and rumen pH. Jugular vein blood samples were collected at the same time from individual animals to determine blood urea nitrogen (BUN) and beta-hydroxy butyrate (BHB). These metabolites were analyzed using the Nutritional Metabolite Kits of the International Atomic Energy Authority (IAEA). Individual body weights were obtained monthly at 0800 hr using an electronic weighing balance (RUDDWEIGH Model: KM-2 S). The data were subjected to analysis of variance procedures.

## RESULTS AND DISCUSSION

*Proximate composition:* The dry matter content of straw was higher ($p<0.01$) than that of grass. As expected urea treatment substantially improved crude protein (CP) content ($p<0.01$) of rice straw (Table 1). The observed increase in CP content of straw was similar to the 3.5% increase in CP of straw obtained by Verma (1984) through urea treatment. The CP and OM contents of grass and straw were comparable to the values reported by Ravindran *et al.* (1987) for *Panicum maximum* Eco type A (Guinea A) harvested at 4 weeks, and by Masaaki *et al.* (1992) for S and UTS.

*Dry matter intake (DMI):* The mean daily dry matter intake (DMI) per kg body weight of the two groups are given in Table 2. Although the grass DMI was not different between the groups, the total DMI of the treatment group was higher ($p\leq0.01$), due to a greater straw DMI ($p\leq0.01$) by this group (Table 2). Similar increases in straw DMI following urea treatment have been reported previously (Saadullah *et al.*, 1981; Wongsrikeao and Wanapat, 1984). The increased DMI of UTS can possibly be attributed to improved palatability and increased CP content (Table 1). Urea treatment is reported to increase the digestibility of straw by enhancing the activity of rumen microflora and facilitating microbial digestion of cellulose (Saadullah *et al.*, 1981). The observed increase in DMI by the treatment group clearly indicated the beneficial effects exerted on the DMI by the improved CP content of roughage.

**Table 1.** Proximate composition of the grass (G), untreated straw (S) and urea-treated straw (UTS) fed to heifers.

| | G | S | UTS |
|---|---|---|---|
| Dry matter (%) | 28.6 ± 5.2* | 67.8 ± 4.6 | 67.7 ± 6.2 |
| Organic matter+ | 88.5 ± 4.0 | 90.0 ± 1.8 | 89.3 ± 2.6 |
| Crude protein+ | 7.9 ± 0.44** | 5.6 ± 0.3 | 9.1 ± 0.2** |

*($P \leq 0.05$) **($P \leq 0.01$) +as % of DM

*Energy intake:* The daily metabolizable energy (ME) intake of the two groups (Table 2) was estimated based on the DMI and ME content of the feed (Ibrahim *et al.*, 1987; Ravindran *et al.*, 1987). Grass ME intake was not different between the groups, because the grass DMI was not different. But the treatment group had a higher ($p \leq 0.01$) total ME intake due to the higher intake ($p \leq 0.01$) of treated straw ME (Table 2). The results suggest that the beneficial effects on energy intake was brought about by improved CP content via the effects on DMI and digestibility. Based on the established values on nutritional requirements for buffaloes (Kearl, 1982), and the observed body weight gain in the two groups of heifers on this experiment (212 *vs* 279 g/d), the daily ME requirements per kg body weight of the two groups were estimated to be 54.6 Kcal (control) and 62.8 Kcal (treatment) respectively. The energy intake (Table 2) of the treatment group (66.1 Kcal) exceeded its requirement, indicating that sufficient energy was available to animals in this group. But the energy intake (54.9 Kcal) and requirement of the control group (54.6 Kcal) were almost equal. Thus, any slight reduction in DMI might have caused minor energy stress in this group. The circulating BHB serves as an indicator of energy stress in ruminants (Lee *et al.*, 1978). The above observations could be further confirmed by examining the circulating BHB values of the two groups. The ME intake values of the two groups reiterated the beneficial effects of using UTS instead of S as a supplementary feed for buffalo.

*Nitrogen intake*: The nitrogen intake (NI) of the two groups (Table 2) were estimated based on the DMI and the N content of the feed. The grass NI was not different between the two groups because the grass DMI of the two groups were similar (Table 2). However, the treatment group had a greater ($p \leq 0.05$) total NI, due to a greater straw NI by this

**Table 2.** Daily intake of dry matter, energy and nitrogen by the two groups of buffalo heifers.

| | Group fed Untreated Straw | Group fed Treated Straw |
|---|---|---|
| **Dry Matter Intake** (g/ kg BW) | | |
| Grass | 27.1 ± 1.9 | 26.4 ± 2.1 |
| Straw | 5.1 ± 1.4$^a$ | 11.0 ± 2.1$^b$ |
| Total | 32.1 ± 3.0$^a$ | 36.5 ± 3.2 |
| **Energy Intake** (Kcal/ kg BW) | | |
| Grass | 46.2 ± 13.1 | 44.8 ± 11.1 |
| Straw | 8.0 ± 0.6$^a$ | 19.3 ± 5.9$^b$ |
| Total | 54.9 ± 9.6$^a$ | 66.1 ± 11.2$^b$ |
| **Nitrogen Intake** (g/ kg BW) | | |
| Grass | 4.56 ± 0.24 | 4.41 ± 0.22 |
| Straw | 5.13 ± 0.11$^a$ | 2.10 ± 0.24$^b$ |
| Total | 5.07 ± 0.21$^a$ | 6.45 ± 0.21$^b$ |

Within a row, means with dissimilar superscripts are significantly different ($p \leq 0.01$).

group (Table 2). The results suggest that supplementary feeding of UTS was successful in improving the NI of heifers. Higher intake of ruminally degradable nitrogen (N) helps to maintain an optimum ammonia concentration in the rumen which favours increased number and activity of rumen microflora (Orskov, 1982) required for better digestibility of the roughage. However, higher intake of N does not necessarily guarantee favourable results, if most of the ammonia N that disappears from the rumen is excreted in the urine as urea, in an attempt to prevent rumen $NH_3$-N toxicity. This is especially true, when poor quality roughage treated or supplemented with urea is given with inadequate levels of available energy. As indicated previously, the energy intake of the treatment group exceeded its energy requirement. Thus, such elimination of N in urine might not have taken place in this group. Since BUN serves as an indicator of N elimination in the urine, this observation could further be confirmed by examining the BUN levels of the two groups.

*Rumen pH:* Rumen pH of the two groups were not significantly different ($p > 0.05$) although the treatment group had slightly higher rumen pH (Table 3). The rumen pH should be maintained at around 6 for optimal microbial protein synthesis. Generally, rumen pH is higher when UTS is fed to ruminants (Masaaki *et al.*, 1992) due to $NH_3$ generating transformational processes taking place in the rumen. Rumen pH is a function of the rate of $NH_3$ production and the buffering capacity of the rumen. In this experiment, the rumen pH was not significantly elevated in response to supplementary feeding of UTS instead of S. This observation points to the existence of a more efficient buffering capacity in the rumen of the treatment animals.

*Rumen ammonia ($NH_3$-N):* As expected, the rumen $NH_3$-N content in heifers of the treatment group was greater ($p \leq 0.05$) than that of the control group (Table 3). Nevertheless, the rumen $NH_3$-N contents of both groups were lower than that required by the rumen microbes (80 mg N/l) for optimal microbial protein synthesis (Hoover, 1986). An optimum ammonia concentration in the rumen favours increased number and activity of rumen microflora (Orskov, 1982) required for better digestibility of the roughage. Since the rumen $NH_3$-N levels of the experimental heifers were less than optimal, the digestibility and utilization of roughage might not have been optimal. But, the greater ME and CP intake (Table 2) as well as the higher rumen $NH_3$-N (Table 3) content of the treatment group heifers, as a result of supplementary feeding of UTS, might have contributed to an improvement of microbial activity in the rumen and greater nitrogen utilization by this group. This hypothesis could be confirmed by comparing the BUN concentrations and body weight gains of the two groups of heifers.

*Beta-hydroxybutyrate (BHB):* Circulating BHB did not vary significantly during the experiment. Mean BHB of the treatment group was not different ($p > 0.05$) from that of the control (Table 3). Circulating BHB serves as an indicator of the energy status of animals, reflecting the balance between fat mobilization and ketone body utilization. Under conditions of inadequate carbohydrate intake, and/or acetonaemia BHB is increased (Lee *et al.*,1978). Reference BHB values for indigenous buffalo have not yet been established. But the observed BHB values (Table 3) were comparable to the reference values established for well-fed non-lactating cattle and sheep (IAEA, 1993). Moreover, the results of another study by the authors (unpublished) indicated the BHB values of well-fed buffaloes were between 0.19 and 0.28 mmol/l. Observed BHB values of both the groups were within that range (Table 3), confirming the hypothesis made previously regarding the non-existence of energy stress in the heifers during the experimental period.

**Table 3.** Rumen parameters and blood metabolites of the two groups of buffalo heifers.

| | Control | Treatment |
|---|---|---|
| **Rumen Parameters** | | |
| pH | $6.8 \pm 0.8$ | $7.5 \pm 0.2$ |
| $NH_3$ - N (mgN/L) | $20.1 \pm 4.4^b$ | $36.5 \pm 4.7^b$ |
| **Blood Metabolites** | | |
| BUN (mmol/l) | $7.23 \pm 1.77$ | $6.49 \pm 1.57$ |
| BHB (mmol/l) | $0.24 \pm 0.06$ | $0.20 \pm 0.04$ |

Within a row, means with dissimilar superscripts are significantly different ($p<0.05$)

*Blood Urea Nitrogen (BUN):* The BUN of the heifers did not vary significantly over time. Mean BUN of the control animals was slightly greater than that of the treatment heifers because more than 80% of the samples of control animals had higher BUN values compared to BUN of the treatment animals. However, the difference was not significant ($p > 0.05$). Mean BUN reflects the balance between ruminally digestible protein and fermentable metabolizable energy in the rumen. For proper microbial function the above ratio is very important. The BUN increases under conditions of excess protein intake, poor quality dietary protein, inefficient protein utilization, carbohydrate deficiency or renal failure. As evidenced by the proximate composition of the diet (Table 1), the nitrogen intake (Table 2) and rumen parameters (Table 3), the control animals did not receive excess protein. The ME intake (Table 2) and BHB values (Table 3) suggest that they were not subjected to severe energy stress. There was no evidence of renal failure in these heifers. Hence, the observation that more than 80% of the control samples contained higher BUN could be due to poor utilization of available nitrogen by the control animals.

*Body Weight Gain (BWG):* During the 40 wk experimental period the mean BW of the control group animals increased from $89.8 \pm 8.9$ to $149.4 \pm 8.1$ kg, while that of the treatment group increased from $89.5 \pm 11.5$ to $180.3 \pm 11.2$ kg. The heifers fed UTS showed higher ($p \leq 0.05$) daily BWG ($212.28 \pm 4.12$ vs $279.28 \pm 6.78$ g), and as a result a higher total BWG ($59.6 \pm 6.31$ vs $80.0 \pm 7.01$ kg). These BWG were comparable to the BWG reported by Wongsrikeao and Wanapat (1984) for buffalo heifers fed urea treated straw.

The results suggest that supplementary feeding of urea-treated straw instead of untreated straw improved protein nutrition, nitrogen utilization and growth performance of indigenous water buffaloes in Sri Lanka.

## Acknowledgements

The authors are grateful to the Swedish Agency for Research Co-operation with Developing Countries, for the funding of the research and the Natural Resources, Energy and Science Authority (NARESA) of Sri Lanka for administering the funds.

## References

A.O.A.C. (1980) *Official Methods of Analysis* (13 Ed.) Association of Official Analytical Chemist, Washington, D.C.

Grass, J.A., Hansen, P.J., Rutledge, J.J. and Hauser, E.R. (1982) Genotype/ environment interactions on reproductive traits of bovine females. I. Age at puberty as influenced by breed, breed of sire, dietary regime and season. *J. Anim. Sci.* **55**, 1441-1457

Hoover, W.H. (1986) Chemical factors involved in ruminal fibre digestion. *J. Dairy Sci.* **69**, 2755-2766

IAEA (1993) Nutritional Metabolite Kit Protocols. Joint FAO/IAEA Programme, Animal Production Unit, IAEA. Vienna.

Ibrahim, M.N.M., Ketelaar, R.S., Tamminga, S., Zemmelink, G. and van der Meer, J. (1987) Nutritive value of some commonly available ruminant feeds in Sri Lanka. *Inst. for Livest. Feed. and Nutr. Res.*(IVVO), Lelystad, The Netherlands. Rpt. 181, 34

Kearl, L.C. (1982) Nutrient requirements of ruminants in developing countries. International Feedstuffs Institute, Utah State Institute, USA.

Lee, A.J., Twardock, A.R, Bubar, R.H., Hall J.E. and Davis, C.L. (1978) Blood Metabolic profiles: Their use and relation to nutritional status of dairy cows. *J. Dairy Sci.* **61**, 1652-1670

Markham, R. (1942) A steam distillation apparatus suitable for microkejldhal analysis. *Biochem. J.* **36**, 790

Masaaki, N., Perera, N., Meiji, O. and Norihito,Y. (1992) Effect of molasses on utilization of urea treated straw. *Res. Bull. Obihiro. Univ.* **17**, 341-347

Orskov, E.R. (1982) Dynamics of nitrogen in the rumen. In: *Protein Nutrition in Ruminants*. E.R. Orskov (Ed.) Academic Press. pp 41-44

Ravindran, V., Perera, H.G.D. and Ravindran, S. (1987) *In vitro* digestibility of some Sri Lankan forage species based on rumen inocula from goats, cattle and buffaloes. *Proc. Sri Lanka Association for Advancement of Science* **45**, 65

Saadullah, M., Haque, M. and Dolberg, F. (1981) Effectiveness of ammonification through urea in improving the feeding value of rice straw in ruminants. *Trop. Anim. Prod.* **6**, 30-36

Verma, M.L. (1984) Urea treatment of straw for higher milk production. *Asian Livestock* **9**, 10-11

Wongsrikeao, W. and Wanapat, M. (1984) The effects of urea treatment of rice straw on the intake and live weight gain of buffaloes. *Proc. 4th Ann. Workshop of the Aust. Asian Fibrous Agricultural Residues Research Network.* pp 81-84

# APPROPRIATE NUTRITIONAL PACKAGES FOR SMALL HOLDER BUFFALO PRODUCTION SYSTEMS IN ASIA

S.K. Ranjhan

*Consultant, Buffalo Rearing Project, Hind Agro Industries Ltd.*
*Friends Colony, New Delhi,*
*INDIA*

## INTRODUCTION

In Asian agriculture, livestock especially buffaloes play an important role in farmer's economy as an integral part of the farming system, whether it is a mixed, arable, specialized farming, backyard or commercial animal production system. The farming systems of South and South East Asian farmers consists of a family, the crop and the livestock. Therefore, in rural project planning, the livestock sector has to be given due importance since it provides not only draught power to the farmer but also additional income. It provides for the use of idle labour between sowing and harvesting of crops, and thereby improves and develops the economic conditions of the small and marginal farmers.

About 97% of the world buffalo population is found in Asia. Riverine buffaloes (2n=50) are found in India and to the west up to Europe (Indian subcontinent, Middle East, Italy, southern Europe and Russia). Many breeds (Murrah, Nili Ravi , Surti, Mehsana, Jaffarabadi, Nagpuri, Bahadawari, *etc.*) famous for milk production are to be found in drier regions of these countries. Swamp buffaloes (2n=48) on the other hand, are found in eastern parts of India extending further to the east up to the Philippines, Thailand, Indonesia, Laos, Pacific Islands, China etc. The low milk producing swamp buffaloes are predominantly found in paddy growing areas, with high rainfall.

# PRODUCTION SYSTEMS

The predominant livestock production system in most Asian countries is the traditional small scale farming involving 1-3 bovines (cattle/ buffaloes). The animals are raised by the small holders primarily for draught power for his small land holding (1- 3 ha) and secondly, for an additional income through milk produced by these animals. When the animals are too old for work or have decreased their milk production, they are slaughtered.

In Asia, the feed resources for ruminants are crop residues (cellulosic wastes), natural grasses (available through grazing) and agro-industrial by-products. Generally, ruminants obtain the major nutrient requirements through grazing in natural grasslands. The pattern of production on range grazing in terms of growth, draught and milk is fairly good during the wet season coinciding with herbage growth. This is followed by a variable decline in production during the dry periods when herbage growth is affected. To sustain optimum production, crop residues and other agro-industrial by-products, concentrates and the like are given as feed supplements during the lean periods depending on their availability. In recent years, the cost of basic ingredients has increased considerably. Thus it has become difficult for many Asian countries to use animal feed ingredients (cakes, fish meal, corn *etc.*) for economic livestock production. Because of this, the use of agro-industrial by-products locally available as livestock feed is increasing at a rapid rate. This paper discusses the strategies for their use in buffalo production with special reference to draught, meat and milk production.

# FEED RESOURCES

## Cellulosic Wastes

In Asia, large quantities of by-products from farm and agricultural processes are available (Table 1). Cereal straws such as rice, maize, wheat, sorghum constitute the major feed resources available for livestock feeding during the dry season. Paddy straw is by far the largest fibrous residue resource available for "on farm" feeding in Asia.

**Table 1.** Potential supply of roughages from some crop by-products in South and Southeast Asia (Thousand metric tonnes).

| | International Feed No. | Southeast Asia [1] | Indian Sub-continent [2] | Total |
|---|---|---|---|---|
| Rice, hulls | 1-08-075 | 11,865 | 17,000 | 28,865 |
| Rice, straw | 1-03-925 | 74,163 | 114,500 | 188,663 |
| Maize, aerial | 1-12-179 | 7,100 | 15,500 | 22,600 |
| Sorghum | 1-04-304 | 2,142 | 33,500 | 35,642 |
| Cassava | 1-10-302 | 155 | n.a | - |
| Sugarcane bagasse | 1-10-768 | 8,126 | 6,264 | 14,390 |
| Sugarcane tops | 2-04-692 | 10,535 | 8,600 | 19,135 |
| Palm press fibre | 2-04-686 | 10,535 | n.a | - |
| Peanut aerial | 1-03-623 | 844 | n.a | - |
| Pineapple, process residue dehydrated | 4-03-722 | 48 | n.a | - |
| Pineapple, process residue | 4-26-968 | 854 | n.a | - |
| Wheat, straw (incl, barley) | 1-05-175 | | 67,000 | 67,000 |

[1]From Khajaren and Khajaren (1980), [2]Calculated from the FAO production figures of 1979, n.a.= not available

A considerable amount of literature is available regarding the characteristics of cellulosic wastes. These have been discussed at many international seminars, workshops and congresses. Reviews of these papers (Ranjhan, 1977; Ibrahim, 1981; Doyle *et al.*, 1985) indicate that crop residues have both physical and chemical constraints limiting their use for optimum animal production.

The physical limitation which results in low voluntary intake (1.5 - 2.0%) is governed by the amount of material in the reticulo-rumen, their rates of digestion and passage of digesta. Therefore, mechanical processes associated with digestion namely, particle size, balance of nutrients and rates of microbial fermentation are important to increase the level of intake. The digestibility of the dry matter is between 37 - 43% with 0% digestible crude protein and 43% TDN.

The chemical limitation of cereal straw is that they are poor in energy, protein, minerals and vitamins. There is considerable variation in the chemical composition which is affected by variety, fertilizer application, stage of harvesting and post harvest management. Lignin and silica tend to limit the digestibility of rice straw. Leguminous straws/residues (peanut, bean, cajanus, *etc.*) are rich in protein ranging from 5-9% and are more palatable.

## Utilization of Crop Residues

Cereal straws (rice, maize, *etc.*) are the main feed resource for buffaloes in Asia specially in the Indian subcontinent, because of the shortage of green fodder during the dry season. At present, the main use of cellulosic wastes in buffalo feeding for growth (meat) and milk production is normally as a basal feed in different proportions along with forage crops (green grass/grazing ) and concentrate feeds (brans, palm kernel, *etc.*). When given in high proportions in diets either for maintenance, growth, or milk production, animals lose production mainly due to low voluntary feed intake. This is further confounded by low digestibility and inadequate supply of protein, minerals and vitamins.

Normally cereal straws are not given as the sole feed. By experience, farmers have learnt that given alone, these would affect the performance of the animals. In a number of experiments, straws have been fed solely (100%) to animals. When fed singly all their limitations in terms of physical and chemical characteristic are manifested in the animal's performance as they are consumed in amounts insufficient to meet the energy and protein requirements of adult and producing animals (Ranjhan, 1977). Mature swamp buffaloes fed straw, lost 80-100 g daily (Castillo *et al.*, 1982; Cheva-isarakul and Cheva-isarakul, 1984; Wanapat *et al.*, 1982).

Leguminous straws/residues fed alone maintain live weights and also sustain limited production. Residual fodder like cowpea, after removal of pods can sustain a growth rate of 400-450 g/d in calves when fed for 120 days (Ranjhan, 1977).

Both the level of intake and the digestibility of the straws can be increased by providing supplementary nutrients. These supplementary feeds provide adequate nutrients to rumen microbes for better fermentation of the cellulosic materials. These nutrients can be provided by supplementation with fermentable nitrogen in the form of urea, ammoniated straw or through supplementary forages (grasses) or concentrated by-products. Under such situations, the responses in growth and milk production will depend on the levels of energy, protein, vitamins and minerals that would be available to the host animals.

## SUGAR CANE TOPS

In many Asian countries, sugar cane is extensively grown in the irrigated areas. Sugar cane tops are available as a feed for buffaloes. There is a potential availability of about 20 million tonnes annually. Tops are very palatable and a rich source of energy. When fed alone, supplementation of protein is necessary as they are deficient.

## GREEN FODDER

In irrigated areas green fodders like maize, cowpea, cajanus, *etc.* can be grown and are excellent feeds for buffaloes. Besides cultivated green fodders, green grasses are also available naturally/grown for feeding buffaloes. These are cenchrus, panicum, para, stylo, *etc.*

## AGRO-INDUSTRIAL BY-PRODUCTS

Few agro-industrial by-products including non-conventional feeds (NCF) are available from the milling/oil extraction industries in Asia which can form part of livestock feeding systems. They are: (a) energy rich supplements like molasses from sugar cane, pineapple wastes and cassava by-products; (b) protein rich supplements like oil cakes (copra meal, palm oil kernel meal, *etc.*) and (c) miscellaneous by-products (rice bran and rubber seeds) which supply energy and protein.

## MOLASSES

Molasses, a by-product of the sugar industry, contains about 35-50% sugar depending on the quality. Molasses is used for production of oxalic acid, lactic acid, butanol, acetone, glycerol, yeast, mono-sodium-glutamate and the like. Molasses has been used also as an inexpensive source of energy in the ration of farm animals.

Molasses has been used mainly in animal feeding at 5-10% level. These levels are used (a) as a carrier for urea impregnation of poor quality roughages like paddy straw and bagasse, (b) as a binder for commercial pelleted feeds for the enrichment and economic feeding of livestock and (c) in the production of urea-molasses-mineral blocks to supply energy, protein and mineral to supplement rations deficient in these nutrients. Liquid urea molasses feeding has also been adopted in few countries such as Cuba.

## APPROPRIATE NUTRITIONAL PACKAGES FOR SMALL HOLDER BUFFALO PRODUCTION

Ruminants in general and buffaloes in particular in Asia are fed cellulosic wastes (straws and stovers) in variable proportions depending on the season and type of animal. These cellulosic wastes are deficient in protein, energy, minerals and vitamins which restrict their intake and digestibility. The most practical and traditional method is to feed straws/stover supplemented with green fodder available from road side grazing, grasses from community grazing grounds, tree leaves and cultivated forages (maize, cowpea, and cajanus) in different proportions depending on the availability of green fodders. Examples of rations based on the above feed resources for growth, milk production and work are presented in Tables 2, 3, and 4 respectively.

**Table 2.** Feeding systems based on straws for meat production.

1. For bull calves, 200 kg body weight (cattle and buffaloes)
   Growth Ration 550 g/day

| | Qty (Kg) | DM (Kg) | DCP (Kg) | TDN (Kg) | ME (Kcal) | Ca (g) | P (g) |
|---|---|---|---|---|---|---|---|
| *A. Paddy straw/ wheat straw/ stover (0,40, 1.4 Mcal/kg) | 4.0 | 3.6 | 0.00 | 1.6 | 5.60 | 8.0 | 2.0 |
| Concentrate[1] mixture (2.5 Mcal/kg) | 2.0 | 1.8 | 0.32 | 1.4 | 5.00 | 4.0 | 10.0 |
| Green grass (1.5, 10, 0.3 Mcal/kg) | 2.0 | 0.4 | 0.03 | 0.2 | 0.80 | 1.2 | 1.0 |
| Total | 8.0 | 5.8 | 0.35 | 3.2 | 11.4 | 13.2 | 13.0 |
| *B. Paddy straw/ wheat straw/ stover | 2 | 1.8 | 0.00 | 0.8 | 2.8 | 4.0 | 1.0 |
| Berseem/ Alfalfa/ Cowpea | | 2.5 | 0.48 | 2.0 | 6.8 | 50.0 | 9.6 |
| Concentrate mixture | | 0.9 | 0.16 | 0.7 | 2.5 | 2.0 | 5.0 |
| Total | 28 | 5.2 | 0.64 | 3.5 | 12.1 | 56.0 | 15.6 |
| **C. Paddy straw | 3 | 2.7 | 0.00 | 1.7 | 4.3 | 6.0 | 1.7 |
| Grazing | 5 | 1.0 | 0.05 | 0.7 | 2.5 | 2.9 | 2.4 |
| Ipil-ipil/ Glyricidia/ Erythrina | 10 | 2.5 | 0.30 | 1.2 | 1.0 | 4.3 | 7.0 |
| Total | 18 | 6.2 | 0.35 | 3.6 | 7.8 | 13.2 | 11.1 |

[1]Concentrate mixture consists of the locally available oil cake (copra meal, sesame cake, groundnut cake, cottonseed cake, *etc.*), bran (rice, wheat, corn *etc.*), chunnies, poultry manure, molasses, *etc.* to supply 15-16 % crude protein and 65-70% TDN.
* Most common on the organized farms
** Most common with the farmers

**Table 3.** Feeding systems based on straws for milk production.

2. For lactating cattle/buffaloes, 450 kg B.W. and 10 litres of milk.

| | Qty (Kg) | DM (Kg) | DCP (Kg) | TDN (Kg) | ME (Kcal) | Ca (g) | P (g) |
|---|---|---|---|---|---|---|---|
| A. Chaffed straw | 6.0 | 5.4 | 0.0 | 2.4 | 8.4 | 12 | 3.0 |
| Concentrate mixture (copra meal, rice bran, corn *etc.*) | 6.0 | 5.4 | 0.9 | 4.4 | 5.6 | 12 | 30 |
| Total | 12 | 10.8 | 0.9 | 6.8 | 24 | 24 | 33 |

B. Chaffed straw + 1.5kg concentrate mixture for maintenance is given. Thereafter for every 3kg milk production in cattle and 2kg of milk in buffalo, 1kg concentrate mixture is fed.

3. For dry animals, 400-500 kg B.W.

| | Qty (Kg) | DM (Kg) | DCP (Kg) | TDN (Kg) | ME (Kcal) | Ca (g) | P (g) |
|---|---|---|---|---|---|---|---|
| *A. Paddy straw/ stover/ wheat straw | 5.0 | 4.5 | 0.00 | 2.00 | 7.00 | 10.0 | 2.5 |
| Concentrate mixture | 1.5 | 1.3 | 0.22 | 1.05 | 3.05 | 3.0 | 7.5 |
| Green grass (grazing/ cut and carry) | 5.0 | 1.2 | 0.05 | 0.70 | 2.5 | 2.9 | 2.4 |
| Total | 11.5 | 7.0 | 0.27 | 3.75 | 12.55 | 15.9 | 12.4 |

[1]Concentrate mixture consists of the locally available oil cake (copra meal, sesame cake, groundnut cake, cottonseed cake, *etc.*), bran (rice, wheat, corn *etc.*), chunnies, poultry manure, molasses, *etc.* to supply 15-16 % crude protein and 65-70% TDN.

* Most common on the organized farms

** Most common with the farmers

**Table 4.** Feeding systems based on straws for draught.

4. For working animals, bullocks, cattle and buffaloes, 450 kg - medium work of 4-6 hr.

| | Qty (Kg) | DM (Kg) | DCP (Kg) | TDN (Kg) | ME (Kcal) | Ca (g) | P (g) |
|---|---|---|---|---|---|---|---|
| *A. Paddy straw/ wheat/ stover | 5.0 | 4.5 | 0.00 | 2.00 | 7.00 | 10.0 | 25 |
| Concentrate[1] mixture (GNC/ copra meal, rice/ wheat bran, molasses) | 1.0 | 0.9 | 0.15 | 0.75 | 2.70 | 2.0 | 5.0 |
| Green grass (grazing/ tethering/ cut and carry) | 10 | 2.4 | 0.10 | 1.40 | 5.00 | 5.8 | 4.8 |
| Total | 16 | 7.8 | 0.25 | 4.15 | 14.7 | 17.8 | 12.3 |
| *B. Paddy straw/ wheat straw/ stover | 5 | 4.5 | 0.00 | 2.00 | 7.0 | 10.0 | 2.5 |
| Mixed green grass available as grazing or cut and carry | 10-15 | 3.6 | 0.15 | 2.10 | 7.5 | 7.7 | 7.2 |
| Total | 13-18 | 8-1 | 0.15 | 4.10 | 14.5 | 17.7 | 9.7 |
| (Not a growth ration but commonly practised - growth rate only 120- 200g) | | | | | | | |
| **C. Paddy straw/ wheat straw | 5.0 | 4.5 | 0.00 | 2.0 | 7.0 | 10.0 | 2.0 |
| Grazing/ tethering | 10.0 | 2.4 | 0.10 | 0.4 | 5.0 | 5.8 | 4.8 |
| GNC/ bran | 0.8 | 0.7 | 0.32 | 0.6 | 2.2 | 1.0 | 4.0 |
| Total | 15.8 | 7.6 | 0.42 | 4.0 | 14.2 | 16.8 | 11.3 |

[1]Concentrate mixture consists of the locally available oil cake (copra meal, sesame cake, groundnut cake, cottonseed cake, *etc.*), bran (rice, wheat, corn *etc.*), chunnies, poultry manure, molasses, *etc.* to supply 15-16 % crude protein and 65-70% TDN.

* Most common on the organized farms

** Most common with the farmers

Straws are either fed dried and long (in many South Asian Countries) or chaffed and soaked (India). Similarly, the concentrate mixture is fed dry or soaked overnight and prepared as 'sani' by mixing the soaked straw with soaked, cooked concentrate mixture and fed to the animals (India).

With leguminous green forages (ipil ipil, cowpea) the ratio of straw to green is 3:1 for maintenance and 1:1 for yearling growing buffalo calves on dry matter basis, growing at a rate of 300-400 g/head per day. Any reduction in the availability of green forage would affect production.

For buffaloes at a production level of 4-5 litres of milk, leguminous fodders and straw are required in a ratio of 1:1 on dry matter basis. When consumed at the rate of 2.5-2.7% of the body weight, the protein and energy requirements for 4-5 litres milk would be satisfied.

## Supplementation with Concentrates

Another strategy in feeding cellulosic wastes to the buffaloes for growth and milk production is the feeding of by-product concentrates. This is commonly practised in many developing countries of Asia such as India, the Philippines, Indonesia and Pakistan. The by-product concentrates commonly available are copra meal, palm oil kernel cake, rice bran, cassava chips, rubber seed meal, *etc*.

The by-product concentrate mixtures prepared and fed at a level 1.5 kg along with crop residues will meet the requirements for maintenance.

## RECOMMENDATIONS

1. Straws (mostly rice followed by wheat) still constitute a major component of the ruminant diet in many countries of the Indian subcontinent and a few Southeast Asian countries.
2. For improving their nutritive value, supplementation with grasses/tree leaves/legumes and concentrates have become traditional feeding practices and are adopted by the farmers. The introduction of new technology of supplementing the straw with urea-molasses impregnation and/or feeding with urea-molasses blocks in the villages need extension efforts by the national governments as has been done in China.

been done in China.

3. Feeding straw mixed with green leguminous fodder/tree leaves like ipil-ipil, glyricidia, erithrina, *etc.*, in ratios of 75:25 and 50:50 are good maintenance and growth/lactation rations, respectively, which can be extended in the villages.

## References

Castillo, L.S., Roxas, D.B., Chavez, M.A., Momongan,V.G. and Ranjhan S.K. (1982) Effect of chopping and soaking rice straw on their voluntary intake by carabaos. *Second Austro-Asian Anim. Prod. Congress,* PICC, Manila, November, 1982.

Cheva-isarakul, B. and Cheva-isarakul, B. (1984) Comparison of the intake and digestibility of different crop residues by sheep, cattle and buffaloes. In: *The Utilization of Fibres Agricultural Residues as Animal Feeds*. Ed. P.T. Doyle, School of Agriculture and Forestry. University of Melbourne, Australia. pp 88-97.

Doyle, P.T., Devendra, C. and Pearce, G.R. (1985) *Rice Straw as a Feed for Ruminants*. Int. Development Programme of Australian Universities and Colleges, Canberra, Australia.

Ibrahim, M.N.M. (1981) Physical, chemical, physio - chemical and biological treatment of crop residues. In: *The Utilization of Fibrous agricultural Residues*. Ed. G.R. Pearce, Australian Government Publishing Service. Canberra. pp 53-68.

Jackson. M.G. (1977) FAO Hqs, Rome.

Khajarern, S. and Khajarern, J. (1980) *Proceeding of the International Workshop on "Studies on Feed Composition, Data Documentation and Feeding Systems in the APHCA Region,* P.86.

Ranjhan, S.K. (1977) *Animal Nutrition and Feeding Practices in India.* Vikas Publ. House, New Delhi.

Wanapat, M. Praserdsuk, S., Chanthai, S. and Sivapragon, A. (1982) Effects on rice straw Utilization of treatment with ammonia released from urea and/ or supplementation with Cassava chips. In: *The utilization of Fibrous Agricultural Residues as Animal Feeds*. Ed. P.T. Doyle, University of Melbourne Printing Service, Victoria, Australia.

# STUDY OF THE GRAZING BEHAVIOUR AND FORAGE UTILIZATION OF FREE RANGE BUFFALO HERDS

H. Peiris and A. Perera

*Veterinary Research Institute,*
*Gannoruwa, Peradeniya,*
*SRI LANKA*

**Abstract:** A study was conducted to investigate the grazing behaviour of free range buffalo herds in relation to the forage quality. Four experiments were conducted under field conditions using twenty four buffaloes, covering both dry and wet seasons of the year and their behaviour in relation to the forage quality was monitored over a twenty four hour period in each study. Results revealed that animals grazing natural forages under coconut spent significantly ($P<0.01$) more time on grazing, selecting feed and ruminating when compared with those grazing *B. milliformis* pastures which had a comparatively higher nutritive value than the natural herbage. Regression analyses indicated that there was a high correlation between the grazing time and the nitrogen content of forages ($r=0.96$) and also between the resting time of the animals and the lignin content of the forages ($r=0.92$).

**Keywords**: Buffalo, grazing behaviour, forage quality.

## INTRODUCTION

Buffalo farming has become very popular among smallholder livestock farmers in Sri Lanka due to many reasons. In general, buffaloes are less affected by parasites and certain bacterial infections such as foot rot and mastitis, and are able to withstand certain adverse environmental conditions better than cattle (Perera *et al.*, 1991). On the other hand buffaloes are identified as dual purpose animals in most parts of the Asian countries.

As far as the management system is concerned, the majority of the animals are reared under free range conditions due to lack of land availability for pasture cultivation. As a result, these animals walk rather long distances for grazing to meet their nutritional needs. In general, these animals derive their feed from natural herbage grown along the road sides, fallowed paddy fields and in coconut plantations. Therefore under the present situation, free range buffaloes are totally dependent on the natural herbage. Since supplementary feeding is not a common practice under small holder systems, it is very important to evaluate the nutritional status of the natural herbage and to study the grazing behaviour of free range buffaloes in relation to the herbage quality.

## MATERIALS AND METHODS

This experiment was carried out at the buffalo research farm, Narangalla located in the dry-intermediate zone of Sri Lanka. Two half an acre grazing fields with natural herbage and cultivated with *B. milliformis* pasture under coconut were selected for the behaviour study and these fields were fenced off separately in order to restrict the movements of the animals during the observation period. Each experimental site had natural facillities for wallowing and drinking. The *B. milliformis* pasture field was included in the present study with a view to comparing the behaviour pattern under improved pasture.

Twenty four buffaloes (12 males and 12 females), with body weights ranging from 160 - 180 kg were selected and an equal number of males and females were randomly allocated into two experimental sites. Each animal was tagged with a luminous number to enable identification in the dark during the behavioural study. The movements and the behaviour of each animal were recorded over a period of 24 hours (from 8 am to 8 am on the following day). The two experiments were carried out concurrently and four such behavioural studies (two in the dry season and two in the wet season) were carried out during two consecutive years.

A separate record sheet was maintained for each animal and their behaviour and movements such as time spent on grazing, walking, ruminating etc. were recorded against time. Herbage samples from both experimental sites were taken before and after each grazing period using a 1x1 metre quadrat for determination of the botanical and chemical composition. A representative sub-sample was taken before and after grazing and separated for identification of different species. A representative sub-sample was taken and dried at 70° C for 48 hours for determination of dry matter (DM). The dried material was then ground to pass through a 2 mm screen and used for determination of crude protein (CP) according to AOAC (1960), fibre (Van Soest, 1963) and mineral analysis (wet digestion). Data were analysed according to 2x2 factorial design discribed by Steel and Torrie, (1960).

## RESULTS AND DISCUSSION

Table 1 shows the behavioural pattern of buffaloes during the wet and dry season under free range conditions. Results revealed that animals spent significantly ($P<0.01$) more time on grazing during the wet season when compared with the dry season. However, animals on *B. milliformis* pasture spent more time grazing and less time resting and ruminating than those grazing native pastures.

Tables 2 and 3 show the nutritive value of herbage sampled during the dry and wet seasons of the year. Results indicate that the native pastures contain significantly higher lignin and lower calcium and phosphorus contents when compared with *B. milliformis* pasture.

The results suggest that animals spent more time ruminating when the herbage contains a higher lignin fraction. This shows that these animals have to spend more time breaking down the forage fraction in the rumen and as a result animals have to utilize more energy for digestion of low quality natural forages than the *B. milliformis* pastures. On the other hand, animals on natural pastures spent more time on walking and less time on grazing suggesting that these animals were more selective when grazing natural pastures when compared to *B. milliformis* pastures. This selectivity is probably associated with the botanical composition of the herbage grazed by the animals (Table 4 and 5), since the percentage of weeds present in natural pastures was significantly higher than that in *B. milliformis*.

**Table 1**. Behaviour of free range buffaloes during the wet and dry seasons over a 24-hour period.

| Forage | % Time spent on | | | | Number of | | |
|---|---|---|---|---|---|---|---|
| | Grazing | Walking | Resting | Ruminating *(% resting) | Drinks | UR | DF |
| **Wet Season** | | | | | | | |
| Native grass | 29.8 | 3.7 | 63.4 | 42.8 | 3 | 2 | 3 |
| Brachiaria grass | 61.1 | 1.1 | 37.1 | 14.1 | 4 | 2 | 4 |
| **Dry Season** | | | | | | | |
| Native grass | 10.6 | 1.2 | 88.2 | 78.8 | 6 | 1 | 2 |
| Brachiaria grass | 34.2 | 0.5 | 65.3 | 57.8 | 5 | 1 | 2 |
| LSD (0.01) | 10.3 | 0.8 | 9.6 | 5.9 | 0.5 | - | - |

UR - urination; DF - defeacation
*the value expressed as a percentage of resting time.

**Table 2**. The organic matter (OM), crude protein (CP), acid detergent fibre(ADF) and total ash content of herbage grazed.

| | OM % | CP % | ADF % | Lignin % | Total Ash |
|---|---|---|---|---|---|
| **Wet Season** | | | | | |
| Native grass | 93.2 | 4.7 | 43.8 | 7.8 | 7.2 |
| Brachiaria grass | 92.2 | 6.9 | 41.6 | 5.6 | 9.8 |
| **Dry Season** | | | | | |
| Native grass | 73.1 | 2.9 | 56.8 | 9.2 | 64.5 |
| Brachiaria grass | 78.9 | 4.1 | 55.7 | 8.9 | 55.3 |
| LSD (0.01) | 3.7 | 0.9 | 5.6 | 1.9 | 1.1 |

**Table 3.** The mineral composition of herbage grazed by buffaloes.

| | Ca (mg/g) | P (mg/g) | Cu (ppm/g) | Zn (ppm/g) | Mg (ppm/g) |
|---|---|---|---|---|---|
| **Wet Season** | | | | | |
| Native grass | 1.49 | 0.98 | 5.92 | 46.92 | 1.99 |
| Brachiaria grass | 2.74 | 1.17 | 8.35 | 44.93 | 2.49 |
| **Dry Season** | | | | | |
| Native grass | 1.18 | 0.81 | 5.61 | 45.71 | 1.81 |
| Brachiaria grass | 2.58 | 1.10 | 8.30 | 43.12 | 2.19 |
| LSD (0.01) | 0.91 | 0.41 | 1.21 | 3.41 | 0.2 |

**Table 4.** Botanical composition of native grazing field.

| Species | % Composition in terms of DM | |
|---|---|---|
| | Wet season | Dry season |
| **Grass** | **60** | **45** |
| Axonopus affinis | 54 | 30 |
| Pogonatherum crinitum | 03 | 07 |
| Chrysopogom aciculatus | 03 | 08 |
| **Weeds** | **40** | **55** |
| Urena lobotes | 12 | 15 |
| Bewila | 10 | 15 |
| Desmodium triflorum | 04 | 05 |
| Mimosa pudica | 04 | 05 |
| Anamirta cocculus | 04 | 05 |
| Phyllanslius debilis | 04 | 05 |
| Desmodium heterophyllum | 02 | 05 |

**Table 5**. Botanical composition of *B. milliformis* grazing field.

| Species | % Composition in tems of DM | |
|---|---|---|
| | Wet season | Dry seson |
| **B. milliformis** | 85 | 70 |
| **Weeds** | 15 | 30 |
| Alysicarpus vaginalis | 02 | 06 |
| Desmodium triflorum | 04 | 06 |
| Mimosa pudica | 09 | 18 |

The behaviour pattern of free range buffaloes has a high correlation with the quality of herbage. The regression equation related to time spent on grazing and the nitrogen content of the herbage shows the following association (r=0.96).

$$Y = 0.012\,X + 0.012$$

Where Y is the time spent on grazing and
X is the nitrogen content of herbage.

A similar high correlation (r=0.92) was observed between the resting time of free range animals and the lignin content of the herbage grazed by the animals. The regression equation relating to the above parameters is given below.

$$Y = 0.072\,X + 3.29$$

Where Y is the resting time and
X is the lignin content of the herbage.

The present study suggests that there is a positive relationship between the quality of pasture and the behaviour of animals under free range conditions. Furthermore, this relationship could be used in the evaluation of forage species under field condition.

In conclusion, the present study provides some information on the relationship between animal behaviour and the quality of the forages eaten by free range buffaloes reared in coconut plantations. Since this management system is very common among small holder livestock farmers in the coconut triangle of Sri Lanka, the animals reared under these conditions do not get sufficient good quality feeds to meet their nutritional needs. They spend more time selecting feed which obviously is an inefficient way of utilizing their energy. Therefore one could not expect to observe any better performances from animals reared under such management conditions. The introduction of a suitable supplementary feeding system for buffaloes reared under free range would be an advantage.

## Acknowledgements

The authors wish to thank Dr.M.C.L.De Alwis, Additional Director of the Department of Animal Production & Health, Peradeniya and Dr. H. Abeygunawardena of Faculty of Veterinary Medicine for providing the facilities at the Buffalo Research Farm at Narangalla and the SAREC/NARESA Buffalo Research Programme for financial support.

## References

AOAC (1960) *Official Methods of Analysis*, 10th edition, ed. W. Horwitz. Association of Official Agricultural Chemists, Washington D.C., pp.832.

Steel, R.G.D. and Torrie, J.H. (1960) *Principles and Procedures of Statistics*. McGraw - Hill Book Company, New York. pp 481.

Perera, B.M.A.O., Ranawana, S.S.E., Fernando, S.T., Jalatge, E., Kuruwita, V.Y., De Alwis, M.C.L. and Weilgama, D.J. (1991) *The Sri Lanka Water Buffalo*. Science Education Series. No 31, NARESA, Colombo.

Van Soest, P.J. (1963) Use of detergents in the analysis of fibrous feeds II. *J. Assoc. Agric. Chem.* **46,** 829.

# THE NUTRITIONAL STATUS OF INDIGENOUS BUFFALOES WITH RESPECT TO SELECTED MICRONUTRIENTS

S.S.E. Ranawana, J. Dharmawardana, A.W.A.S. Abeysekara, A.A.J. Rajaratne, G.D.J.K. Gunaratne and E.M.C. Ekanayake

*Veterinary Research Institute,*
*Gannoruwa, Peradeniya,*
*SRI LANKA*

**Abstract:** An earlier survey of the mineral status of buffaloes on smallholdings in Sri Lanka based on analysis of blood taken in a single visit revealed inadequacies of several essential minerals. The present follow-up investigation was designed to study these minerals in more depth and to include the fat-soluble vitamins A and E which, according to other available evidence, are likely candidates for deficiencies. Ancillary information was also collected so as to relate the status to indices of production and health.

Five locations representative of the main buffalo production systems, namely, Rambukkana, Kuliyapitiya/Pannala, Mihintale, Tisssamaharama and Thanamalwila were visited and up to 10 farmers were selected from each area. From each herd, one cow and its calf were chosen for the study. The visits were planned to coincide with a major change in the annual calendar of buffalo management; for example, in Rambukkana, they were visited just after paddy was planted, after harvest and just before the cultivation season so as to complete an annual cycle. Apart from taking blood samples, observations were made at intervals on other ancillary information, particularly in relation to feed availability.

Altogether 33 field visits were made over a period of 24 months, of which 18 were in dry seasons and 15 during wet. Feed availability was found to be limited at 7 visits but adequate at other times. A total of 365 blood samples were collected, of which 186 were from calves and the balance from cows. Most of the blood samples have been analysed for PCV, haemoglobin and for the selenium enzyme, glutathione peroxidase. Analysis of plasma for the different minerals and vitamins is not yet complete.

*Nutritional status of selected micronutrients*

Measurements of chest girth showed that in the dry zone locations of Mihintale and Tissamaharama/Thanamalwila, the animals lost weight in August/September. Haematocrit values, an index of general body condition, were also low in cows at Tissamaharama during this time. Values for PCV were below normal in many of the calves at Mihintale. Activity levels of the enzyme glutathione peroxidase were inadequate in only 9 out of 159 measured; of these 7 were calves.

Examination of the animals revealed abnormalities in the skin and coat in 60% of the calves and 40% of the cows. The lesions on the calves consisted mainly of excessive hair growth or dry, scaly skin. In cows, abnormalities manifested themselves mainly as a dry and scaly skin, often with a rash. A significant number also had depigmentation of hair, particularly on the face. Once the plasma analyses are completed, the status for the different micronutrients will be related to these findings.

**Keywords**: Buffalo, nutrition, micronutrients

## Session III- Nutrition and Feeding

## Panel Discussion

**Q:** C. Chantalakhana - The term strategies means not only nutritional. I think you have already presented excellent data on nutritional improvement of fibrous feeds. In my experience, when one talks of strategies in nutrition other dimensions like collection of fibrous residues, transport and storage could be included. I would like to hear your views, Dr. Thu on strategies of utilization of byproducts. Generally, when there is a greater demand for one byproduct like rice straw, the cost may increase and it would not be economical to use such products. Then there is also competition for its use in other industries, for example, the use of rice straw in the production of paper pulps etc. Thailand exports dried sugar cane tops to Japan and the cost in Thailand is high.

**A:** N.V. Thu - Rice straw is collected, chopped and stored by the farmers for feeding of animals. However, if it is left in the field, it will go waste. It is very difficult to collect and store good rice straw during rainy season. In Vietnam, studies have been done on methods of storage.

**Q:** T.N. Jayatilake - One of your transparencies shows that the overall utilization of fibrous feed residues by farmers is disappointing. Can you elaborate on this?

**A:** N.V. Thu - In Vietnam there in ample rice straw available, but the farmers let it go waste and as a result there is even pollution of canals. The farmers generally keep buffaloes for work in Vietnam. They do not benefit from feeding urea treated rice straw. We did research on treated straw for a long time but this is not being used in the field.

**Q:** C. Devendra - Sri Lanka has a good track record of nutrition research on utilization of fibrous residues. Has the time come to shift the emphasis away from the laboratory to the farm? Ideally, the emphasis should now be 80% research at farm level and 20% at the laboratory level. Are you going to continue to pursue fundamental research which really will not cater to the needs of poor farmers? The second question is, if there are problems at farm level, I think we must address these. Is there lack of understanding on such problems or are there some faults in project design? I like to get some feedback.

**A:** A.N.F. Perera - We have been doing a lot of fundamental research. The university has limited resources to go out and work with farmers. Extension is not a function of the University. We collaborate with Department of Animal Production and Health and National Livestock Development Board who have a strong linkage with the farmers.

**Q:** H. Abeygunawardena - I would like to know what are the feeding systems easily applicable to the farmers? What is the optimum level of feeding rice straw and *ipil-ipil* for different genotypes and different production systems? Can these be incorporated into feeding packages that can be extended to a poor farmer?

**A:** S.K. Ranjhan - I entirely agree with Dr. Devendra because we have conducted so many research studies. However, we have not conducted on-farm research so as to get participation of the farmers. If the farmer participates in the trials, then he will readily accept those technologies. Otherwise the technologies that we generate in the institutes or the universities may not be accepted because the farmer has not participated in those trials.

**Q:** D.K. Singh - In India 39,000 on-farm trials have been conducted. The farmers recognize that there is a marginal benefit. As long as trials are conducted and funded by someone else, farmers are ready to accept on-farm trials. But when we checked subsequently after the trials have ended, the farmers have discontinued to use the technologies.

**Q:** K.K. Pathirana - It would have been more useful if you had looked at the changes in body weight. We are indiscriminately using the terms 'urea treatment' and 'urea supplementation' and actually what you have done in your study is supplementation. In urea treatment a high concentration of urea is used to affect a change in the chemical nature of the roughage.

**A:** R. Sivakanesan - In fact, one of the tables present a decline in body weight. In both the control and urea supplemented groups we observed a decline in body weight, post-calving when compared to the pre-calving situation. The supplemented group had a lesser decline in body weight even though it was not statistically significant.

**Q:** I.D. Silva - Could not the normal BUN level indicate the normal kidney function? What is the normal BUN level in buffalo?

**A:** E.R.K. Perera - Normal levels are not known for buffaloes but these levels are comparable with those established for cattle.

**Q:** C. Devendra - Is Sri Lanka ready to address the problem of feeding domestic ruminants throughout the year? How can we shift the emphasis to on-farm trials so that we could adopt the research findings to the farms.

**A:** S.K. Ranjhan - I agree with Dr.Devendra. First, we have to study what are the feeding systems in Sri Lanka with regard to different agro-climatic zones. What are the constraints and how with the known technologies could one intervene to obtain optimum results with minimum inputs? Then conduct on-farm trials and see the results where the farmer is participating. If the farmer gets any benefit out of it then he will go for it. We have as yet not developed the appropriate technologies to suit different agro-ecological zones. It is high time that we think of our future approach towards nutrition research.

**Q:** B.M.A.O. Perera - I wonder whether a nutritionist could comment on this?

**A:** M.N.M. Ibrahim - I think what we have been adopting was a lab to land technology. During the last four-five years, there has been resource based studies in Sri Lanka but in my opinion what is lacking is that we have not looked into what is needed in the field. On the other hand, we have ample technologies and what we should do is to comprehensively review the data that are available. If we identify the needs of the farmers, then we will have tailor-made kits for farmers. However, we should test these on farms.

**Comment:** J.A. de S. Siriwardene - There is a communication gap between the researcher, extension worker and the farmer. We know that farmers need to feed during the dry season and farmers are demanding information on how to feed their animals during the dry season. We have developed very good technologies. I think there is something missing in the linkage between the researcher and the farmer. With regard to straw feeding, straw is available in the fields which are located at a distance from the homestead. During the harvesting season farmers are busy and they are not able to spend time in collecting, transporting or storing straw.

**Comment:** Sujatha Premaratne - Our buffalo farmers keep buffaloes as a source of supplementary income. They do not use treated straw as it is expensive and they are not prepared to spend time on such activities. Urea supplementation is possible at 2% level. What we should be doing is to use fodders that are freely available even in the dry zone as feed supplements.

**Comment:** A.N.F. Perera - I have a different opinion. No matter how good the research and extension services are, if the farmer is not ready to accept it, even with most appropriate technologies available, it will not be possible to convince the farmer. We know that some dairy farmers ask for help but some are not willing to accept it, even if you go to their doorstep.

**Comment:** H. Abeygunawardena - It is clear from the discussion that we have failed to transmit new technologies to Sri Lankan farmers. Probably this could be attributed to a wrong approach. In the light of this experience, in our program what we have been doing is to work through the existing extension network and provide continuing education with the hope of effectively disseminating our extension message to the farmers. I would like to invite all the nutritionists present here to work with us to carry out on-farm trials in the Mahaweli area during next three years.

**Comment:** N.H. Shah - The linkage between researcher and extension worker is not there in most of the developing countries. In Pakistan we are trying to establish proper linkages between the researcher, extension worker and the farmer. We have to recognize that there are different types of farmers. Subsistence farmers are very often reluctant to adopt new technologies.

**Comment:** C. Devendra - Clearly a methodological approach that will distinctly deliver technologies to farmers is missing. Somehow there is a failure to address how research can be driven over time, that can be translated and delivered. Need for solidarity of different disciplines is required and this could be addressed by adopting agricultural system based education.

**Comment:** N.H. Shah - Basic research is required to understand the buffalo properly and this type of research should be carried out under controlled conditions.

**Q:** N.H. Shah - India is the leading country in upgrading buffalo. Is India doing anything regarding conservation of germ-plasm?

**A:** S.K. Ranjhan - We are a part of the FAO project that is aimed at preservation of indigenous breeds.

**Q:** C. Chantalakhana - In China farmers adopted new technology of treated straw widely. Use of treated straw increased from 3 thousand to 11 million metric tonnes over the years. Could you give reasons for this fast adoption of technology?

**A:** S.K. Ranjhan - I think it was started under a UNDP project and farmers have found it to be useful and also being a socialist country it would have been little easier to transfer the technology.

**Q:** S.S.E. Ranawana - In India about 60% of forage fed to ruminants consist of crop residues. Is the figure right? Is that based on energy or weight?

**A:** S.K. Ranjhan - It is based on production and consumption. However, these figures have been arrived at by doing random surveys. The quantities that are fed to animals depend on the physiological state of the animal also.

**Q:** V. Momongen - Do the animals graze more during night time?

**A:** H. Peiris: Yes, but I have not indicated it here.

**Q:** N.U.Horadagoda - Did you look at the intestinal parasites in the calves you described in your paper?

**A:** S.S.E. Ranawana - We did not look at the intestinal parasites. However, most of these animals had been dewormed and also these signs were seen in 50-60% of the animals.

**Q:** R. Sivakanesan - On what basis did you conclude that the levels were deficient especially in the absence of norms?

**A:** S.S.E. Ranawana - We used the values published for cattle. As we had a large sample, we could even use the survey to work out normal values.

NA - 143 - 289

# THE IMPORTANCE OF REPRODUCTIVE EFFICIENCY IN SUCCESSFUL BUFFALO DAIRY FARMING

S. N. H. Shah

*Center of Animal Biotechnology*
*P.O.Box No.121, Veterinary Research Institute, Peshawar, PAKISTAN*

**Abstract:** The buffalo is an important dairy animal in third world countries. In India, Pakistan and Egypt, the buffalo is the principal dairy animal producing a high quantity of milk with a high fat percentage when compared to Zebu cows. The economy of milk production depends largely on the reproductive efficiency of the concerned animals. The financial loss caused by sub-optimal reproductive performance of dairy buffalo herds is an important issue for large and small farmers. Education of the farmers has a favourable effect ($P<0.05$) on reproductive management. A herd health program focussed on fertility control was initiated in several buffalo herds and resulted in the improvement of buffalo dairy farming.

**Keywords:** Buffalo, reproduction, herd fertility, small-farms, Pakistan.

## THE BUFFALO AS A DAIRY ANIMAL

The buffalo is a very important dairy animal in Pakistan. More than half the rural population depends at least in part on the buffalo for their livelihood. Milk is a highly valued and commonly appreciated food item of the Pakistani diet. This includes fluid milk as well as traditional milk products such as ghee (clarified butter fat) cheese, lassi and khoa (a coagulated milk product which forms the basis of indigenous sweets). In Asia, the increase in dairy production over the 10 year period from 1978 to 1988 has been around 50%, while in the rest of the world this was only 15%. About 80% of Asia's milk is produced in India and Pakistan, where the increase during this period was almost 60% (Pronk, 1992). The buffalo population is less than that of cattle in India and

Pakistan, but they contribute 52% and 71% of the total milk production in the two countries respectively (Table 1).

**Table 1.** Buffalo milk as a percentage of total milk production in India and Pakistan.

| Country | Population (millions) | | Buffalo milk (%) | Reference |
|---|---|---|---|---|
| | Cattle | Buffalo | | |
| India | 200 | 75 | 52% | Pronk, 1992 |
| Pakistan | 16.8 | 13.5 | 71% | Shah, 1991 |

In Pakistan buffaloes are kept only for milk production. Peri-urban commercial milk production has developed rapidly in response to growing urban milk demand, and also because of difficulties of collecting and transporting fluid milk from rural production areas. The trend in milk production during the past several years has been strongly in favour of buffaloes, mainly because of the consumer preference for buffalo milk.

In Pakistan about 71% of the total milk is produced by buffaloes. Seventy percent of the total 13 million buffalo population is owned by landless and small farmers with land holdings ranging from 0.5 to 5.5 hectares. The average number of buffaloes per household is 2 to 3 animals. Some commercial buffalo production units of 20 to 200 heads have been established, and many others are under consideration. At the start most of them have a high percentage of female buffaloes in production, but with time this percentage declines due to reproductive failures, resulting in a considerable share of the total feed being consumed by dry buffaloes and their progeny.

The average milk yield of first three lactations of registered and recorded Nili-Ravi dairy buffaloes at four Government livestock farms ranged from 1602 to 4353 kg, while the yield of rural buffaloes which have been registered, identified and production recorded under the program titled "Studies required for development of progeny tested bulls to improve milk production in Punjab" ranged from 1618 to 4535 kg. The average milk yield of 1243 rural buffaloes recorded in 17 villages was found to be 2061 kg (Shah, 1991), and that of buffalo dairy farms under the Herd Health Program of the Center of Animal Biotechnology was 7.5 litres per buffalo per day (Shah, 1994).

Nili-Ravi and Kundi are the two important breeds of dairy buffalo in Pakistan. About four decades back, the Nili and Ravi were considered two separate breeds as described by Indian Imperial Council of Agricultural Research (1941) and they derived their names from the Blue (Nili) water of the Sutlej River and the Valley of the Ravi River, respectively. However due to frequent movements of men and animals both breeds lost their distinctive attributes and experts decided to group Nili and Ravi strains as one breed under the common name Nili-Ravi.

The Kundi breed derives its name from the shape of its horns. A typical specimen of this breed would possess horns in the shape of a curve, thick at the base and pointed at the tip forming a well-shaped ring called "Kundi". It was first briefly described as Kundi by Ware (1942). Ulvi (1927) described these animals as Murrah-Sindhi buffaloes. They closely resemble the Murrah and are considered a geographical type of the same.

## REPRODUCTION

It is noted that average calving intervals are usually high (from 471 to 585 days) in many countries where buffaloes are reared (Shah, 1990). The losses due to prolonged calving intervals are summarized as follows: loss of milk, excessive additional feed costs, delay in replacement of stock, disinterest of the farmer in open and dry buffaloes resulting in further deterioration of the animals, culling of potentially productive animals, and the fact that the farmer is compelled to sell the animal at a low price.

Shah *et al.* (1989) observed that on average a buffalo is able to complete 4.8 lactations. However, as only 17.2% of the buffaloes have calving intervals of less than 400 days, only this limited number of buffaloes can complete 6 lactations during their lifetime. It is a tremendous loss to the herd's economy. Shah *et al.* (1991) observed that in Nili-Ravi buffaloes the annual milk yield decreased by an average of 2 kg per day when the calving interval increased from 365 to 635 days. They recommended that initiation of fertility control programs should be a high priority by the Veterinary services of the country.

A survey was conducted in 100 livestock farms of cattle and buffalo in 13 villages of Dera Ismial Khan district to investigate the status of farmer education, land utilization pattern, herd size and composition, nutrition of the animals, reproductive management and fertility problems (Shah, 1995). There were 366 cattle and 477 buffaloes in these farms. Among the cattle population, 60% were of local non-descriptive type, while among the buffaloes Nili-Ravi was the predominant breed. Majority of the farmers (61%) used their own land for fodder cultivation ranging from 0.5 to 6.25 acres.

Farmer education had a favourable effect ($P<0.05$) on reproductive/health management. The following criteria were used for assessing the reproductive management practised by farmers in the project area; heat detection methods; time of detection; correct mating time, preference for restricted mating in suitable seasons, culling and herd replacement practices; and keeping of reproductive records.

Based on the above information the management was divided into the following three categories: Good (70-100% successful adaptation of the above parameters); Fair (50-70% successful adaptation); and Poor (below 50% successful adaptation).

Good, fair and poor categories of reproductive management were found in 22, 45, and 33% of the farmers, respectively. These management practices had significant effects on the incidence of anoestrus in both cows ($P<0.05$) and buffaloes ($P<0.001$).

## HERD HEALTH PROGRAMME (HHP)

A Herd Health Programme (HHP) which is unique in Pakistan has been initiated in the North-West Frontier Province by the Center of Animal Biotechnology (CAB), Veterinary Research Institute, Peshawar in March 1992. The programme has been designed to provide Veterinary and advisory services to the registered farmers for solving problems in health and reproduction.

The programme is mainly focussed on fertility control. In the HHP more emphasis is placed on preventive medicine such as regular vaccination, fertility control, nutrition and management. Non-pregnant animals are identified by examination *per rectum* and treated to get them pregnant within 60 days of the previous parturition. Fertility management is supported by the milk progesterone EIA test. All the animals are ear tagged and record cards are maintained at CAB with information on the reproduction, health and production of each milking animal. A chart for reproduction and health data and a register for milk production printed in Urdu language are supplied to the farmers for keeping records of their animals.

In the first year of the programme, fertility status of the registered farms was judged by the following formula recommended by Shah *et al.* (1989):

$$F.S = \frac{(FSCR)-(PC-160)}{NC}$$

| | | | |
|---|---|---|---|
| Where: | F.S. | = | Fertility status |
| | FSCR | = | First service conception rate |
| | NC | = | No. of services per conception |
| | PC | = | Parturition to conception interval |

Normally values ranged from 0 to 95. Farms having the ideal level of reproductive efficiency will have a value of 95. We observed that only three farms had ideal values. Overall average F.S. of all registered herds was 30. This indicates that reproductive status of the buffalo dairy farms registered under HHP was very low. The data is under analysis but preliminary results indicated that after adoption of the HHP average F.S. increased to 50 and average calving interval of registered farms decreased from 517 to 480 days.

## References

Indian Imperial Council of Agriculture Research (ICAR) (1941) A brief survey of some of the important breeds of cattle in India. Part II. Prize winners at the first All-India Cattle Show, Delhi. *Miscellaneous Bulletin* No. 24.

Pronk, F. (1992) Trends in livestock dairy production and policies in Asia. In: *International Workshop on Livestock Production in Rural Development,* IAC Wageningen, The Netherlands. pp 82-93.

Shah, N.H. (1995) A Study on fertility problems in small village herds of cattle and buffaloes in D.I. Khan District. *MSc Thesis,* Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, NWFP Agriculture University, Peshawar, Pakistan.

Shah, S.K. (1991) *Buffaloes of Pakistan.* Pakistan Agriculture Research Council, Islamabad, Pakistan.

Shah, S.N.H. (1994) *Annual Progress Report of Center of Animal Biotechnology,* P.O.Box No.121, Peshawar, Pakistan.

Shah, S.N.H., Dijkhuizen, A.A., Willemse, A.H. and van de Wiel, D.F.M. (1991) Economic aspects of reproductive failure in dairy buffaloes of Pakistan. *Preventive Veterinary Medicine* **11,** 147-155.

Shah, S.N.H., Willemse, A.H., van de Wiel, D.F.M. and Engel, B. (1989) Influence of season and parity on several reproductive parameters of Nili-Ravi buffaloes. *Animal Reproduction Science* **21,** 177-190.

Shah, S.N.H. (1990) Prolonged calving intervals in the buffalo. *PhD Thesis,* Utrecht University, The Netherlands.

Ulvi, A.M. (1927) Murrah Buffaloes in Sind. *Bombay Department of Agriculture Bulletin* **134,** 6.

Ware, F. (1942) A further survey of some important breeds of cattle and buffaloes in India. Delhi Imperial Council of Agriculture Research, *Miscellaneous Bulletin* **54,** 16.

NA - 143 - 297

# A COMPARATIVE STUDY OF REPRODUCTION AND PRODUCTIVE CHARACTERISTICS OF INDIGENOUS SWAMP AND EXOTIC RIVER BUFFALOES IN SRI LANKA

H. Abeygunawardena, W.D. Abayawansa and B.M.A.O. Perera

*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, SRI LANKA*

**Abstract**: The multipurpose, indigenous swamp buffalo (Lanka buffalo) forms an integral component of rural crop-livestock production systems. With a view to increasing the dairy characteristics of the indigenous buffalo population, exotic river buffaloes were imported into the country from time to time. They are maintained in state livestock farms and the progeny, primarily males, are issued to small holders for upgrading their indigenous animals. A retrospective study was undertaken by analyzing farm records to assess the reproductive and productive performance of the three main exotic river types, Surti (S), Murrah (M), and Nili-Ravi (N) with those of the Lanka (L) buffalo. The mean values recorded for S, M, N and L respectively were: birth weight (kg) 27.1, 27.6, 30.1 and 17.9; prepubertal growth rate (kg/d) 0.25, 0.46, 0.52 and 0.16; age at first calving (months) 51.8, 55.0, 52.1 and 44.9 and calving interval (days) 482, 510, 491 and 507. S, M and L showed a clear seasonal calving pattern with 69%, 68% and 68% of calvings respectively occurring during the period September to January. The mean lactation yield (litres) and lactation length (days) respectively were: 1003 and 267 in S; 1052 and 287 in M; and 1761 and 309 in N. In conclusion, all three exotic river type buffaloes maintained in large farms performed below the expected levels for the genotype in milk production as well as in reproduction. Of the three exotic breeds, N showed higher ($P<0.05$) milk production; the reproductive performance did not differ among breeds. This poor performance may be due to the sub-optimal management conditions, augmented by seasonal shortages of pasture and fodder. The indigenous buffaloes appear to reach sexual maturity early, suggesting that they attain optimum body weight at an earlier age than the river types.

**Keywords:** Reproductive performance, milk yield, Lanka buffalo, river buffaloes.

## INTRODUCTION

Domestic water buffaloes (*Bubalus bubalis*) are widely distributed in Asia. They are multipurpose agricultural animals and serve as a major source of milk, meat and farm power (Cockrill, 1974). In Sri Lanka, the buffalo population is estimated to be about 0.8 million and over 99% of them are of the indigenous (Lanka) type (de Silva *et al.*, 1985). They are reared primarily by subsistence farmers, managed extensively with minimum inputs (Kumaratilake and Buvanendran, 1979; de Silva *et al.*, 1985) and account for nearly one fourth of the milk collected by the organized milk collecting network (Ministry of Agricultural Development and Research, 1991/92).

In order to improve the productivity of Lanka buffaloes, whose milk production level is only about 384 litres per lactation (Kumaratilake and Buvanendran, 1979), three breeds of exotic river type buffaloes were imported to the country from time to time for cross-breeding programmes (Ministry of Rural Industrial Development, 1986/87). These animals were kept in state owned livestock farms and maintained as nucleus herds.

The objectives of this study were to compare the calving pattern, calving interval, birth weight, prepubertal growth rate, age at first calving, calving to first service period, lactation yield and lactation length of Surti, Murrah, Nili-Ravi and Lanka buffaloes maintained on institutional farms in Sri Lanka.

## MATERIALS AND METHODS

Four livestock farms (Melsiripura, Nikaweratiya and Narangalla in the Kurunegala district and Kotaliya in the Polonnaruwa district) maintained under the National Livestock Development Board (NLDB) were visited. The dates of calvings and services, birth weights, body weights of calves during the prepubertal period, lactation yields and lactation lengths for each calving were collected for Surti (1980 to 1993) from Melsiripura, for Murrah (1975 to 1993) from Kotaliya, for Nili-Ravi (1991 to 1993) from Nikaweratiya and for Lanka buffaloes (1980 to 1993) from Narangalla. All the Surti, Murrah and Lanka buffaloes were locally bred, while the Nili-Ravi were imported. All animals were grazed during the day with stud bulls present in the herd, and paddocked at night. All exotic breeds were routinely milked while the indigenous animals were under free suckling.

Data analysis was done using the computer software package, Quattro Pro. The simple arithmetic mean, standard deviation and percentiles for variables were calculated. Comparison of means for variables among the four buffalo breeds was performed using ANOVA.

## RESULTS

*Calving pattern:* Surti, Murrah and Lanka buffaloes showed a clear seasonal variation in calvings with the majority occurring from September to January (69%, 68% and 68.5% respectively). The calving pattern for Lanka buffaloes and the rainfall at the location are shown in Figure 1. Calvings commenced with onset of North-East monsoon rains in September and peak calvings occurred during the period November-December. Nili-Ravi buffaloes did not show a clear seasonal pattern in calvings but showed year to year variation; in 1991 the majority of calvings occurred from July to September, in 1992 from February to March, and in 1993 throughout the year.

*Calving interval:* The average calving intervals are given in Table 1. They ranged from 318 to 1604 days for Surti, 308 to 1604 days for Murrah, 311 to 1061 days for Nili-Ravi and 323 to 1117 days for Lanka buffaloes. There was no difference between the breeds ($P>0.05$). A significant proportion of calving intervals were over 500 days for all breeds (Table 2).

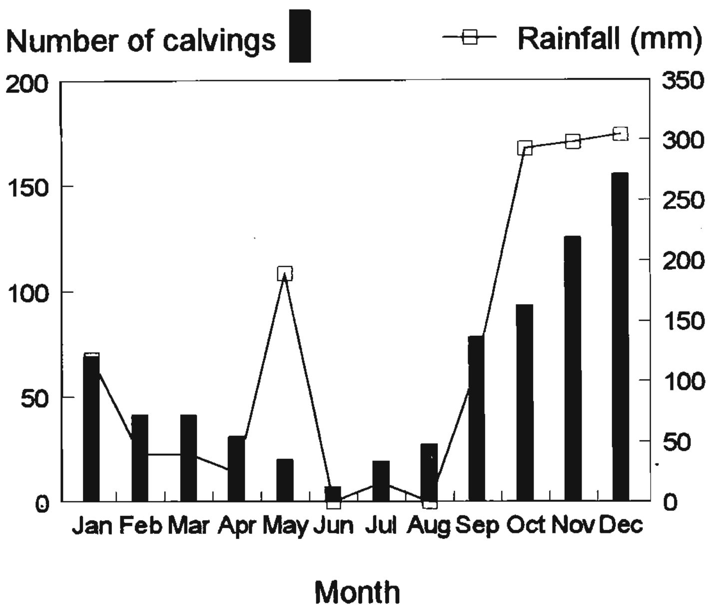



**Figure 1.** Monthly calving pattern in Lanka buffaloes and rainfall at the Narangalla Buffalo Research Station.

**Table 1.** Estimates of reproductive and productive parameters of Surti, Murrah, Nili-Ravi and Lanka buffaloes.

| Parameter | Surti | Murrah | Nili-Ravi | Lanka |
|---|---|---|---|---|
| Birth weight (kg) | 27.1±4.0 | 27.6±2.7 | 30.1±5.1 | 17.9±2.8 |
| Prepubertal growth rate (kg/day) | 0.25±0.13 | 0.46±0.74 | 0.52±0.34 | 0.16±0.18 |
| Age at first calving (months) | 51.8±11.2 | 55.0±8.0 | 52.1±9.6 | 44.9±5.1 |
| Calving to first service (days) | 149±79.4 | 177±83.2 | 137±90.7 | – |
| Calving interval (days) | 482±157.7 | 510±183.6 | 491±126.0 | 507±158 |
| Lactation yield (litres) | 1003±391 | 1052±496 | 1761±581 | – |
| Lactation length (days) | 267±72.0 | 287±92.0 | 309±97.3 | – |

**Table 2.** Distribution of calving intervals (CI) in Surti, Murrah, Nili-Ravi and Lanka buffaloes.

| C I | Surti | | Murrah | | Nili-Ravi | | Lanka | |
|---|---|---|---|---|---|---|---|---|
| (Days) | No. | % | No. | % | No. | % | No. | % |
| 300-365 | 88 | 20.3 | 122 | 18.6 | 24 | 29.1 | 16 | 8.6 |
| 366-400 | 73 | 16.8 | 110 | 16.8 | 14 | 11.1 | 37 | 19.8 |
| 401-465 | 89 | 20.5 | 102 | 15.6 | 20 | 15.9 | 48 | 25.7 |
| 466-500 | 35 | 8.1 | 50 | 7.6 | 16 | 12.7 | 11 | 5.9 |
| >500 | 149 | 34.3 | 272 | 41.5 | 52 | 41.3 | 75 | 40.1 |

*Birth weight:* The average birth weights of different breeds are presented in Table 1. The weights were different among the breeds ($P<0.05$) with the highest weight recorded in Nili-Ravi buffaloes and the lowest in Lanka buffaloes. No difference ($P>0.05$) was found between the birth weights of Murrah and Surti calves.

*Prepubertal growth rate:* The average growth rates are given in Table 1. They were different ($P<0.05$) among the breeds; the highest growth rate was recorded in Nili-Ravi and the lowest in Lanka buffaloes. In all breeds, some fluctuations in the growth rate were seen during the prepubertal period (Table 3).

**Table 3.** Prepubertal growth rate (kg/day) at different ages of Surti, Murrah, Nili-Ravi and Lanka buffaloes.

| Age (months) | Surti | Murrah | Nili-Ravi | Lanka |
|---|---|---|---|---|
| 1 - 6 | -- | 0.37 | 0.43 | 0.22 |
| 6 - 12 | 0.39 | 0.15 | 0.42 | 0.13 |
| 12 - 18 | 0.30 | -- | 0.41 | 0.11 |
| 18 - 24 | 0.33 | 0.86 | 0.26 | 0.15 |
| 24 - 30 | 0.16 | 0.36 | 0.47 | 0.37 |
| 30 - 36 | 0.19 | 0.27 | -- | 0.11 |
| 36 - 42 | 0.25 | 0.35 | -- | 0.32 |

*Age at first calving:* The average ages at first calving for different breeds are given in Table 1. There was no difference ($P>0.05$) in the age at first calving among Surti, Murrah and Nili-Ravi breeds but it was lower ($P<0.05$) in Lanka buffaloes. The majority of Surti, Murrah and Nili-Ravi buffaloes calved first when they were over 48 months old but the majority of Lanka buffaloes calved before 48 months (Table 4). The coefficient of variation for the age at first calving was lowest in Lanka buffaloes compared to that for other breeds.

**Table 4.** Distribution of age at first calving in Surti, Murrah, Nili-Ravi and Lanka buffaloes.

| Age | Surti | | Murrah | | Nili-Ravi | | Lanka | |
|---|---|---|---|---|---|---|---|---|
| (months) | No. | % | No. | % | No. | % | No. | % |
| < 36 | 8 | 4.9 | 3 | 1.9 | 0 | 0 | 5 | 12.8 |
| 36 - 48 | 58 | 35.4 | 34 | 21.0 | 7 | 41.2 | 30 | 76.9 |
| 48 - 60 | 67 | 40.8 | 70 | 43.2 | 7 | 41.2 | 4 | 10.3 |
| > 60 | 31 | 18.9 | 55 | 33.9 | 3 | 17.6 | 0 | 0.0 |

*Calving to first service period:* The average intervals from calving to first service are given in Table 1. Majority (43.8%) of Nili-Ravi animals were served first before 100 days after calving, while the majority of Surti and Murrah animals were served first at 100 to 200 days (Table 5). Dates of services were not available for Lanka buffaloes.

**Table 5.** Distribution of intervals from calving to first service in Surti, Murrah and Nili-Ravi buffaloes.

| Interval | Surti | | Murrah | | Nili-Ravi | |
|---|---|---|---|---|---|---|
| (days) | No. | % | No. | % | No. | % |
| <100 | 11 | 26.2 | 3 | 13.6 | 39 | 43.8 |
| 100 -200 | 22 | 52.4 | 11 | 50.0 | 27 | 30.3 |
| 200 -300 | 6 | 14.3 | 7 | 31.8 | 17 | 19.1 |
| >300 | 3 | 7.1 | 1 | 4.5 | 6 | 6.7 |

**Table 6.** Percentage distribution of lactation yield (litres) in Surti, Murrah and Nili-Ravi buffaloes.

| Yield (litres) | Surti (%) | Murrah (%) | Nili-Ravi (%) |
|---|---|---|---|
| <100 | 2.8 | 3.7 | 2.9 |
| 100 - 500 | 10.1 | 10.8 | 1.5 |
| 500 - 1000 | 36.2 | 35.5 | 7.6 |
| 1000 - 2000 | 50.5 | 47.4 | 57.3 |
| 2000 - 3000 | 0.5 | 2.2 | 29.0 |
| >3000 | -- | 0.5 | 2.3 |

*Lactation yield and length:* The lactation yields for Surti, Murrah and Nili-Ravi buffaloes are given in Table 6. In Nili-Ravi buffaloes, 29% of lactation yields were above 2000 litres per lactation, whereas in Murrah and Surti, the percentage of lactation yields above this level were 2.2% and 0.5%, respectively. There were variations in the lactation yield within a breed as well as among the breeds and the variation was high in Nili-Ravi.

As shown in Table 1, the lactation length was different ($P<0.05$) among Surti, Murrah and Nili-Ravi buffaloes. The longest was in Nili-Ravi and the shortest in Surti. The data for lactation yield and length were not available for Lanka buffaloes in this study.

## DISCUSSION

A seasonal pattern of calving has been reported in several previous studies in Sri Lanka in Murrah (Jalatge and Buvanendran, 1971; Lundström *et al.*, 1982), Surti (Goonawardena *et al.*, 1983) and Lanka buffaloes (Kumaratilake and Buvanendran, 1979; de Silva *et al.*, 1985). In this study too, all except Nili-Ravi buffaloes, showed strong seasonality in calvings. In all locations except at Nikaweratiya, calvings commenced with the onset of North-East monsoon rains and peak calvings occurred in the months of November and December. The previous studies have implicated feed availability, through its effects on onset of post-partum ovarian activity and subsequent conception, as the primary factor responsible for this seasonality (Perera *et al.*, 1984). The pasture and fodder availability is strongly related to the seasonal rainfall in all locations of the present study where the precipitation occurs through the North-East monsoon rains during September to December each year. Although seasonal rainfall occurs at Nikaweratiya, the lack of clear seasonality in Nili-Ravi buffaloes may be due to several reasons. They were imported animals and were therefore given optimum management conditions which ensured year around adequate levels of nutrition. Furthermore, they were imported as pregnant animals and would therefore be out of synchrony with the local seasonal trends. It would be interesting to study whether, with time, this herd will eventually synchronize its conceptions, and consequently its calvings, with the seasonal rainfall.

The birth weight and growth rate of farm animals are traits with high heritability and repeatability (Gianola and Tyler, 1973). This implies that the values of these traits depend primarily on genetic composition and selection intensity. In the current study, it is seen that the lowest birth weight and growth rate were in Lanka buffaloes which have been maintained over the years as closed herds without selection for reproduction and production traits. The birth weights of Surti and Murrah animals were lower than values reported for animals maintained in the Indian subcontinent (Johari and Bhat, 1979; Dobson and Kamonpatana, 1986). This could well be due to lack of introduction of genetic material to the nucleus herd periodically and also due to lack of selection for these traits. The birth weight and growth rate of Nili-Ravi buffaloes were highest among the exotic breeds. This could be due to the fact that they were recently introduced from Pakistan, with a degree of selection imposed prior to importation.

The growth rate of Murrah buffaloes in this study is also much lower than the values reported by Tilakaratne *et al.* (1979) for this breed in Sri Lanka under experimental conditions with supplementary feeding. This suggests that the low growth rate is due to limitations of nutrient supply along with lack of selection for this trait.

The ages at first calving in all the breeds, except in Lanka buffaloes, were well over four years. Further, the values observed for exotic buffaloes maintained in state farms were longer than the values reported for the same breeds in the Indian subcontinent (Johari and Bhat, 1979; Raut *et al.*, 1974; Reddy and Mishra, 1980). This suggests that the exotic buffaloes maintained in state farms show delayed sexual maturity and hence later first calving. It has been shown that the age at puberty in buffaloes is related to the birth weight and growth rate (McCool *et al.*, 1987) and it commonly occurs as the animal reaches two thirds of its mature body weight. As the Lanka animals are low in mature body weight they reach the critical body weight earlier than the exotic breeds. Hence the delay in puberty is conceivably due to poor growth rate among the exotic buffaloes, and partly due to poor management conditions in nucleus herds.

The average calving interval among the four breeds was well over 500 days and this was highly variable within the breed rather than between the breeds. The calving interval is a function of the calving to first oestrus interval, the number of services for conception and the gestation length. The gestation lengths of exotic and Lanka buffaloes are well over 10 months (Perera *et al.*, 1984) and sometimes as long as 330 days. As such, they would not be able to maintain the ideal calving interval of one calf per year. Further, as their postpartum ovarian activity is significantly influenced by seasonal availability of pasture and fodder every year, a proportion of buffalo cows calving down during the latter part of the calving season experience delay in the commencement of postpartum ovarian activity. This may be the reason for long calving intervals in all four breeds. Repeat breeding and other reproductive disorders are unlikely to be important factors, as previous studies have shown that buffaloes served by stud bulls conceived mostly at the first postpartum oestrus.

The length of lactation of the three exotic breeds studied here are in agreement with an earlier report for Murrah buffaloes in Sri Lanka (Rajamahendran *et al.*, 1980-81). The total lactation yield was greatest in Nili-Ravi, followed by Murrah, with Surti being the lowest. These values were well below those reported for the same breeds in the Indian subcontinent (Gudi, 1971; Bhat *et al.*, 1980; Gill, 1980; Patel, 1980). The milk yield and the lactation length also are traits with high heritability and repeatability and hence the low lactation yields are probably due to poor genetic potential of the exotic breeds resulting from lack of selection for this trait. The total lactation yield reported for Lanka buffalo was about 364 kg (Wijeratne, 1962) and the low yield is conceivably due to poor genetic potential of this breed.

In conclusion, this study showed that the exotic buffalo breeds, namely Murrah, Surti and Nili-Ravi maintained under state farm conditions perform below that expected for each genotype. In comparison, the Lanka buffalo is an extremely poor producer. Reproductive performance of all four breeds is low and characterized by delayed age at first calving, long calving intervals and seasonal calving pattern.

## Acknowledgements

We thank the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for providing financial support for this study, and the International Atomic Energy Agency, Vienna, for technical assistance. We also thank the Managers and staff of the livestock farms at Kotaliya, Melsiripura, Nikaweratiya and Narangalla, and Mr. Janaka Herath and Miss. Ayesha Senarath Bandara for their assistance.

## References

Bhat, P.N., Bhat, P.P., Khan, B.V., Goswamy, U.B. and Singh, B. (1980) Animal genetic resources in India. *Proc. SABRAO Workshop*, Tropical Agricultural Research Centre, Japan. p 134.

Cockrill, R.W. (1974) The working buffalo. In: *The Husbandry and Health of the Domestic Buffalo,* Food and Agriculture Organization, Rome. pp 313-328.

de Silva, L.N.A., Perera, B.M.A.O., Tilakaratne, N. and Edqvist, L-E (1985) Production systems and reproductive performance of indigenous buffaloes in Sri Lanka. *Monograph,* Swedish University of Agricultural Sciences, Uppsala, Sweden.

Dobson, H. and Kamonapatana, M. (1986) A review of female cattle reproduction with special reference to a comparison between buffalo cows and zebu. *J. Reprod. Fert.* **77,** 1-36.

Gianola, D. and Tyler, W.J. (1973) Influence of birth weight and gestation period of Holstein-Friesian cattle. *J. Dairy Sci.* **57,** 235-240.

Gill, S.S. (1980) Buffalo improvement research. Its impact on socio economic conditions of milk producers. In: *Summer Institute on Buffalo Management Systems.* Eds. R.N. Pal and N.S.R. Sastry. Haryana Agricultural University, Hissar. pp 18-24

Goonawardena, L.A., Tilakaratne, N. and Jalatge, E.F.A. (1983) A study of calving frequency in the water buffalo. In: *Current Development and Problems in Swamp Buffalo Production.* Ed. H. Shimizu, University of Tsukuba, Japan. pp 141-145.

Gudi, A.K. (1971) Some observations on productive and reproductive characteristics of Meshana buffaloes at Aarey milk colony, Bombay. *Indian vet. J.* **48,** 210-211.

Jalatge, E.F.A. and Buvanendran, V. (1971) Studies on some production traits of Murrah buffaloes in Ceylon. I. Characters associated with reproduction. *Trop. Anim. Hlth. Prod.* **3,** 114-124.

Johari, D.C. and Bhat, P.N. (1979) Effect of genetic and non-genetic factors on reproductive traits in Indian buffaloes. *Indian J. Anim. Sci.* **49,** 1-6.

Kumaratilake, W.L.J.S. and Buvanendran, V. (1979) A survey of production characteristics of indigenous buffaloes in Sri Lanka. *Ceylon vet. J.* **27,** 10-13.

Lundström, K., Abeygunawardena, H., de Silva, L.N.A. and Perera, B.M.A.O. (1982) Environment influence on calving interval and estimates of its repeatability in the Murrah buffalo in Sri Lanka. *Anim. Reprod. Sci.* **5,** 99-109.

McCool, C.J., Townsend, M.P., Wolfe, S.G. and Entwistle, K.M. (1987) Endocrinological studies on pregnancy, postpartum anoestrus and seasonal variation of ovarian activity in the Australian swamp buffalo cow. *Buffalo J.* **2,** 181-194.

Ministry of Agricultural Development and Research (1991/92) *Sri Lanka Livestock Statistics.* Ministry of Agricultural Development and Research, Sri Lanka.

Ministry of Rural Industrial Development (1986/87) *Sri Lanka Livestock Statistics.* Ministry of Rural Industrial Development, Sri Lanka.

Patel, R.K. (1980) Economics of dairying with buffaloes as dairy animals. In: *Summer Institute on Buffalo Management Systems*. Eds. R.N. Pal and N.S.R. Sastry. Haryana Agricultural University, Hissar. pp 29-82.

Perera, B.M.A.O., de Silva, L.N.A. and Karunaratne, A.M. (1984) Studies on reproductive endocrinology and factors influencing fertility in dairy and draught buffaloes in Sri Lanka. In: *Proceedings of the Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division*. Manila, Philippines.

Rajamahendran, R., Ravindran, V. and Kumaragunaratnam, S. (1980-81) A study on the performance of Murrah buffaloes at Polonnaruwa in Sri Lanka. *J. Nat. Agric. Soc. Ceylon* **17-18,** 123-128.

Raut, K.C., Singh, S. and Choudhary, M.L. (1974) Some factors influencing the production of buffalo milk. *Indian J. Anim. Sci.* **44,** 713-716.

Reddy, K.M. and Mishra, R.R. (1980) Effect of age at first calving on lactation components and calving distribution in Murrah buffalo. *Indian J. Dairy Sci.* **33,** 3.

Tilakaratne, N., Matsukawa, T., Buvanendran, V. and Thangarajah, P. (1979) Growth, feed conversion and carcass characteristics of cattle and buffaloes feed grass and concentrates. *Ceylon vet. J.* **24,** 9-12.

Wijeratne, M.V.S. (1962) Some of the production statistics of the Ceylon buffalo. *Ceylon vet. J.* **10,** 48-49.

# SEASONAL VARIATIONS IN SEMINAL AND TESTICULAR CHARACTERISTICS IN BUFFALO BULLS

D. Gunarajasingam[1], H. Abeygunawardena[1], V.Y. Kuruwita[1], E.R.K. Perera[2] and B.M.A.O. Perera[1]

[1]*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya,*
[2] *Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** Studies were conducted in buffalo bulls (*Bubalus bubalis*) to determine whether seasonal factors influence testicular, endocrine and seminal characteristics. In study 1, three Nili-Ravi bulls maintained as semen donors at the Central Artificial Insemination Centre at Kundasale were used. Semen samples (n=132) were collected using an artificial vagina at weekly intervals for twelve months and evaluated for volume, colour, density, mass activity, motility and concentration. Monthly collections (n=36) were used to study live and dead status (nigrosin-eosin stain), sperm morphology (unstained or stained with modified William's stain) and cells other than sperms (haematoxylin-eosin). The monthly average volume of semen ranged from 1·1 ± 0·1 to 7·0 ± 2·2 ml/ejaculate (mean 2·9 ± 1·9) and concentration from 607 ± 186 to 2050 ± 353 million/ml (mean 1389 ± 567). The colour of semen varied from cloudy to creamy and density from 2D to 4D. Mass activity and motility of sperms ranged from 2+ to 4+ and 70 to 90% respectively. Percentage dead sperms ranged from 2 to 24 (mean 8·2 ± 5). Percentage of head, mid-piece and tail abnormalities were 3·3 ± 1·8, 3·2 ± 1·6 and 7·0 ± 4 respectively. Desquamated epithelial and spermatogonial line cells were also found (1 to 2 cells per microscopic field). Motility percentage showed significant monthly variation ($P<0.05$) with three peaks during January (88%), May (89%) and August (90%). No significant variations were observed in the other characteristics.

In study 2, seasonal changes in testicular morphology were examined in indigenous buffalo bulls during hot (August) and wet (December) seasons. Testes were removed at 3 - 4 years of age (n = 3 per season), tissue samples taken from three locations, preserved in Bouin's solution, processed and stained with H-E stain. The mean diameters of seminiferous tubules (n = 30 per testis) during the months of August and December respectively were 177·2 ± 15·2 μm and 193·7 ± 14·2 μm, and this difference was significant ($P<0{\cdot}01$). However, no differences due to season were detected in the relative frequencies of the seminiferous epithelial cycle.

**Keywords:** Buffalo bulls, semen, testicular characteristics, seasonal changes

## INTRODUCTION

Artificial breeding has been adopted as a primary tool for improving productivity of indigenous cattle and buffaloes in Sri Lanka. The success of the artificial breeding programme depends on many factors and among them the quality of semen used plays a very important role. The quality of semen is assessed conventionally by examining the colour, density and mass activity, motility, morphology and the live and dead percentages of sperms. These characteristics are assessed using a number of techniques, which have been developed over the past fifty years and adopted or modified by various laboratories. The techniques have their own merits and demerits (Galloway and Norman, 1980; Rajamahendran and Dharmasena, 1984). In the present study, several standard as well as modified techniques were used to study the monthly variation in the semen quality of Nili-Ravi buffalo bulls reared in the mid-country region of Sri Lanka. An attempt was also made to determine whether seasonal changes occur in the seminiferous tubules of the testis in indigenous buffaloes.

## MATERIALS AND METHODS

### Study I: Monthly Variations in Seminal Characteristics

*Animals*: Nili-Ravi buffalo bulls (n = 3) maintained as semen donors at the Central Artificial Insemination Centre, Kundasale in the mid-country intermediate zone of Sri Lanka were used. The ages of the bulls ranged from 5 to 6 years and their weights ranged from 680 - 800 kg. They were stall-fed with grass, fodder and concentrates. Splashing of water over the body was done once a day for 15 minutes as a substitute for wallowing. At this location, the rainfall ranged from 24.2 mm (August) to 325.1 mm (December), the temperature from 18.7°C (February) to 31.0°C (May) and the relative humidity from 77 to 91%.

*Semen collection:* Semen samples were collected between 0800 - 0900 hours at weekly intervals over a twelve month period using artificial vagina (AV). The AV was assembled and maintained at 42°C until used for collection. Prior to semen collection each bull was allowed three false mounts on a restrained male dummy. At the fourth mount semen was collected into the graduated collection tube attached to the AV which was covered with an insulator to prevent cold shock. Semen samples were maintained at 37°C in a water bath during the initial evaluation. The ejaculate obtained from each bull during the fourth week of each month was used for detailed evaluation.

*Semen evaluation:* The volume of semen collected was directly read from the graduated collection tube and colour and density were visually judged. The colour was graded from transparent to thick creamy and density on a scale from D to 4D. Mass activity was assessed by placing a drop of semen on a warm slide (37°C) soon after collection and examining at x100 magnification using a microscope fitted with a warm stage, set at 37°C. The activity was graded on an ascending scale from 1+ to 4+. Percentage motility was assessed after diluting 1-2 drops of fresh semen in 2 ml of skimmed milk diluent. A drop of diluted semen was placed on a warm slide at 37°C, covered with a coverslip and examined under a phase contrast microscope. Sperm concentration was estimated by adding 25 µl semen to 4975 µl of sodium bicarbonate buffer (1:200 dilution), charging this solution in a Neubauer' type counting chamber, and counting the sperms under phase contrast microscope at x400 magnification. The concentration was expressed in millions per ml of semen.

For assessing live and dead sperms, a drop of fresh semen was placed on a warm glass slide (37°C), 4 to 5 drops of nigrosin-eosin (N/E) stain were added and incubated at the same temperature for 2 minutes. A thin smear was made, allowed to dry at room temperature and two hundred sperms were counted. Live and dead sperms were expressed as percentages. To detect head abnormalities modified William's stain was prepared by adding 10% carbol fuchsin solution and 10% eosin-B solution in 10% ethyl alcohol in a 1:1 ratio. A thin smear was made from a drop of fresh semen, dried, immersed in 100% ethyl alcohol for 2 minutes and allowed to dry in air. The slide was placed in William's stain for 8-10 minutes and then washed in distilled water and allowed to dry at room temperature. Two hundred sperms were counted and the head abnormalities were expressed as a percentage. For assessing acrosomal, mid-piece and tail abnormalities one to two drops of fresh semen was added to one ml of formal saline, a drop placed on a glass slide, covered with a cover slip and examined under a phase contrast microscope at x1000 magnification. Two hundred sperms were counted and the different abnormalities were expressed as percentages. Cells other than sperms were counted after placing a drop of fresh semen on a glass slide, making a thick smear, fixing the dried smear in graded concentrations of ethyl alcohol, staining with Ehrlich's haematoxylin for 20 minutes and counterstaining with 1% eosin Y. These were expressed as cells per microscopic field.

## Study II: Monthly Variations in Testicular Characteristics

*Animals and samples:* Six indigenous (Lanka) buffalo bulls aged 3 - 4 years raised at the Narangalla Buffalo Research Station located in the low-country intermediate-zone were used in this study. During daytime they were allowed to graze on improved pasture in a coconut plantation and allowed to wallow in a pool of water. The bulls were randomly selected and castrated in two batches of three each in August and December, which corresponds to the driest and wettest seasons at this farm. The testes and epididymides were collected and trimmed. Three samples of 1.5 x 1.0 x 0.5 cm were obtained from the upper, central and lower parts of each testis and placed in Bouin's fluid for twelve hours. They were then stored in 70% ethyl alcohol until processed for histology. Using standard techniques, 5 µm sections were made and stained with haematoxylin and eosin-Y stains. Ten slides were prepared from each sample.

*Seminiferous tubular diameter (STD):* Measurements were made using an ocular graticule with a linear scale consisting of 100 divisions. Using a calibrating scale it was determined that each division of the graticule corresponded to 6.50 µm when used at a magnification of x100. Two diameters were measured for each cross-section at right angles to each other, of 10 intact seminiferous tubules, from each of the three locations of the testis.

*Seminiferous epithelial cycle:* One hundred seminiferous tubules were examined per testis from each of the three locations (upper = 33, central = 34 and lower = 33) at x1000 magnification. Each cross section of the tubule was classified into its appropriate stage based on the cellular contents, according to the criteria described by Ortavant *et al.* (1977).

*Statistical analysis:* The Minitab computer software package was used for statistical analysis.

## RESULTS AND DISCUSSION

### Study 1

The seminal characteristics of Nili-Ravi buffalo bulls are summarized in Table 1. The mean ejaculate volume of 2.97 ml is in agreement with that reported by Kumar *et al.* (1993), but lower than that reported by Dixit *et al.* (1984) for Murrah bulls. In comparison, the volume of the ejaculate in cattle is higher, ranging from 1 to 15 ml

(Galloway and Norman, 1980). In the present study the overall monthly mean volumes of semen showed three peaks, during December (3.62 ± 3.20 ml), June (3.43 ± 2.06 ml) and July (3.44 ± 2.11 ml). However, the monthly variation was found to be not significant (P>0.05) and is in agreement with that observed by Dixit *et al.* (1984). On the other hand, Gill *et al.* (1974) noted monthly variations in semen volume.

**Table 1.** Semen characteristics observed in Nili-Ravi buffalo bulls.

| Parameters | Mean ± SD | Range |
|---|---|---|
| Semen volume (ml) | 2.97 ± 1.96 | 0.5 - 9.0 |
| Colour | --- | Cloudy - Creamy |
| Density | --- | 2D - 4D |
| Mass activity | --- | 2+ - 4+ |
| Sperm concentration ($10^6$/ml) | 1389 ± 567 | 440 - 2915 |
| Sperm motility (%) | 86.3 ± 5.3 | 70 - 90 |
| Dead sperm (%) | 8.2 ± 5.0 | 2 - 24 |

The mean sperm concentration of Nili-Ravi bulls in this study was 1389 ± 567 million per ml semen (Table 1) and was lower than the values reported by Rajamahendran and Dharmasena (1984) for local, Surti and Murrah buffaloes, but greater than the values reported by Kumar *et al.* (1993), Dixit *et al.* (1984) and Zafar *et al.* (1988) in Nili-Ravi and Murrah buffaloes. The monthly mean concentrations showed two peaks during April (1581 ± 443) and July (1529 ± 642), but the differences were not significant (P>0.05).

The motility of sperms ranged from 70 to 90% with a mean of 86.3% (Table 1). This is higher than the mean values of 74.6% reported by Dixit *et al.* (1984) and 77.8% reported by Kumar *et al.* (1993) in Murrah bulls. This could be due to the fact that in the present study motility was assessed after addition of a semen extender, resulting in the dilution of motility inhibiting factors present in the seminal plasma (Bass *et al.*, 1983). The monthly mean values for sperm motility were lower during December (81%), March (78%) and September (85%) than during other months (86 to 90%), and the differences were significant ($P<0.05$). Similar variations have been reported by Gill *et al.* (1974).

The colour and the density of the semen varied from cloudy to creamy and 2D to 4D respectively. The mass activity ranged from 2+ to 4+. As these values are subjective, attempts were not made to compare with other reports.

During the experimental period, the dead sperms ranged from 2 to 24% with a mean of $8.2 \pm 5.03$ (Table 1). Rajamahendran and Dharmasena (1984) observed 14 and 17% dead sperms in Murrah and Surti buffalo bulls respectively, while Dixit *et al.* (1984) observed 10%, which is in close agreement to the mean value obtained in the present study. Most studies reported a seasonal variation in the percentage of dead sperms (Gill *et al.*, 1974; Rajamahendran and Dharmasena, 1984; Dixit *et al.*, 1984), although no such variation was observed in the present study.

The microscopic characteristics of buffalo spermatozoa are similar to that of cattle except in the shape of the sperm head (Mukherjee, 1966), which is shorter and broader than that of cattle. The morphological abnormalities are summarized in Table 2. The mean percentages of head, mid-piece and tail abnormalities were 3.31, 3.16 and 7.00 respectively. The comparable values observed in Nili-Ravi bulls by Ahmad *et al.* (1987) were 6.5, 4.5 and 3.4 and by Saeed *et al.* (1989) were 7.82, 0.34 and 5.58 respectively. In the present study the head abnormalities ranged from 1.7% (August) to 6.3% (September) and appeared in three peaks during June (5.7%), September (6.3%) and November (4.3%) while mid-piece abnormalities occurred in three peaks during March (4%), August (4%) and November (4%), and the tail abnormalities in four peaks during February (8%), June (9%), August (9%) and November (8%). However the monthly variations observed in these abnormalities were not significant ($P>0.05$). There is insufficient published information on this breed to determine the upper and lower limits of various abnormalities to be used as a guideline in semen evaluation.

**Table 2.** Sperm abnormalities found in the semen of Nili-Ravi bulls:

| Location | Abnormality | % Mean ± S.D |
|---|---|---|
| Head | Pear shaped | 0.11 ± 0.16 |
| | Narrow at base | 0.42 ± 0.49 |
| | Abnormal contour | 0.22 ± 0.22 |
| | Undeveloped | 0.67 ± 0.45 |
| | Narrow | 0.25 ± 0.49 |
| | Loose | 1.44 ± 0.54 |
| | Abaxial | 0.11 ± 0.22 |
| | Overall | 3.21 ± 1.83 |
| Mid-piece | Proximal cyto. droplets | 2.61 ± 0.60 |
| | Distal cyto. droplets | 0.25 ± 0.32 |
| | Abnormal mid piece | 0.31 ± 0.30 |
| | Overall | 3.16 ± 1.61 |
| Tail | Single bent | 3.58 ± 1.06 |
| | Double bent | 0.75 ± 1.02 |
| | Coiled under head | 2.22 ± 0.83 |
| | Others | 0.44 ± 0.61 |
| | Overall | 7.00 ± 4.07 |

Single and polynucleated spermatogonial cells and epithelial cells were also observed but were very few in number (1-2 cells per microscopic field), which indicates that the testicular functions were in a healthy state in these animals.

## Study 2

*Seminiferous tubular diameter (STD):* The mean and range for STD in indigenous buffalo bulls during the months of August and December are given in Table 3. Statistical analysis indicated that the mean difference of STD between the two periods was significant ($P<0.01$). The overall mean for indigenous bulls in the present study was lower than that reported for swamp buffalo bulls in Malaysia, where the STD showed a decreasing trend from 258.6 to 226.5 μm with increasing age from 26 to 32 months (Nordin *et al.*, 1986; Vidyadaran *et al.*, 1989). The mean values of 225 μm reported for rams (Courot and Ortavant, 1981) are also higher than in the present study. In horses an increase in STD occurs during the breeding season (Squires and Thompson, 1983).

**Table 3.** Diameter of seminiferous tubules during August and December in indigenous buffalo bulls.

| Month | Animal | Seminiferous tubular diameters (μm) | | |
|---|---|---|---|---|
| | | Minimum | Maximum | Mean±SD |
| August | A | 150.42 | 228.90 | 178.67 ± 16.94 |
| | B | 143.88 | 210.26 | 173.90 ± 15.88 |
| | C | 153.96 | 209.28 | 176.58 ± 12.04 |
| | Overall | | | 177.22 ± 15.20 |
| December | X | 163.50 | 215.83 | 191.00 ± 13.89 |
| | Y | 163.50 | 215.83 | 194.73 ± 16.47 |
| | Z | 163.50 | 222.36 | 195.40 ± 13.92 |
| | Overall | | | 193.71 ± 6.36 |

*Seminiferous epithelial cycle:* The frequencies observed in the testicular sections in the seminiferous epithelial cycle during August and December are given in Table 4. They were similar to the eight stages reported by Ortavant *et al.* (1977) in cattle, rams, boars and horses. McCool *et al.* (1989) identified eight stages in buffalo bulls, and also further subdivided stages I, II, IV, VIII into sub-stages. No attempt was made in the present study to identify these sub-stages. Most stages, including VIII of the cycle which consists of mature spermatids, had similar frequencies during August and December.

**Table 4.** Frequencies of stages of the seminiferous epithelial cycle during the months of August and December in indigenous buffalo bulls.

| Month | Animal | Stage of cycle (% frequency) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII |
| August | A | 25 | 19 | 21 | 5 | 2 | 4 | 9 | 15 |
| | B | 23 | 22 | 22 | 5 | 2 | 2 | 10 | 14 |
| | C | 26 | 19 | 20 | 6 | 1 | 10 | 8 | 10 |
| | **Mean** | 25 | 20 | 21 | 5 | 2 | 5 | 9 | 13 |
| December | X | 25 | 21 | 18 | 4 | 1 | 8 | 10 | 13 |
| | Y | 26 | 14 | 17 | 7 | 3 | 7 | 12 | 14 |
| | Z | 25 | 17 | 22 | 7 | 4 | 5 | 5 | 15 |
| | **Mean** | 25 | 17 | 19 | 6 | 3 | 7 | 9 | 14 |

Semen of Nili-Ravi buffaloes is extensively used in cross-breeding programmes for upgrading indigenous stock in Sri Lanka. This study has provided data on normal parameters to evaluate semen samples collected under local conditions. The values reported are in close agreement with reports available for this breed in other Asian countries. Some seasonal changes observed in semen in other countries were not detected in the present study, possibly due to the mild nature of climatic changes during the year at the location of Study 1 in the mid-country of Sri Lanka.

In Study 2, differences were apparent between the months of August and December in the STD, but not in the frequencies of the stages of the seminiferous epithelial cycle. Since the two studies were conducted at different locations, it is not possible to correlate these findings directly. However, STD is known to be influenced by a variety of factors such as breed, age, endocrine and nutritional status as well as environmental factors, and could be a sensitive index of testicular function. It would therefore be interesting to study the relationship between STD, the frequencies of the stages of the seminiferous epithelial cycle, and the seminal characteristics in more detail at the same location.

## Acknowledgements

We thank the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for providing financial support for this study, and the International Atomic Energy Agency, Vienna, for technical assistance. We also thank the Department of Animal Production and Health for providing facilities at the Central Artificial Insemination Station (CAIS), Kundasale, and Dr. W.W. Abeygunawardena and his staff at CAIS for their co-operation. The assistance of Mr. Rex Fernando at the Buffalo Research Farm, Narangalla, is gratefully acknowledged.

## References

Ahmad, M., Latif, M. and Ahmad, M. (1987) Morphological abnormalities of spermatozoa of Nili-Ravi buffalo. *Buffalo J.* **2,** 153-160.

Bass, J.A., Molan, P.C. and Shannon, P. (1983) Factors in seminal plasma of bulls that affect the viability and motility of spermatozoa. *J. Reprod. Fert.* **68,** 275-280.

Courot, M. and Ortavant, R. (1981) Endocrine control of spermatogenesis in the ram. *J. Reprod. Fert.* **30,** 47-60.

Dixit, N.K., Agarwal, S.P., Agarwal, V.K. and Dwaraknath, P.K. (1984) Seasonal variation in serum levels of thyroid hormones and their relation with seminal quality and libido in buffalo bulls. *Theriogenology* **22,** 497-507.

Galloway, D.B. and Norman, J.R. (1980) Laboratory examination of semen as a diagnostic aid. *Proc. 9th Int. Congr. Anim. Reprod. and Artif. Insem.* Madrid, Spain, **IV,** 714-717.

Gill, R.S., Gangwar, P.C. and Takkar, O.P. (1974) Seminal attributes in buffalo bulls as affected by different seasons. *Indian J. Anim. Sci.* **44,** 415-418.

Kumar, S., Sahni, K.L. and Bistha, G.S. (1993) Cytomorphological characteristics of motile and static semen of buffalo bulls. *Buffalo J.* **2,** 117-127.

McCool, C.J., Entwistle, K.W. and Townsend, M.P. (1989) The cycle of the seminiferous epithelium in the Australian Swamp buffalo. *Theriogenology* **31,** 399-417.

Mukherjee, D.P. (1966) Study of the quantitative characteristics of live and dead spermatozoa of bulls, buffalo bulls, rams and goats. *World Rev. Anim. Reprod.* **1,** 74 - 82.

Nordin, W., Azmi, T.I. and Bongso, T.A. (1986) Light and electron microscope studies on testicular development in swamp buffalo bulls (*Bubalus bubalis*). *Proc. Buffalo Seminar.* Bangkok, Thailand. pp. 287-293.

Ortavant, R., Courot, M. and Hochereau de Reviers, M.T. (1977) Spermatogenesis in Domestic Mammals. In: *Reproduction in Domestic Animals*. Eds. Cole, H.H. and Cupps, P.T. pp. 203-225.

Rajamahendran, R. and Dharmasena, L. (1984) Preservation of buffalo semen in citric acid, whey and tris buffer extender at -196°C. *J. Nat. Sci. Council Sri Lanka* **12,** 45-51.

Saeed, A., Chaudhry, I.H., Khan, I.H. and Khan, N.U. (1989) Studies on morphology of buffalo bull semen of different age groups. *Buffalo J.* **1,** 99-102.

Squires, E.L. and Thompson Jr., D.L. (1983) Spermatogenesis, testicular composition and the concentration of testosterone in equine testis as influenced by season. *J. Reprod. Fert.* **20,** 451-457.

Vidyadaran, M.K., Azmi, T.I. and Nordin, W. (1989) *Seminar on Buffalo Genotypes for Small Farms in Asia,* Serdang, Malaysia. pp. 201-214.

Zafar, A.H., Ahmad, N. and Shah, S.K. (1988) Effects of seasonal variation on semen production of Nili-Ravi bulls. *Buffalo J.* **1,** 61-68.

NA - 143 - 321

# EFFECTS OF DIFFERENT SUCKLING REGIMES ON POSTPARTUM FERTILITY OF BUFFALO COWS AND GROWTH AND MORTALITY OF BUFFALO CALVES

H. Abeygunawardena, V.Y. Kuruwita and B.M.A.O. Perera

*Department of Veterinary Clinical Studies, Faculty of Veterinary, Medicine and Animal Science, University of Peradeniya, Peradeniya, SRI LANKA*

**Abstract:** Low annual calving rates in indigenous buffaloes have been shown to be due to prolonged postpartum anoestrus, caused primarily by seasonal fluctuation of quantity and quality of pasture and fodder and by free suckling of calves. This study was conducted to determine the effects of different suckling regimes on postpartum fertility of buffalo cows and growth and mortality of calves. Six suckling managements were tested: free (*ad-libitum*) suckling (FS); FS and weaning by day 45 (FS+W45); FS and weaning by day 60 (FS+W60); FS and weaning by day 90-120 (FS+W90); once a day suckling from day 7 (OS); and twice a day suckling from day 7 (TS). Different combinations of these were tested during 4 successive calving seasons. Calves were weighed at monthly intervals. Conception date was calculated by subtracting the mean gestation length of 301 days from the actual calving date. The pregnancy rates at 90, 120 and 150 days were: 19, 20 and 23% in FS (n=74); 54, 73 and 73% in FS+W45 (n=11); 6, 31 and 37% in FS+W60 (n=16); 5, 17 and 27% in FS+W90 (n=18); 38, 59 and 59% in OS (n=29); and 31, 45 and 59% in TS (n=22). The calf mortality in these groups were 5.4, 55, 31, 33, 6.8 and 4.5% respectively. Free suckling resulted in the lowest pregnancy rates. Weaning after 60 days did not improve postpartum fertility. Calf mortality was high with all three weaning ages tested. Suckling once or twice a day improved fertility and, although the former resulted in slower growth rates during the first 3 months, there was no difference from 4 months onwards. In conclusion, the results show that once a day as well as twice a day suckling can be adopted as suitable regimes to improve postpartum fertility in indigenous buffaloes.

**Keywords:** Buffalo, suckling regimes, fertility, calf growth, calf mortality

## INTRODUCTION

Indigenous buffaloes form about 95% of the total buffalo population of 0.8 million in the country (Sri Lanka Livestock Statistics, 1986/87). They remain the most important multipurpose agricultural animal since inhabitation of the island in the 6th and 5th century BC (Siriweera, 1982) and have played a quiet but vital role in rural crop-livestock mixed production systems. Valuable baseline information on indigenous buffaloes and the rural production systems was provided by de Silva *et al.* (1985) and Perera *et al.* (1987). Indigenous buffaloes are largely concentrated in North-western and South-eastern parts of the country and are managed extensively on communal grazing lands with minimum inputs, usually along with cattle. They are primarily used by small-holders for land preparation for rice cultivation and also for threshing of paddy after the harvest. In some areas, particularly where the production of "curd" is popular, buffaloes are also milked. The milk yield is low, ranging from 1 to 2 liters per day over a 4 to 6 month lactation period (de Silva *et al.*, 1985).

Research has revealed that many physiological and biochemical aspects of reproduction in buffaloes closely resemble those of cattle (Dobson and Kamonpatana, 1986; Abeygunawardena *et al.*, 1994). In both species the period from parturition to the commencement of oestrous cycles, which is referred to as the postpartum acyclic period, is influenced by many biological and environmental factors. Nutrition and suckling have been identified as two of the major factors which determine postpartum fertility, while breed, age of the dam, season of calving, milk yield and postpartum infections are also known to play a role (Short *et al.*, 1990). Previous work on indigenous as well as exotic buffaloes in Sri Lanka have shown that age at first calving is well over 48 months and annual calving rates are often less than 60% (Lundstrom *et al.*, 1982; de Silva *et al.*, 1985). The primary determinant of low calving rate in extensively managed buffaloes was shown to be prolonged postpartum anoestrus, with conception occurring soon after resumption of oestrous cycles (Perera *et al.*, 1984). Seasonal fluctuations in nutrient supply (Lundstrom *et al.*, 1982) and suckling (Perera *et al.*, 1987; Mohan *et al.*,

1990) were identified as the two major factors which cause extended postpartum anoestrous periods. Similar effects on postpartum reproduction have been demonstrated elsewhere in river and swamp buffaloes (El-Fouly *et al.*, 1976; Usmani *et al.*, 1985; Lubis and Fletcher, 1987) and in cattle (Short *et al.*, 1972, Laster *et al.*, 1973; Reeves and Gaskins, 1981; Randel, 1981; Wettermann *et al.*, 1978; Wettermann *et al.*, 1986).

Reducing the cow-calf interaction by complete or temporary weaning, or limited suckling, has been shown to remove the suckling-induced suppression of gonadotrophin secretion (Edwards, 1985), and resulted in early return to oestrus (Wettermann *et al.*, 1986) as well as increased pregnancy rates (Laster *et al.*, 1973). Previous work by our group has demonstrated that marked differences exist in the reproductive performance of indigenous buffaloes at different locations under traditional management conditions in Sri Lanka, and that this can be partly attributed to different suckling practices adopted by the farmers (de Silva *et al.*, 1985; Perera *et al.*, 1987; Mohan *et al.*, 1990).

Therefore the objectives of the present study were to test the effects of different suckling regimes on postpartum reproductive functions, calf growth and mortality, with the aim of developing practically feasible calf management strategies which would improve the reproductive performance of buffaloes.

## MATERIALS AND METHODS

*Location, animals and management*: The studies were conducted at the SAREC/NARESA Buffalo Research Station at Narangalla, located in the low-country intermediate zone of Sri Lanka. The station receives an average rainfall of 2057±405 mm/annum and precipitation is primarily during North-East (October to January) and South-West (April to June) monsoon seasons. The daily average ambient temperature was 31.3±1.6°C and the relative humidity ranged from 64 to 80%. The herd of about 200 indigenous buffaloes were extensively managed with free grazing during the day on natural and improved pasture under coconut and paddocked at night. A stud bull was always run with the herd and the cows were not milked.

*Suckling regimes:* The following six suckling management regimes were tested in four trials during four successive calving seasons: (1)free (*ad libitum*) suckling (FS); (2) FS and weaning by day 45 postpartum (FS+W45); (3) FS and weaning by days 60 postpartum (FS+W60); (4) FS and weaning by days 90-120 postpartum (FS+W90); (5) restricted suckling once a day (OS) and (6) restricted suckling twice a day (TS). The distribution of regimes between trials were: Trial 1 (1989/90) - FS, FS+W45 and OS; Trial 2 (1990/91) - FS, FS+W60 and OS; Trial 3 (1991/92) - FS and FS+W90; and Trial 4 (1992/93) - FS and TS. Animals were randomly assigned to the treatments at calving, the cows were allowed to run with a stud bull and the subsequent calving dates were recorded. Calves in OS and TS groups were kept in a shed away from the dams while the latter were either on the grazing land or in the night paddock. They had free access to water and were also allowed access to young pasture from 45 days of age. The OS group was allowed to suckle their dams for 30 to 45 minutes in the morning, while the TS group was allowed to suckle them a second time in the afternoon. No attempts were made to observe oestrus or services as the animals were managed extensively.

*Calf growth and mortality:* Growth of calves was monitored in Trials 2 and 4 only. Body weights were recorded at birth and at monthly intervals thereafter. Mortality among the calves was recorded in all four trials. No attempts were made to rescue sick calves.

*Pregnancy rates:* Following subsequent calvings, the approximate conception dates were calculated by subtracting the estimated gestation length of 301 days (Wijeratne *et al.*, 1962) from the calving interval. The pregnancy rates at 90, 120 and 150 days postpartum were calculated based on the estimated conception dates.

*Statistical analysis:* Pregnancy rates of dams and mortality and body weights of calves in different treatments within trials were statistically analyzed to determine the effect of the suckling treatment on postpartum fertility and calf performance.

## RESULTS

The effect of suckling management regimes on pregnancy rates and calf mortality in each individual trial are given in Table 1 and the combined results from all four trials for the different treatments are illustrated in Figure 1.

**Table 1.** Pregnancy rates at 90, 120 and 150 days postpartum in buffalo cows and mortality rates in calves subjected to different suckling regimes.

| Trial and Treatment | n | Pregnancy rate | | | Calf mortality % |
|---|---|---|---|---|---|
| | | 90 days | 120 days | 150 days | |
| *Trial 1* | | | | | |
| Free Suckling (FS) | 09 | 33.3 | 33.3 | 33.3 | 1.1 |
| FS+Weaned at 45 d | 11 | 54.5 | 72.7 | 72.7 | 54.5 |
| Once a day suckling | 09 | 66.6 | 66.6 | 66.6 | 1.1 |
| *Trial 2* | | | | | |
| Free Suckling | 10 | 0.0 | 10.0 | 30.0 | 0.0 |
| FS+Weaned at 60 d | 15 | 6.6 | 33.3 | 40.0 | 33.3 |
| Once a day suckling | 20 | 25.0 | 55.5 | 55.5 | 5.0 |
| *Trial 3* | | | | | |
| Free Suckling | 31 | 25.8 | 25.8 | 25.8 | 3.2 |
| FS+Weaned at 90-120 d | 16 | 6.3 | 18.7 | 41.2 | 37.5 |
| *Trial 4* | | | | | |
| Free Suckling | 21 | 14.2 | 14.2 | 14.2 | 4.7 |
| Twice a day suckling | 22 | 31.8 | 45.4 | 59.0 | 7.1 |

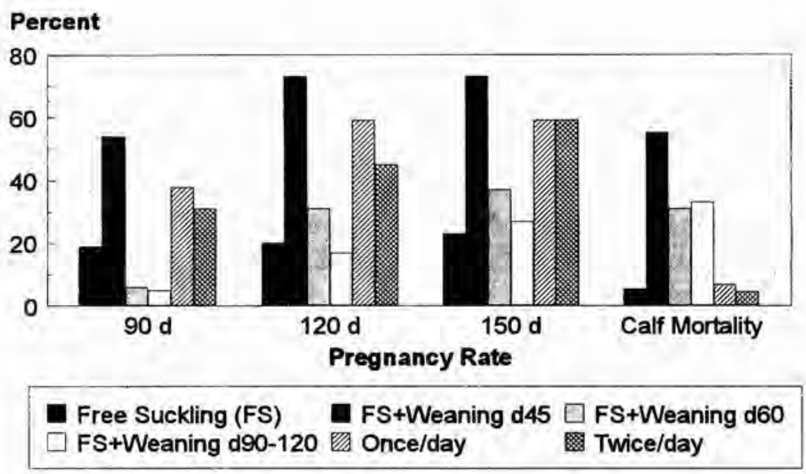



**Figure 1.** Combined results from four trials showing pregnancy rates in cows at 90, 120 and 150 days postpartum and calf mortality for the six suckling regimes.

Free suckling resulted in the lowest pregnancy rates, while weaning by day 45 resulted in the highest. The latter regime, however, led to the highest calf mortality. Weaning at later stages (60 to 120 days) did not improve pregnancy rates markedly over that of the free suckled group and mortality remained high. Restricted suckling once or twice a day had beneficial effects on pregnancy rates and the calf mortality was similar to that in the free suckling group.

The birth weights and monthly body weights of calves in FS and OS groups in Trial 2 and in FS and TS groups in Trial 4 are given in Table 2. The mean growth rates for the different groups are illustrated in Figure 2. Although the growth rates were slightly lower in the OS group than in the FS group, the difference was not statistically significant as there was a high variation of body weights within the group. The difference in body weight was less with advancing age. There were no differences in body weights and growth rates between TS and FS calves.

**Table 2.** Mean birth weights and monthly body weights of calves subjected to free, once a day or twice a day suckling.

| Trial and Treatment | Birth Weight (kg) | Body Weight (kg) | | | |
|---|---|---|---|---|---|
| | | 1mo | 2 mo | 3 mo | 4 mo |
| *Trial 2* | | | | | |
| Free suckling | 20.0±2.3 | 36.2±8.1 | 47.4±12.3 | 53.0±13.6 | 64.8±3.6 |
| Once a day suckling | 19.3±1.9 | 30.6±6.7 | 36.4± 8.8 | 47.8±9.0 | 55.5±14.7 |
| *Trial 4* | | | | | |
| Free suckling | 17.3±3.0 | 29.7±6.4 | 39.5±9.5 | 47.9±11.4 | 55.6±11.8 |
| Twice a day suckling | 18.3±2.3 | 28.6±4.0 | 37.4±6.4 | 46.1±7.7 | 53.8±9.3 |

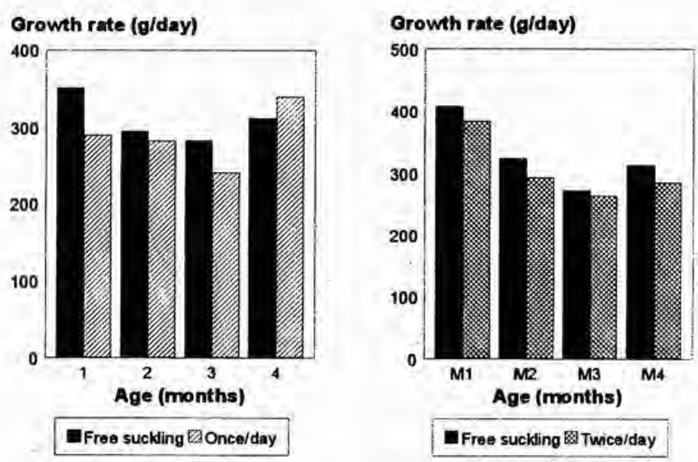



**Figure 2.** Mean growth rates of calves subjected to free, once a day or twice a day suckling.

## DISCUSSION

It has been well established that the low fertility often seen in indigenous buffaloes of Sri Lanka is not due to an inherent characteristic of the breed, but is more to do with environmental, nutritional and management factors (de Silva *et al.*, 1985; Perera *et al.*, 1987). These studies have identified fluctuating nutrient supply resulting from seasonal rainfall, together with free suckling by calves, as key factors causing prolonged postpartum acyclicity and thereby leading to long calving intervals.

The events occurring during the postpartum period, which are critical for the overall efficiency of the reproductive process, have been subjected to detailed study over the last two decades in many species (Lamming *et al.*, 1981; Dobson and Kamonapatana, 1986; Short *et al.*, 1990). After parturition, which in itself is considered a stressful event to the mother, lactation commences and the previously gravid uterus undergoes an extensive repair process. The hypothalamo-pituitary-gonadal axis is initially inactive and gradually regains its cyclic activity. The speed with which this occurs is determined by many factors, chief among which is the balance between the demand for metabolic substrates for lactation and body maintenance on the one hand, and their availability from stored reserves and daily feed intake on the other. Factors such as interactions between the dam and offspring, including suckling, are also known to influence the return to ovarian cyclicity (Short *et al.*, 1972; Peters *et al.*, 1981).

In the present study, free suckling by calves in all the trials resulted in low pregnancy rates between 90 and 150 days postpartum. Since the cows were run with intact bulls, the delay in conception is most likely due to delayed commencement of ovarian activity. Studies in cattle have demonstrated that weaning facilitates only the onset of ovarian activity and does not influence conception *per se* (Reeves and Gaskins, 1981; Wettermann et al., 1986). This also corroborates previous studies on extensively managed buffaloes which showed that conception usually follows immediately after resumption of ovarian activity (Perera *et al.*, 1984). Our results are in general agreement with those reported by Mohan *et al.* (1990) in Lanka buffaloes, El-Fouly *et al.* (1976) in Egyptian buffaloes, Jainudeen *et al.* (1982/83) in Malaysian buffaloes and Wagner and Hameel (1969), Short *et al.* (1972) and Laster *et al.* (1973) in cattle.

Much research effort has been directed towards elucidating the mechanisms underlying the suckling-induced anoestrus in cattle (Wagner and Oxenreider, 1972; Short *et al*, 1972; Carruthers and Hafs, 1980; Carruthers *et al.*, 1980). It has been shown that the suckling-induced inhibition of postpartum reproduction can occur independently of postpartum nutrition (Short *et al.*, 1972). Further, the mechanical act of suckling alone may not be sufficient to fully inhibit postpartum reproduction, but the complex interplay between the cow and calf which normally occurs may need to be present over a period of time (Williams *et al.*, 1984). Many previous studies have also clearly demonstrated that the resumption of episodic release of gonadotrophins, particularly LH, is a prerequisite for the establishment of ovarian cyclicity following calving (Peters *et al.*, 1981; Schams *et al.*, 1978; Walters *et al.*, 1982 a, b and c). Suckling appears to delay the appearance of episodic release of LH (Smith *et al.*, 1981; Peters *et al.*, 1981) and cause anomalies in FSH secretion (Williams *et al.*, 1983).

Suckling and cow-calf interactions appear to suppress episodic release of GnRH from the hypothalamus (Walters *et al.*, 1982 a, b and c), decrease responsiveness of the pituitary to GnRH (Smith *et al.*, 1981), decrease the positive feedback responsiveness of the hypothalamo-pituitary axis to estrogen (Smith *et al.*, 1981) and increase its sensitivity to the negative feedback inhibitory effects (Acosta *et al.*, 1983). How these changes are brought about by suckling is not clear but, there are several hypotheses which implicate opioid neuronal pathways in the central nervous system (Whisnant *et.al.*,1986), cortisol (Wagner and Oxenreider, 1972; Dunlap *et al.*, 1981; Padmanabhan *et al.*, 1983; Abeygunawardena *et al.*, 1985) and prolactin (Williams and Ray, 1980; Wheeler *et al.*, 1982). Recent studies, however, suggest that cortisol and prolactin, which are secreted in large quantities in response to suckling, may play only a modulating role rather than being direct mediators (Short *et al.*, 1990).

Early studies aimed at developing practical calf management methods to improve postpartum fertility showed that cows which have their calves weaned at birth have shorter postpartum anoestrous periods than those that are suckled (Short *et al.*, 1972; Bellows *et al.*, 1974). In beef cattle, weaning calves 1 to 2 weeks prior to the beginning of the breeding season resulted in higher pregnancy rates (Laster *et al.*, 1973). Reeves and Gaskins (1981) demonstrated that once a day suckling from 21 days postpartum allows early return to oestrus, while Edwards (1985) showed that short-term calf separation for 72 hours caused increased mean LH concentration and LH pulse frequency. Wettermann (1986) further demonstrated that short-term calf separation is effective only in cows with good body condition, while Randel (1981) showed that restricting suckling to once a day from 30 days after calving does not interfere with growth of the calf.

In our study weaning by day 45 and 60 resulted in higher pregnancy rates, but weaning at later stages was less effective. This suggests that the hypothalamo-pituitary axis may be more sensitive to inhibition by suckling during the early postpartum period, and that beyond this period other limiting factors such as nutrition may play a dominant role. Alternatively, the calf may be suckling the mother less frequently by this stage, thereby causing less inhibitory effects.

All weaning ages tested (45-120 days) resulted in increased calf mortality. This suggests that the buffalo calf may be dependant on the dam for an extended period. Possibly, free suckling discourages the calf from picking up roughages at an early stage, thereby delaying the development of fermentative functions in the rumen. Hence, when weaning results in the abrupt cessation of milk intake, the calf may be unable to adapt quickly to a forage-based diet. However, in view of the beneficial effects of early weaning on fertility seen in the present study, it would be justified to examine whether provision of supplementary feeds such as milk replacers can reduce the high calf mortality in a cost-effective manner.

Both regimes of limited suckling tested, once or twice a day, had beneficial effects on postpartum fertility. Although calves subjected to once a day suckling had an initial reduction in body weights and growth rates, this was not seen in those subjected to twice a day suckling. Even in the former group, there was a gradual recovery of this loss with time and their body weights did not differ significantly from those of free suckled calves from four months onwards. Further, most importantly, neither treatment resulted in the high calf mortality seen in the weaned groups.

The findings of this study offer a perfectly viable management option for extensively managed buffalo herds in Sri Lanka. In practice most herds are milked only once a day in the morning and the herd (including calves) is then taken to grazing lands, usually some distance away from the farmers dwellings, and the calves are separated from dams only when the herd is brought back in the evening to the paddocks. Limited suckling could easily be incorporated into this system, by encouraging the farmers to leave the calves behind in a separate paddock with sufficient bedding and water. Provision of hay or grazing for a few hours a day would also be very beneficial for reducing the initial inhibition of growth, and would permit early development of ruminal function. It must be stated here that this system of management is in fact being practiced traditionally by village farmers in a few remote locations in Sri Lanka (Perera *et al.*, 1987). These farmers, however, have evolved this system not as a method of improving fertility, but as a means to reduce calf losses from predators such as leopards and crocodiles which are present in such areas. Under these situations, Perera *et al.* (1987) observed that the postpartum anoestrous period was significantly shorter, and the annual pregnancy rates higher, than under the more prevalent traditional systems with free suckling. Our study provides the scientific evidence for the beneficial effects of restricted suckling regimes on postpartum fertility.

In conclusion, the results of the present study indicate that free suckling is a cause of poor reproductive performance in buffaloes, and that limited suckling either once or twice a day advances conception and thereby reduces the calving interval, with no major inhibitory effect on calf survival and growth. Limited suckling, once or twice a day, can therefore be recommended as a suitable calf rearing system for extensively managed buffalo herds in Sri Lanka.

## Acknowledgements

We thank the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for providing financial support for this study, and the International Atomic Energy Agency, Vienna, for technical assistance and supply of RIA kits. We also thank the Manager and staff of the Narangalla Buffalo Research Station. The assistance of Messrs. A.M. Karunaratne, Janaka Herath and Rex Fernando and Ms. Ramani Ruwanpathirana is gratefully acknowledged.

## References

Abeygunawardena, H., Weston, P.G., Wagner, W.C. and Hixon, H. E. (1985) Influence of adrenal glands on episodic and GnRH-induced release of luteinizing hormone in suckled cows. *J. Anim. Sci.* (Suppl.) **61,** 415.

Abeygunawardena, H., Kuruwita, V.Y., Karunaratne, A.M. and Abayawansa, W.D. (1994) Reproductive characteristics of indigenous buffaloes in Sri Lanka under experimental conditions. In: *Proceedings of Fourth Word Buffalo Congress*, 26-30 June 1994, Sao Paulo, Brazil.

Acosta, B., Tarnarsky, G.K., Platt, T.E., Hamernik, D.L., Brown, J.L., Schoenemann, H.M. and Reeves, J.J. (1983) Nursing enhances the negative effect of estrogen on LH release in the cow. *J. Anim. Sci.* **57,** 1530-1536.

Bellows, R.A., Short, R.E., Urick, J.J. and Pahnish, O.F. (1974) Effect of early weaning on postpartum reproduction of the dam and growth of calves born multiple or single. *J.Anim. Sci.* **39,** 589-594.

Carruthers, T.D. and Hafs, H.D. (1980) Suckling and four-times daily milking: Influence on ovulation, oestrus and serum luteinizing hormone, glucocorticoid and prolactin in postpartum Holsteins. *J. Anim. Sci.* **50,** 919-925.

Carruthers, T.D., Convey, E.M., Kesner, J.S., Hafs, H.D. and Cheng, K.W. (1980) The hypothalamo-pituitary gonadotrophic axis of suckled and non-suckled dairy cows postpartum. *J. Anim. Sci.* **51,** 949-957.

de Silva, L.N.A., Perera, B.M.A.O., Tilakaratne, L. and Edqvist, L.E. (1985) *Production Systems and Reproductive Performances of Indigenous Buffaloes in Sri Lanka.* Monograph, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Dobson, H. and Kamonpatana, M. (1986) A review of female cattle reproduction with special reference to comparison between buffaloes, cows and zebu. *J. Reprod. Fert.* **77,** 1-36.

Dunlap, S.E., Kiser, T.E., Rampacek, G.B., Kraeling, R.R. and Thompson, F.N. (1981) Effect of suckling on cortisol, progesterone and luteinizing hormone in postpartum beef cows. *Theriogenology* **16,** 185-193.

Edwards, S. (1985) The effects of short-term calf removal on pulsatile LH secretion in postpartum beef cows. *Theriogenology* **23,** 777-785.

El-Fouly, N.A., Kotby, E.A. and El-Sobhy, H.E. (1976) Postpartum ovaraian activity in suckled and milked buffaloes. *Theriogenology* **5,** 69-81.

Jainudeen, M.R., Bongso, T.A. and Tan, H.S. (1982/83) Postpartum ovaraian activity and uterine involution in the suckled swamp buffaloes (*Bubalus bubalis*). *Anim. Reprod. Sci.* **5,** 181-190.

Lamming, G. E., Wathes, D.C. and Peters, A.R. (1981) Endocrine patterns of the postpartum cow. *J.Reprod. Fert.* (Suppl) **30,** 155-170.

Laster, D.B., Glimp, H.A. and Gregory, K.E. (1973) Effect of early weaning on postpartum reproduction of cows. *J. Anim. Sci.* **36,** 734-740.

Lundstrom, K., Abeygunawardena, H., de Silva, L.N.A. and Perera, B.M.A.O. (1982) Environmental influence on calving interval and estimates of its repeatability in the Murrah buffalo in Sri Lanka. *Anim. Reprod. Sci.* **5,** 99-109.

Lubis, A. and Fletcher, I.C. (1987) Postpartum reproduction in swamp buffalo cows at two feeding levels. *Anim. Reprod. Sci.* **13,** 183-189.

Mohan, V., Kuruwita, V.Y., Perera, B.M.A.O. and Abeygunawardena, H. (1990) Effect of suckling on the resumption of ovarian activity in buffaloes. *Trop. Agric. Res.* **2,** 306-315.

Padmanabhan, V., Keech, C. and Convey, E.M. (1983) Cortisol inhibits and adrenocorticotrophin has no effect on luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells in vitro. *Endocrinology* **112,** 1782-1787.

Perera, B. M.A.O., de Silva, L.N.A. and Karunaratne, A.M. (1984) Studies on reproductive endocrinology and factors influencing fertility in dairy and draught buffaloes in Sri Lanka. In: *The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia.* International Atomic Energy Agency, Vienna. pp.13-28.

Perera, B.M.A.O., de Silva, L.N.A., Kuruwita, V.Y. and Karaunaratne, A.M. (1987) Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka. *Anim. Reprod. Sci.* **14,** 115-127.

Peters, A.R., Lamming, G.E. and Fisher, M.W. (1981) A comparison of plasma LH concentrations in milked and suckling post--partum cows. *J. Reprod. Fert.* **62,** 567-573.

Randel, R.D. (1981) Effect of once-daily suckling on postpartum interval and cow-calf performance of first-calf Brahman x Hereford heifers. *J. Anim. Sci.* **53,** 755-757.

Reeves, J. J. and Gaskins, C.T. (1981) Effect of once-a-day nursing on rebreeding efficiency of beef cows. *J. Anim. Sci.* **53,** 889-891.

Schams, D., Shallenberger, E., Menzer, ch., Stangl, J., Zottemeier, K., Hoffman, B. and Karg, H. (1978) Profile of LH, FSH and progesterone in postpartum dairy cows and their relationship to commencement of cyclic functions *Theriogenology* **10,** 453-468.

Short, R. E., Bellows, R.A., Moody, E.L. and Howlands, B.E. (1972) Effects of suckling and mastectomy on bovine postpartum reproduction. *J. Anim. Sci.* **34,**70-74.

Short, R.E., Bellows, R.A., Staigmiller, R.B., Berardinelli, J.G. and Custer.E.E. (1990) Physiological mechanisms controlling anoestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* **68,** 799-816.

Siriweera, W.I. (1982) Water resources and buffalo and cattle rearing in Sri Lanka: A historical perspective. *Occasional Publication 30,* Agrarian Research and Training Institute, Colombo, Sri Lanka.

Smith, J. F., Payne, E., Trevit, H.R., McGowan, L.T., Fairclough, R., Kilgour, R. and Goold, P.G. (1981) The effect of suckling upon the endocrine changes associated with anoestrous in identical twin dairy cows. *J. Reprod. Fert.* (Suppl) **30,** 241-249.

Sri Lanka Livestock Statistics (1986/87) Ministry of Rural Industrial Development, Colombo, Sri Lanka.

Usmani, R.H., Ahamed, M., Inskeep, E.K., Dailey, R.A., Lewis, P.E. and Lewis, G.S. (1985) Uterine involution and postpartum ovarian activity in Nili Ravi buffaloes. *Theriogenology* **24,** 435-448.

Wagner, W.C. and Hansel, W. (1969) Reproductive physiology of postpartum cow: I. Clinical and histological findings. *J. Reprod. Fert.* **18,** 493-500.

Wagner, W. C. and Oxenreider, S.L. (1972) Adrenal function in the cow. Diurnal changes and the effects of lactation and neurophyseal hormones.*J.Anim. Sci.* **34,** 630-635.

Walters, D.L., Kaltenbach, C.G., Dunn, T.G. and Short, R.E. (1982a) Pituitary and ovarian function in postpartum beef cows: I. Effect of suckling on serum and follicular fluid hormone and follicular gonadotrophin receptors. *Biol. Reprod.* **26,** 640-646.

Walters, D.L., Short, R.E., Convey, E.M., Staigmiler, R.B., Dunn, T.G. and Kaltenbach, C.C. (1982b) Pituitary and ovarian function in postpartum beef cows: II. Endocrine changes prior to ovulation in suckled and non-suckled postpartum cow compared to cycling cows. *Biol. Reprod.* **26,** 647-654.

Walters, D.L., Short, R.E., Convey, E.M., Staigmiller, R.B., Dunn, T.G. and Kaltenbach, C.C. (1982c) Pituitary and ovarian function in postpartum cows: III. Induction of oestrus, ovulation and luteal function with small dose injection of GnRH. *Biol. Reprod.* **26,** 655-662.

Wettermann, R.P., Turman, E.J., Wyatt, R.D. and Totusek, R. (1978) Influence of suckling intensity on reproductive performance of range cows. *J. Anim. Sci.* **47,** 342-346.

Wettermann, R.P., Hill, G.M., Boyd, M.E., Spitzer, J.C., Forrest, D.W. and Beal, W.E. (1986) Reproductive performance of postpartum beef cows after short-term calf separation and dietary energy and protein supplementation. *Theriogenology* **26,** 433-441.

Wheeler, M.B., Anderson, G.B., Munro, C.J. and Stabenfeldt, G.H. (1982) Prolactin response in beef cows and heifers suckling one or two calves. *J. Reprod. Fert.* **64,** 243-249.

Whinsnant, C.S., Kiser, T.E., Thompson, F.N and Barb, C.R. (1986) Opioid inhibition of luteinizing hormone secretion during the postpartum period in suckled beef cows. *J. Anim. Sci.* **63,** 1445-1448.

Wijeratne, W.V.S. (1962) Some of the production statistics of Ceylon buffaloes. *Ceylon vet. J.* **10,** 48-49.

Williams, G.L.and Ray, D.E. (1980) Hormonal and reproductive profiles of early postpartum beef heifers after prolactin suppression or induced luteal function. *J. Anim. Sci.* **50,** 906-918.

Williams, G.L., Talavera, F., Peterson, B.J., Kirsch, J.D. and Tilton, J.E. (1983) Coincident secretion of follicle stimulating hormone and luteinizing hormone in early postpartm cows: effect of suckling and low level increase of systemic progesterone. *Biol. Reprod.* **29,** 362-373.

Williams, G.L., Kirsch, J.D., Post, G.R., Tilton, J.E. and Slanger, W.D. (1984) Evidence against chronic teat stimulation as an autonomous effector of diminished gonadotropin release in beef cows. *J. Anim. Sci.* **59,** 1060-1069.

# EFFECTS OF EXOGENOUS HORMONES ON FERTILITY OF POSTPARTUM ANOESTROUS BUFFALOES

H. Abeygunawardena, V.Y. Kuruwita and B.M.A.O. Perera

*Department of Veterinary Clinical Studies, Faculty of Veterinary, Medicine and Animal Science, University of Peradeniya, Peradeniya, SRI LANKA*

**Abstract:** Seasonal calving and low calving rate, caused by long postpartum anoestrus, have been identified as the major factors which limit the productivity of buffaloes in Sri Lanka. As a management strategy to improve fertility of buffaloes during the postpartum period, the effects of the following regimes of exogenous hormone treatments were tested in anoestrous animals; (a) two injections of 250 μg of GnRH (Receptal, Hoechst) 24 h apart; (b) two injections of 4 mg of FSH (Intervet) given 12 h apart, followed 12 h later by treatment (a); (c) progesterone releasing intravaginal device (PRID, Sanafi) for 11 days and 500 IU of PMSG (Folligon, Intervet) at the time of coil removal and (d) progesterone releasing silastic ear implant (Synchromate B, Intervet) for 11 days and 500 IU of PMSG at the time of implant removal. An untreated group of animals remained as control (e). After the hormonal treatments they were allowed to run with stud bulls. Subsequent calvings were recorded. The number (and percentage) of animals which became pregnant within 90 days of treatment in groups a, b, c, d and e were: 0/10 (0%), 0/12 (0%), 19/23 (83%), 32/44 (73%) and 15/45 (33%), respectively. In a separate trial, 9 cows were given treatment (d), kept away from bulls and observed for oestrous signs. Plasma progesterone was measured by RIA in blood samples collected at frequent intervals up to 45 days. All treated animals showed distinct signs of oestrus following implant removal and 8 (89%) had ovulations as indicated by plasma progesterone. However, two animals had abnormal luteal phases and relapsed into acyclicity. The lack of response to GnRH treatment could be due to refractoriness of the ovary and pituitary, and the lack of tertiary follicles. The combination of progesterone and PMSG given sequentially appears to be capable of overcoming this refractoriness, possibly through the build up of LH reserves and stimulation of follicle development, and appears to be an effective method for inducing oestrus and improving pregnancy rates in anoestrous buffaloes. However, more studies are needed to determine the factors which influence the success rate.

**Keywords:** Buffalo, anoestrus, post-partum, hormones, treatment

## INTRODUCTION

Seasonal calving and low calving rate have been identified as two major factors which limit the productivity of buffaloes in Sri Lanka (Lundstrom *et al.,* 1982; de Silva *et al.,* 1985). Majority of calvings occur with the commencement of North-East monsoon rains in October/November, and analysis of calving and conception patterns in relation to rainfall data suggest that the seasonality of calving may be the result of entrainment of postpartum ovarian activity by the seasonal fluctuations in pasture and fodder availability which are determined by seasonal rainfall. Animals which calve early in the calving season have the benefit of an abundance of natural pasture and fodder during the early postpartum period while those that calve late in the season experience hot and humid weather conditions along with scarcity of feed.

This prolonged postpartum acyclicity makes buffalo farming uneconomical to small holders as well as at state farms. It is imperative that various management and therapeutic strategies should be developed to overcome this problem. Apart from supplementary feeding (Dziuk *et al.,* 1983), early weaning (Laster *et al.,* 1973) and limited suckling (Randel, 1981; Reeves and Gaskin, 1981; Bastidae *et al.,* 1984), exogenous hormones have been used to improve the pregnancy rate of cattle as well as buffaloes in many countries (Kesler *et al.,* 1982; Odde, 1990; Narasimha Rao and Venkatramiah, 1991). Further, the hormonal induction and synchronization of oestrus would be a very useful aid for planned breeding in upgrading of indigenous buffaloes with genetically superior exotic river buffaloes.

The objectives of this study were to determine the effects of various hormonal preparations in inducing oestrus in anoestrous buffaloes, to assess subsequent pregnancy rates, and to characterise the hormonal profiles and external signs after induction of oestrus.

## MATERIALS AND METHODS

### Trials at Research Farm

These were conducted at the SAREC/NARESA/NLDB Buffalo Research Station at Narangalla, located in the intermediate zone of Sri Lanka. The annual rainfall is 1583 ± 910 mm, and occurs primarily during October to December (North-East monsoon) and April to June (South-West monsoon). The ambient temperature fluctuates between 20 and 33°C with a mean of 25°C. Average relative humidity fluctuates between 60 to 87 % with a mean of 78%.

Indigenous (Lanka) and cross-bred buffalo cows were managed extensively on natural and improved pasture under a coconut plantation, grazed for 8 - 10 hours during daytime and paddocked in the night. They were allowed to wallow once a day and the calves allowed free suckling. Stud bulls were always with the herd. Calvings were seasonal with peak calving in October to January. Cows which were more than 100 days after calving were examined by rectal palpation and those with no palpable ovarian structures were selected for the study.

*Hormonal Treatments:* The following regimes were used: (a) two injections of 250 μg of Gonadotrophin Releasing Hormone (GnRH, "Receptal", Hoechst) 24 h apart; (n=9): (b) two injections of 4 mg of Follicle Stimulating Hormone (FSH, Intervet) given 12 h apart, followed by treatment (a) 12 h later; (n=12): (c) Progesterone Releasing Intravaginal Device (PRID, Sanafi) for 11 days and 500 IU of Pregnant Mare Serum Gonadotrophin (PMSG, "Folligon", Intervet) at the time of coil removal; (n=23): (d) progesterone releasing silastic ear implant ("Synchromate B", Intervet) for 11 days and 500 IU of PMSG at the time of implant removal; (n=12); and (e) untreated controls (n=22). After treatment the cows were allowed to run with stud bulls, subsequent calvings were recorded and the pregnancy rate at 90 days after treatment was calculated.

*Intensive Sampling Trial:* In a separate trial, 9 cows were given treatment (d), kept separate from bulls and observed for oestrous signs for 5 days after treatment. Blood samples were collected daily between days 1 - 5, 18 - 23 and 38 - 46, and at 3 day intervals during the intervening periods. Blood was centrifuged at 1000 rpm for 10 minutes, plasma separated and stored at -20°C until assayed for progesterone by radioimmunoassay (RIA).

*Hormone Assay:* Plasma progesterone was quantified by RIA using antibody pre-coated tubes and $^{125}I$ progesterone as tracer (supplied by the Joint FAO/IAEA Division, Vienna). The sensitivity of the assay was 0.3 nmol/l and intra-and inter-assay coefficients of variation were 9.7% and 13.7%, respectively.

*Statistical Tests:* Differences among the treatments were tested for statistical significance by Chi-square test.

## Trial at Village Location

This was conducted at Hambantota in the Dry Zone of Sri Lanka, where the annual rainfall is between 875 and 1875 mm, with the primary precipitation occurring during the North-East monsoon from September to December. The animals were of indigenous type and managed extensively with grazing during the day on communal lands and paddocking at night. They were milked once in the morning, with calf separation during the night only. Calvings were seasonal with the majority occurring between September and January. The trial was conducted during January to March. Pluriparous cows which were more than 100 days after calving were examined by rectal palpation and those with no palpable ovarian structures were selected for the study. Treatment (d) was given to 32 cows while 23 were kept as controls. They were allowed to run with stud bulls, subsequent calvings were recorded and the pregnancy rate at 90 days after treatment was calculated.

# RESULTS

## Trials at Research Farm

*Hormonal Treatments:* The pregnancy rates at 90 days after the different treatments are given in Table 1. GnRH treatment, with or without FSH pre-treatment, was ineffective in inducing oestrous cycles in any of the treated animals. With both progesterone releasing devices, the retention rate was 100%. There was some tissue reaction to the vaginal coil (PRID) with a discharge of purulent material at the time of coil removal, but there was little reaction to the ear implant.

**Table 1.** Number and percentage of buffalo cows pregnant at 90 days after treatment.

| Treatment | n | Number pregnant | Pregnancy rate (%) |
|---|---|---|---|
| a. GnRH | 10 | 0 | 0 |
| b. FSH + GnRH | 12 | 0 | 0 |
| c. PRID + PMSG | 23 | 19 | 83 |
| d. Ear Impl. + PMSG | 12 | 11 | 91 |
| e. Control | 22 | 7 | 32 |

*Intensive Sample Trial:* In the intensive sampling trial, all 9 treated animals showed signs of oestrus following implant removal. Vulval swelling, redness and mucus discharge were observed commencing from 12 hours, and they were maximal between 48 to 60 hours after implant removal. The vulval temperature ranged from 98 to 102°F and the variations in the vulval temperature were more related to the time of the day than to the time after treatment. The majority of animals showed restlessness and homosexual behaviour while a few had increased frequency of urination.

The plasma progesterone profiles showed that although all nine treated animals exhibited signs of oestrus, only 8 had ovulations. As shown in the selected plasma progesterone profiles in Figure 1, the synchrony of ovulations was poor and appeared staggered over several days. One animal which ovulated had a very short luteal phase (Figure 2) and did not continue cyclicity, while another animal which had an apparently normal luteal phase also relapsed into acyclicity. In the other 6 animals oestrous cycles were of variable lengths and they continued to cycle.

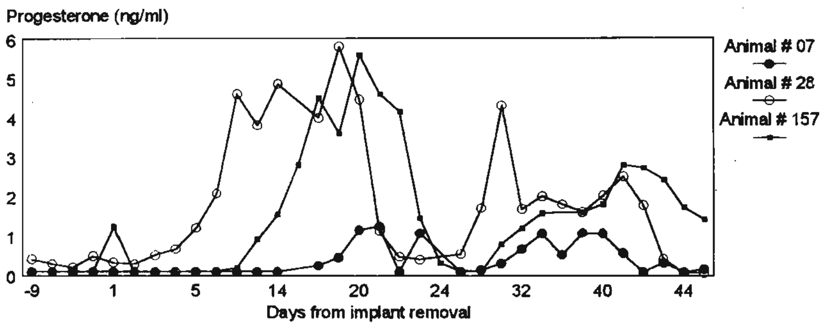



**Figure 1.** Plasma progesterone profiles in three animals after treatment with progesterone ear implant and PMSG.

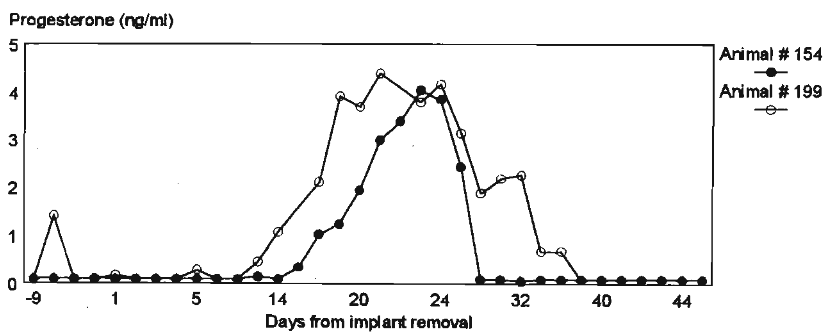



**Figure 2.** Plasma progesterone profiles from two animals after treatment with progesterone ear implant and PMSG, showing short (No. 154) and normal (No. 199) luteal phases and subsequent acyclicity.

## Trial at Village Location

The pregnancy rate at 90 days after treatment with progesterone ear implant and PMSG was 66% (21/32), while the pregnancy rate in the control group was 35% (8/23). The retention rate of the ear implant was 94%.

# DISCUSSION

Reappearance of episodic bursts of LH following calving has been shown to be a prerequisite for the resumption of postpartum ovarian activity in cattle (Peters *et al.*, 1981) and to be true also for buffaloes (Mohan *et al.*, 1990). Intermittent injections of low doses of GnRH at frequent intervals have successfully induced ovulation in postpartum cows (Riley *et al.*, 1981; Walters *et al.*, 1982; Vorstermans and Walten, 1985). This effect has been shown to be associated with increase in responsiveness of the pituitary to GnRH (Foster, 1978), increase in LH and FSH pulse frequency and amplitude (Walters *et al.*, 1982; Spicer *et al.*, 1986; Jagger *et al.*, 1987) and stimulation of follicular steroidogenesis and induction LH receptors (Spicer *et al.*, 1986). As the administration of frequent injections is practically not possible, effects of bolus injections have been tested by many workers and the effect of double injections of GnRH on the release of gonadotrophins and induction of oestrus have been demonstrated in cattle (Fonseca *et al.*, 1977; Webb *et al.*, 1977) and buffaloes (Barkawi and Aboul-Ela, 1987). These trials also demonstrated that intermittent injections besides being cumbersome had little advantage over the bolus injections (Vorstermans and Walten, 1985).

In our study, bolus injections of GnRH into anoestrous buffaloes did not result in increased pregnancy rate. Poor fertility after use of GnRH in postpartum anoestrous buffaloes was also reported by Barkawi and Aboul-Ela (1989) and Narasimha Rao and Venkatramiah (1991). Several studies have shown that the responsiveness of the postpartum cow to exogenous GnRH is dependent on many factors such as days postpartum (Foster *et al.*, 1980; Irvin *et al.*, 1981), pre-treatment oestradiol concentration which is determined by ovarian follicular development (Kesler *et al.*, 1977; Fernandes *et al.*, 1978; Jagger *et al.*, 1987) and suckling (Troxel *et al.*, 1980). Pretreatment oestrogen concentration also affects the responsiveness of the pituitary to GnRH by influencing the number and affinity of receptors (Leung *et al.*, 1984). It is therefore likely that the poor response obtained in our study, where the animals were also subjected to free suckling, was due either to the refractoriness of the pituitary to GnRH, or the lack of response of the ovaries due to absence of developing follicles.

Therefore in the next trial, FSH was used to stimulate follicular development prior to GnRH injections. The ability of exogenous FSH to stimulate follicular development has been demonstrated in many species including buffaloes (Karaivanov, 1986). However, pre-treatment with two 4 mg injections at 12 hours apart failed to improve the effectiveness of exogenous GnRH. This does not, however, invalidate this hypothesis but may reflect an inadequacy of either the dose given or the time allowed between the FSH and GnRH injections to ensure sufficient follicular development.

The use of progesterone in the induction and synchronization of oestrus has been studied in many species and two hypotheses have been used to explain the effects. One proposes that exogenous progesterone inhibits the release of gonadotrophins, thus building up reserves for release upon withdrawal (Breuel *et al.*, 1993). Another is that the progesterone may sensitize the ovaries to the action of gonadotrophins (Gonzalez-Padilla *et al.*, 1975; Smith *et al.*, 1979). In our study with progesterone, induction of oestrus was successful in all treated animals. However, plasma progesterone profiles indicated that 8 out of 9 animals responded with an ovulation. Of the responders, however, two did not continue to cycle; even in those that did cycle, the interval from implant withdrawal to rise in progesterone was not very uniform, suggesting that oestrus, LH surge and ovulation may not be well synchronized. This was also reported by others (Rentfrow *et al.*, 1987). The variation in the response to exogenous progesterone treatment can be due to several factors, including age of the animal, interval from calving to treatment, nutritional status and the pre-treatment ovarian status. Studies in cattle have shown that luteal dysfunction is one of the causes of reduced fertility following synchronized and induced oestrus (King *et al.*, 1986; Favero *et al.*, 1988).

Many workers have attempted to improve pregnancy rates following progesterone treatment by including oestrogens and/or gonadotrophins in the regime. For example, oestrogen pretreatment with progesterone implant for 10-11 days followed by PMSG injection has been reported to give high pregnancy rates (Chupin *et al.*, 1977; Mikisch *et al.*, 1978). In our studies conducted at the research station as well as under village conditions, the 90 day pregnancy rates were above 70%. Similar high fertility rates have been reported among anoestrous as well as cycling buffaloes (Virakul *et al.*, 1988). Studies in cattle have yielded first service conception rates ranging from 33 to 66% (Odde, 1990). In general, previous studies indicate that fertility can be high when cycling animals are synchronized, whereas it is low when induction of oestrus is done in anoestrous animals (Brink and Kiracope, 1988; Brown *et al.*, 1988).

In the present study, buffaloes treated with progesterone and PMSG expressed good signs of oestrus over a period of 1 to 2 days. Since they were run with bulls, it is likely that they were served several times during the induced oestrus, thus contributing to the high fertility rate obtained. On the other hand, if artificial insemination is to be used, it is necessary to determine the optimum time for insemination after induced oestrus following this regime of hormonal treatment.

In conclusion, the results of this study show that short term progesterone treatment in conjunction with PMSG appears to be very promising in inducing oestrus and improving the pregnancy rate in indigenous buffaloes during the post-partum period. However, more studies are needed to determine the factors which affect the success rate in order to improve the efficiency of treatment. Further, time sequences in relation to implant removal, LH surge, ovulation and oestrus need to be determined precisely in order to use this treatment for planned breeding with artificial insemination.

## Acknowledgements

We thank the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for providing financial support for this study, and the International Atomic Energy Agency, Vienna, for technical assistance and provision of the RIA kits. We also thank the farmers in Hambantota for their co-operation. The assistance and dedicated work of Dr. W.D. Abayawansa, Mr. A.M. Karunaratne, Mr. Janaka Herath, Mr. Jayadasa Weeratunga, Mr. Rex Fernando and Ms. Ramani Ruwanpathirana are gratefully acknowledged.

## References

Barkawi, A.H. and Aboul-Ela, M.B. (1987) Response of acyclic and cyclic buffaloes to treatment with GnRH analogue. *Buffalo J.* **2,** 169-179.

Barkawi, A.H. and Aboul-Ela, M.B. (1989) The postpartum response of suckling buffaloes to treatment with GnRH analogue. *Buffalo J.* **2,** 183 - 189.

Bastidae, P., Troconiz, J., Verde, O. and Silva, O. (1984) Effect of restricted suckling on pregnancy rates and calf performance in Brahman cows. *Theriogenology* **21,** 289-293.

Brink, J.T. and Kiracofe, G.H. (1988) Effect of oestrus cycle stage at Synchromate B treatment on conception and time to oestrus in cattle. *Theriogenology* **29,** 513.

Breuel, K.F., Lewis, P.E., Inskeep, E.K. and Butcher, R.L. (1993) Endocrine profiles and follicular development in early weaned postpartum beef cows. *J. Reprod. Fert.* **97,** 205 - 212.

Brown, L.N., Odde, K.G., Lefeur, D.G., King, M.E. and Newbauer, C.J. (1986) Norgestomet-alfaprostal or Synchromate B for oestrous synchronisation in beef cows. *J. Animal Sci.* **60,** Suppl. 1, 383 (abstract).

Chupin, D., Pelot, J. and Mauleon, J. (1977) Control of oestrus and ovulation in dairy cows. *Theriogenology* **7,** 339-347.

de Silva, L.N.A., Perera, B.M.A.O., Tilakaratne, N. and Edqvist, L-E (1985) Production systems and reproductive performance of indigenous buffaloes in Sri Lanka. *Monograph,* Swedish University of Agricultural Sciences, Uppsala, Sweden.

Dziuk, P.J. and Bellows, R.A. (1983) Management of reproduction of beef cattle, sheep and pigs. *J. Anim. Sci.* **57,** Suppl. 2, 355-379.

Favro, R.J., Faulkner, D.B., Kesler, D.G. (1988) Oestrous synchronization in beef females with Synchromate B. Efficiency and factors that restrict optimal pregnancy rate. *Theriogenology* **29,** 245.

Fernandes, C.L., Thatcher, W.W., Wilcox, C.J. and Calb, E.P. (1978) Luteinizing hormone release in response to GnRH during postpartum period of dairy cows. *J. Anim. Sci.* **46,** 430.

Fonseca, F.A., Kosugiyama, M., Britt, J.H. and Ritchie, H.D. (1977) Ovulation, oestrous cycle and endocrine response after GnRH in suckled cows. *J.Anim. Sci.* **45,** Suppl. 1, 158.

Foster, J.P., Lamming, G.E. and Peters, A.R. (1980) Short-term relationships between plasma LH, FSH and progesterone concentrations in postpartum dairy cows and the effect of GnRH injection. *J. Reprod. Fert.* **59,** 32 327.

Foster, J.P. (1978) Plasma concentrations after single or double injections of synthetic LH-RH in dairy cows. *J. Reprod. Fert.* **54,** 119-121.

Gonzalez-Padilla, E., Wiltbank, J.N. and Niswender, G.D. (1975) Puberty in beef heifers. I. The interrelationship between pituitary, hypothalamic and ovarian hormones. *J. Anim. Sci.* **40,** 1091.

Irvin, H.J., Zaied, A.M., Day B.N. and Garverick, H.A. (1981) GnRH-induced LH release in suckled beef cows: 1. The effects of days postpartum and oestradiol 17ß concentrations on the release of LH following administration of GnRH. *Theriogenology* **15,** 443-448.

Jagger, J.P., Peters, A.R. and Lamming, G.E. (1987) Hormonal response to low-dose GnRH treatment in postpartum beef cows. *J. Reprod. Fert.* **80,** 263-269.

Karaivanov, Ch. (1986) Comparative studies on the superovulatory effect of PMSG and FSH in water buffalo (*Bubalus bubalis*). *Theriogenology* **26,** 51-59.

Kesler, D.J., Cmarik, G.F., Weichenthal, B.A., Thompson, L.H., Lode, T.F. and Ott, R. (1982) The effect of short term calf removal in combination with GnRH treatment and the effect of limited nursing on reproductive performance of postpartum suckled beef cows administered $PGF_{2\alpha}$ for ovulation control. *Theriogenology* **18,** 87-93.

Kesler, D.J., Garverick, H.A., Youngquist, R.S., Elmore, R.G. and Bierschwal, C.J. (1977) Effect of days postpartum and endogenous reproductive hormones on GnRH - induced release of LH in dairy cows. *J. Anim. Sci.* **45,** 797 - 803.

King, M.E., Odde, K.G., LeFever, D.G., Brown, L.Y. and Neubauer, C.J. (1986) Synchronization of oestrus in embryo transfer recipients receiving demi-embryos with Synchromate B or Estrumate. *Theriogenology* **26,** 221.

Laster, D.B. Glimp, H.A. and Gregory, K.E. (1973) Effects of early weaning on postpartum reproduction of cows. *J. Anim. Sci.* **36,** 734-740.

Leung. K., Padmanabhan, V., Convey, E.M., Short, R.E. and Staigmiller, R.B. (1984) Relationship between pituitary responsiveness to GnRH and number of GnRH - binding sites in pituitary gland of beef cows. *J. Reprod. Fert.* **71,** 267-277.

Lundstrom, K., Abeygunawardena, H., de Silva, L.N.A. and Perera, B.M.A.O. (1982) Environmental influence on calving interval and estimates of its repeatability in the Murrah buffalo in Sri Lanka. *Anim. Reprod. Sci.* **5,** 99-109.

Mikisch, E.D., Mukembo, G., Lefever, D.G. and Wiltbank, J.N. (1978) Synchronization of oestrus in beef cattle. II The effect of injections of norgestomet and an oestrogen in conjunction with a norgestomet implant in heifers and cows. *Theriogenology* **10,** 201.

Mohan, V., Kuruwita, V.Y., Perera, B.M.A.O. and Abeygunawardena, H. (1990) Effect of suckling on the resumption of postpartum ovarian activity in buffaloes. *Trop. Agric. Res.* **2,** 306-315.

Narasimha Rao, A.V. and Venkatramiah, P. (1991) Induction and synchronization of oestrus and fertility in seasonally anoestrous buffaloes with GnRH and a PGF analog. *Anim. Reprod. Sci.* **25,** 109-113.

Odde, K.G. (1990) A review of synchronization of oestrus in postpartum cattle. *J. Anim. Sci.* **68,** 817-830.

Peters, A.R., Lamming, G.E. and Fisher, M.W. (1981) A comparison of plasma LH concentrations in milked and suckled postpartum cows. *J. Reprod. Fert.* **62,** 567.

Rajamahendran, R. and Taylor, C. (1991) Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Hoestein heifers treated with norgestomet. *J. Reprod. Fert.* **92,** 461-467.

Randel, R.D. (1981) Effect of once-daily suckling on postpartum interval and cow-calf performance of first calf Brahman x Hereford heifers. *J. Anim. Sci.* **53,** 755-757.

Reeves J.J. and Gaskin, G.T. (1981) Effect of once-a-day nursing on rebreeding efficiency of beef cows. *J. Anim Sci.* **53,** 889-891.

Rentfrow, L.R., Randel, R.D. and Neuendorff, D.A. (1987) Effect of oestrous synchronization with Synchromate B on serum luteinizing hormone, progesterone and conception in Brahman cows. *Theriogenology* **28,** 355-362.

Riley, G.M., Peters, A.R. and Lamming, G.E. (1981) Induction of pulsatile LH release, FSH release and ovulation in postpartum cyclic beef cows by repeated small doses of GnRH. *J. Reprod. Fert.* **63,** 559-565.

Smith, M.F., Burrell, W.C., Shipp, C.D., Sprott, L.R., Songster, W.N. and Wiltbank, J.N. (1979) Hormone treatments and use of calf removal in postpartum beef cows. *J.Anim. Sci.* **48,** 1285-1994.

Spicer, L.J., Convey, E.M., Tucker, H.A. and Echternkamp, S.E. (1986) Effects of intermittent injections of LHRH on specific binding of $^{125}I$ - labelled gonadotrophin to granulosa and theca, and concentration of steroid in serum and ovarian follicles during postpartum anovulation in suckled beef cows. *J. Anim. Sci.* **62,** 1324-1331.

Troxel, T.R., Kesler, D.J., Noble, R.C. and Carlin, S.E. (1980) Ovulation and reproductive hormones following steroid treatment, calf removal and GnRH in postpartum suckled beef cow. *J. Anim. Sci.* **51,** 652-659.

Virakul, P., Chantraprateep, P., Lohachit, C., Prateep, P., and Demakan, T. (1988) Synchronization of oestrus in swamp buffalo by using norgestomet and norgestomet plus PMSG. *Buffalo J.* **1,** 95-98.

Vorstermans, J.P.M. and Walten, J.S. (1985) Effect of intermittent injections of gonadotrophin releasing hormone at various frequencies on the release of luteinizing hormone and ovulation in dairy cows. *Anim. Reprod. Sci.* **8,** 335-347.

Walters, D.C., Short, R.E., Convey, E.M., Staigmiller, R.B., Dunn, T.G. and Keltenbach, C.C. (1982) Pituitary and ovarian function in postpartum beef cows III. Induction of oestrus, ovulation and luteal function with intermittent small dose injections of GnRH. *Biol. Reprod.* **26,** 655-662.

Webb, R., Lamming, G.E., Haynes, N.B., Hafs, H.D. and Manns, J.G. (1977) Response of cyclic and postpartum cows to injections of synthetic LH-RH. *J. Reprod. Fert.* **50,** 203-210.

NA-143-351

## Session IV - Reproduction

### Panel Discussion

**Q:** B.M.A.O. Perera - What is the farmer demand for herd fertility programmes from commercial dairies and small farms, and what is the method used by farmers for record keeping?

**A:** N.H. Shah - There are five categories of farmers, based on herd size: (a) 1 - 5 milking cows (small farms); (b) 5 - 10; (c) 10 - 20; (d) 20 - 50; and (e) > 50. Within these categories the demand for herd fertility investigation is elicited only by the c, d and e categories (i.e. the large scale commercial farmers). The demand from the small farmers are therefore poor. Farmers keep simple records on fertility parameters such as milk production, services etc. The major reports are computerized and kept by the project's para-professional staff who carry out extension.

**Comment:** N.H. Shah - In Pakistan the buffalo is the main source of milk (71%) and low reproductive efficiency such as the increase in calving interval from 365 days to 635 days decreases the annual milk yield by 2 kg per day. Herd fertility is also reduced due to heat stress, which is seen mainly when high temperature occurs together with dry weather.

**Q:** H. Abeygunawardena - The Nili Ravi buffaloes shown in slides appear to have good body condition. Is this a result of feeding or effective culling?

**A:** N.H. Shah - Since nutrition is an important factor for reproduction, animals are kept at the desired level of nutrition by monitoring the respective blood metabolite levels, using assays developed in Pakistan. Assays in excess of 3000 are being carried out. In addition rigorous culling is also in place among small farms. A high culling rate is due to reproductive problems despite appreciable milk yields giving economic returns.

**Q:** H. Abeygunawardena - In Sri Lanka culling is based on age and not on reproductive performance. The Nili Ravi performance here has been disappointing. Could this be due to environmental factors?

**A:** N.H. Shah - The poor performance of Nili Ravi buffaloes in Sri Lanka cannot be due to humidity, as in Pakistan the reproductive performance is only affected by high temperature combined with dryness.

**Q:** D.K. Singh - What is the major cause of infertility? Is it due to malnutrition or infections?

**A:** N.H. Shah - Nutrition and seasonal heat stress are major factors, but infections are also prevalent.

**Q:** S.K. Ranjhan - You mentioned that buffalo contributes to 71% of milk production. What is their contribution to meat production?

**A:** N.H. Shah - Meat from cattle is about 32%, and meat extracted from buffaloes appears to be 42%. The main area which requires more research is consideration of the dressing percentages and population estimates of buffaloes.

**Q:** P. Bunyawejchevin - At what age was the measurement of prepubertal growth rate done?

**A:** H. Abeygunawardena - At 6 to 12 months.

**Comment:** I. Settergren - The data presented is very interesting and can be applied in herd health programmes to maintain reproductive performance and to implement preventive measures. Buffalo reproduction has become a very strong component of this programme. The results obtained over the years in Sri Lanka could be important for the comparison of reproductive performance in cross breeding dairy buffaloes. The results would have been more meaningful if all the buffalo breeds had been maintained in the same farm.

**Comment:** C. Chantalakhana - It is important to emphasise the concepts of sustainable agriculture that are upheld by CGIAR. Production inputs of Draft Animal Power are very important *vis a vis* commercial production to optimise utilization of available resources. Conservation of natural resources, biodiversity and indigenous genetic resources such as cattle and chicken should be strengthened to prevent dependency on outside influences. A holistic approach for research is therefore essential.

**Comment:** S.K. Ranjhan - Farmers practise sustainable agriculture but the scientists have overlooked their mixed farming practices. There should be more emphasis to study their systems and to try and optimise productivity using research results to increase sustainability. The indigenous animal population cannot meet the demands of the increasing human population. Therefore it is necessary to have commercial producers to meet the demand. Cross breeding has so far failed to improve the productivity of the indigenous population. Therefore what is essential is to improve productivity through selection and proper feeding.

**Q:** C. Chantalakhana - Will the farmers be able to adopt a restricted suckling regime of one or two sucklings at village level?

**A:** H. Abeygunawardena - In many farming systems in Sri Lanka once a day milking is practised and the calf is not allowed to follow the cow. For this system it is recommended to allow suckling for 30 - 45 minutes after milking, and keep the calf separated when the cow is sent to graze. In situations where milking is done twice a day, this can be repeated. The procedure can be easily incorporated to the existing farming practices.

**Comment:** N.H. Shah - Research results indicate significant improvement (73%) of fertility rates. But calf mortality is high, which is a limitation. The possibilities of controlling calf mortality was discussed in the paper. Although this could be done with calf starters, etc. it cannot be recommended, as small farmer's economic returns are poor, and adoption will be difficult at this stage.

**Comment:** C. Chantalakhana - I agree with Dr. Shah's comment. In India also it has been found difficult to get farmers to give calf starters.

**Comment:** D.K. Singh - Experience in South India has shown that calf mortality is around 30 - 40%, but by improving management methods, eg. colostrum feeding 1/2 to 1 hour after birth, etc., farmers were able to reduce mortality. Therefore early weaning was possible without calf mortality and fertility levels increased.

**Comment:** H. Abeygunawardena - The recommendation of early weaning is valid for improvement of indigenous farming systems, particularly under semi-extensive farming in the mid and intermediate zones.

**Q:** N.H. Shah - How was the anoestrous condition defined? If the animals were truly anoestrous GnRH should have a better response. If they were suboestrous, then progesterone implants would give a better response. Is it possible that the animals would have been in suboestrous and not true anestrous?

**A:** H. Abeygunawardena - Animals were defined as anoestrous when they had not shown any heat signs within a period after parturition, and the ovaries did not show any functional activities on palpation.
I do not agree with your comments. GnRH acts on the pituitary to

stimulate FSH/LH release for follicular development in the ovaries. Progesterone inhibits GnRH release at hypothalamic level and its withdrawal brings about a preferential release of LH and FSH that stimulates non functional ovaries. Experience with cattle and other species indicate better response to GnRH in animals with functional ovaries (suboestrous animals) rather than in anestrous animals.

**Comment:** N.H. Shah - Since anestrous condition was determined by rectal palpation diagnostic error could confuse results.

**Q:** Prof. Settergren - What is the number of calving cows that come to heat within the 90 day postpartum period, without treatment and what is the cost of the most effective treatment?

**A:** H. Abeygunawardena - A 55% calving rate had been reported. A majority calved during the early period of the calving season. Therefore treatment requires to be directed only to those that calve during the latter period or during unfavourable environmental conditions due to the reduced fertility observed. The cost of treatment was estimated at Rs. 500.00 and as small producers obtain 5-6 litres milk per day, this is affordable.

**Comment:** S.K. Ranjhan - A cheap cost effective oestradiol vaginal sponge treatment is used in Indonesia with results over 75% for blanket AI after 72 hours.

NA - 143 - 355

# DISEASE PREVENTION AND RECENT INNOVATIONS IN THE CONTROL OF BACTERIAL AND VIRAL DISEASES OF BUFFALOES

D.K. Singh

*Principal Scientist & Head, Biotechnology Laboratory*
*National Dairy Development Board, Anand - 388 001*
*INDIA*

**Abstract:** Buffalo holds greatest promise for production as a good source of milk, meat and draught power. Therefore, the present 148 million world buffalo population is likely to increase at the rate of 10 per cent per annum compared to the projected 5 per cent increase in cattle population. Most of the diseases occurring in cattle are also recorded in buffalo. However, there are differences in their severity and manifestations.

Disease prevention strategies depend on their economic importance, benefit-cost ratio, availability of financial resources, human health hazards etc. Diseases such as rinderpest and Foot and Mouth Disease (FMD), with bearing on international trade, require control as a priority. Financial resources of a country, willingness of the farmers to cooperate, availability of infrastructure and technical know-how effect the efforts made in prevention of diseases: Creation of a National Animal Production and Health Information System (NAPHIS), establishment of disease diagnostic laboratories and development of communication infrastructure are prerequisites for the implementation of an effective disease prevention programme.

Rinderpest, FMD, infectious bovine rhinotrachietis, rabies and calf scours are the important viral diseases of buffaloes which require prevention and control on priority. Recent innovations made for their diagnosis and prevention include development of DNA probes and recombinant vaccines against rinderpest and rabies. Haemorrhagic septicaemia, brucellosis, tuberculosis and paratuberculosis are important bacterial diseases of buffaloes which require immediate control measures. Development of ELISA and double emulsion adjuvanted vaccine for HS; ELISA, DNA probes and PCR for the diagnosis of brucellosis; gamma-interferon ELISA for tuberculosis and PCR for paratuberculosis are major innovations made towards their diagnosis and control.

**Keywords**: Buffalo, bacterial and viral diseases, prevention, control, strategy.

## INTRODUCTION

According to Cockrill (1994), of all the domestic animals, water buffalo holds greatest promise and potential for production. The value of the buffalo as a source of superior milk, meat and as a work animal is now being increasingly recognised. Therefore, buffalo is considered a multipurpose animal. Even then, as per Food and Agriculture Organisation of the United Nations, buffalo has been the most neglected animal. It is only during the last few decades that research efforts have concentrated on the buffalo. The world buffalo population lies between 130 to 155 million, of which about 60 million are in India. Due to several virtues, the annual increase in buffalo population is likely to be 10 per cent.

The general impression is that diseases of buffaloes are identical to those of cattle and that buffaloes are resistant to many of the infections occurring in cattle. Therefore, studies on buffalo diseases have been neglected. While buffaloes are resistant to certain protozoan and viral infections that are serious diseases in cattle, there are others that are more serious in buffaloes. *Toxocara vitulorum* infection for example, is much more serious in buffalo calves than in cattle. Further, metabolism of drugs, their pharmacokinetics and efficacy, significantly differs in buffaloes. A classical example is triclabendazole, which requires much higher dose rates for achieving therapeutic levels in buffaloes (Sanyal, 1995).

## DISEASE PREVENTION STRATEGIES

Diseases are a major impediment to the management of livestock. A wide variety of information is necessary before formulating any disease control strategy. Economic analysis of losses caused by various diseases helps to justify investments required for their prevention and control.

Information regarding incidence rates, losses due to individual diseases, projected benefits over costs for the control of each disease, availability of financial resources and technical know-how about individual diseases, their bearing on international movement and trade of livestock and livestock products will also assist in the prioritization of preventive measures. Analysis of above mentioned parameters must satisfy the farmers and Governments, that the cost and inconvenience likely to be caused by the control programme will be far exceeded by the benefits following control of disease(s).

Apart from economic considerations, human health hazards due to certain diseases such as rabies and brucellosis may help in justifying control/prevention programmes. Cost of creating infrastructure for control, requirement of legislative support, availability of diagnostic reagents and vaccine production facilities for infectious diseases and category of the diseases in accordance to international organisations such as the Office International des Epizooties (OIE) also have a bearing on prioritization of diseases to be prevented. Control of certain OIE list A diseases such as FMD and rinderpest may require priority, as these affect international trade in livestock. Analysis of risk due to presence or introduction of an infectious disease, perception of the farmers, their willingness to cooperate in a programme and probabilities of success of a programme should also be considered while prioritizing the diseases for intervention.

Prevention may be required on a farm or in a locality, state or in a country as a whole. If a disease such as brucellosis which is prevalent in most countries, and is also widely prevalent in the country under reference, its prevention only on a farm maintaining bulls for semen production may be necessary in the form of regular seromonitoring and preventing entry of reactors to the farm. If a country is free from a disease, preventive measures will have to be directed towards avoiding entry into the importing country of carrier animals/animal products from other countries. Emphasis, therefore, will be on confirming freedom in the exporting country or testing of animals including quarantine, before entry into the importing country and restrictions on animal movement at the international borders.

The creation of a National Animal Production and Health Information System (NAPHIS) for monitoring production of animals, their population, incidence of diseases and losses due to these diseases, will be an important first step towards formulating disease prevention strategies. A well established disease diagnostic laboratory and an efficient communication and data processing network is also essential for their implementation.

## INNOVATIONS IN THE CONTROL OF BUFFALO DISEASES

### Viral Diseases

*Foot and Mouth Disease:* This is a febrile, highly contagious disease of all cloven footed animals. The incubation period is 7-10 days, depending on the dose of infection. In buffaloes, the disease is mild, compared to cattle and the duration is shorter. Severity varies with age, state of immunity and nutritional status. Mouth lesions are quite severe and are comparable to those in cattle.

In Asia and the Middle-East, FMD virus types O, A, C and Asia - 1 are prevalent, whereas in West, Central and East African countries, South African Type (SAT) I, II and O, A, C types and in South Africa SAT -I, II and III are recorded. Within each type there are several strains, which differ antigenically. All the secretions and body tissues are positive for the virus. Milk and semen can have virus four days before symptoms appear (Donaldson, 1993). Recovered animals are persistent carriers of virus present in the oesopharyngeal region. Material from pharynx can be used for demonstrating virus by PCR (Donn, 1993).

Tissue culture grown, formalin or BEI inactivated and suitably adjuvanated vaccines incorporating most prevalent strains of each type of the virus are used. There appears to be no cross protection between the types. Calves are given primary vaccination between 8 and 10 weeks of age followed by second vaccination after one month followed by booster vaccinations every 4-6 months.

Large scale FMD control programmes implemented by the Dairy Board of India in the southern peninsular India, indicate that immunity following immunization persists for a period of four months. Therefore, for optimum results, large scale regular vaccination at four monthly intervals, is essential in order to maintain herd immunity. Regular seromonitoring following vaccination, restriction on animal movement in the control area, monitoring prevalence of the virus types, their strain differentiation and incorporation in vaccine, whenever necessary, are essential components of any FMD control strategy.

Cost benefit ratio of FMD control programme is 1:6 but could go up to 1:20, depending on the vaccination coverage. Higher the coverage, greater are the gains. FMD control and prevention programmes are expensive, time consuming and require adequate legislative support for compulsory vaccination of all susceptible species such as cattle, buffaloes, sheep, goats and pigs. Once the incidence becomes sporadic, vaccinations have to be followed by disposal of infected animals and ring vaccinations.

At a recently held workshop on the diagnosis and epidemiology of FMD in South East Asia, an urgent need for establishing a control programme between countries of the SAARC (South Asian Association for Regional Cooperation) was emphasised by Pandit (1995). It was felt necessary to create vaccine banks and undertake surveillance and monitoring of FMD on a regional basis (Copland *et al.*, 1993), irrespective of political boundaries.

*Rinderpest (RP):* Rinderpest is an acute and sometimes subacute, febrile, highly contagious and fatal disease of cattle, buffalo, sheep and goats caused by morbilli virus and characterised by severe haemorrhagic catarrh, formation of diphtheric membranes and ulcers in mucous membranes.

Opinions vary about the degree of susceptibility of buffaloes to rinderpest. Lacrimation is the first sign followed by fever on day two, post infection. Sudden drop of milk yield, erythema and ulcers in the buccal mucosa, dental pad and papular or pink rash affecting skin of the perineum, scrotum and in the inner thigh are common signs, leading either to recovery or death. Diarrhoea, leucopenia with lymphocytopaenia are commonly recorded.

Culture attenuated Kabette 'O' strain of RP virus is extremely safe and is used in most RP control programmes. It is not transmitted to other animals and confers life long immunity. The only problem is its sensitivity to heat thus requiring the maintenance of an expensive cold chain.

Protection against RP is possible with vaccinia virus recombinants expressing the hemagglutination (HA) or fusion (F) gene (Yilma *et al.*, 1988). The use of vaccinia virus as a vector which expresses heterologous viral antigen allows its use as a live thermostable vaccine for RP.

RP has been eradicated from many countries and epizootics occurring in western, central and eastern Africa during the 1980s had undergone remission. However, during 1990-94 sporadic outbreaks were again reported in Kenya, Sudan and Uganda, whereas the disease is enzootic in Ethiopia. In Turkey the disease appeared in 1991, after a lapse of 20 years. Whereas outbreaks are reported in Lebanon, Oman, Saudi Arabia, and in the United Arab Emirates. In Asia, RP is present in Pakistan, Russia, Sri Lanka and in the Southern states of India.

A series of internationally funded eradication campaigns such as the Pan Africa Rinderpest Eradication Campaign (PAREC) in Africa, the South Asia Rinderpest Eradication Campaign (SAREC), and the West

Asia Rinderpest Eradication Campaign (WAREC) in Asia are in operation. Presently, the Government of India and the European Economic Commission (EEC) are implementing Operation Rinderpest Zero (ORZ). This programme envisages discontinuation of vaccination and seromonitoring in the northern states of India which are free from RP and vaccination in the southern states where isolated cases of RP are reported.

Accurate and rapid diagnosis is the key to success of any control strategy. Agar gel precipitation and serum neutralization tests are commonly used. Competitive ELISA using monoclonal antibodies are useful for differentiating the related viral infections. Hybridization techniques using cDNA are useful in early and specific diagnosis (Diallo *et al.*, 1989). Reverse transcription and polymerase chain reaction (RT-PCR) developed by Barret *et al.* (1993) has greatly facilitated differential diagnosis between RP, pes des petis ruminant and canine distemper.

*Ephemeral fever* (Three days sickness): It is characterized by febrile reactions, stiffness of muscles, inappetance and recovery usually occurs within a few days. Affected buffaloes show sudden rise of temperature, rigors, watery eyes, swelling of eye lids and lameness including difficulty in deglutination. The disease is present in several countries, including Australia and is transmitted by blood sucking insects during warm moist climate. Control of insects is the best strategy. Recently in Australia tissue culture attenuated vaccines have been developed.

*Infectious Bovine Rhinotracheitis (IBR):* IBR is a clinical disease of cattle and buffaloes characterised by tracheitis, rhinitis, enteritis and fever. The virus can also cause conjunctivitis, vulvovaginitis and abortion. In buffaloes vulval eruptions and abortions are reported. Seropositivity for IBR in buffaloes in India is high and virus has been isolated from semen and aborted foetus. Infection can be transmitted during natural breeding and artificial insemination (AI). Infective status can be confirmed by serological tests such as IHA, virus neutralization and ELISA. Positive bulls should not be used for breeding.

*Rabies:* Rabies is a fatal disease of all warm blooded animals. The disease is usually transmitted by the bite of rabid dogs and foxes, therefore, it is more common in grazing buffaloes. In Brazil most cases of rabies are caused due to the bite of vampire bats (*Desmodus rotundus*) (Lau, 1994). Buffaloes generally show symptoms of ataxia, incordination, tympany, grinding of the teeth and constipation. Once suspected, treatment should be immediately stopped and disease allowed to take its course, where death should occur within 6-7 days of first appearance of symptoms. Control of rabies includes destruction of dogs and post exposure vaccination. Animals bitten by suspected rabid dogs or foxes are given postbite vaccination with vaccines containing formalin treated rabid sheep brain tissue suspension, daily for 14 days. Recently, vaccines of tissue culture origin have been developed. These are less painful and require a smaller dose and a fewer number of inoculations.

Widespread distribution of bait containing capsules of antirabies vaccine to wild life, either manually or by aircraft have been used in Austria, Belgium, France, Germany, Switzerland, Finland, Poland, Slovakia and Slovenia for the control of sylvatic rabies (Anon, 1995a). Destruction of stray dogs, vaccination of pets and oral vaccination of wild animals through bait may be the best strategy for control.

*Buffalo pox:* The disease is specific to buffaloes and has been reported in epidemic forms in India, Pakistan, Indonesia and Italy. It is enzootic and seems to be related to cowpox. Milkers may get infected from affected teats. Vero cell culture attenuated buffalo pox virus vaccine developed by Mohanty and Rai (1988) is reported to be quite effective.

*Calf scours:* Neonatal calf diarrhoea due to rotavirus and enterotoxigenic *Escherichia coli* (ETEC) infection in buffalo calves is associated with yellowish white diarrhoea. Clinically the disease is mild to severe and most cases occur between 0 to 28 days. Rotavirus infection in buffalo calves is more prevalent during winter months (Khattar and Pande, 1988). The presence of virus in faeces can be confirmed by various serological tests such as AGPT, CIE and ELISA. Polyacrylamide gel electrophoresis studies indicate the occurrence of two distinct types.

Occurrence of rotavirus has been reported in cattle, buffaloes and human babies as the main cause of enteritis in many countries including those in Europe (McNulty and Logan, 1983).

ELISA for demonstrating ETEC is efficient. Using fast protein liquid chromatography for separation of K-99 antigen of ETEC and their subsequent use for development of specific antibodies for use in a coagglutination test, is a recent development towards diagnosis of colibacillosis in calves in India (Anon, 1995b).

**Bacterial Diseases**

*Haemorrhagic septicaemia:* The term Haemorrhagic septicaemia (HS) is now used to describe an acute fatal disease, primarily caused by two serotypes of *Pasteurella multocida* in cattle and buffaloes. Earlier the *P. multocida* were classified based on somatic and capsular antigens. Currently, Carters typing is most popular. Serotype 6:B and 6:E are the serotypes of *P. multocida* associated with HS in cattle and buffaloes in Asia and Africa, respectively (Carter, 1961).

In most areas where HS occurs, it ranks as the most important disease of buffaloes and is prevalent in South East Asia, in Southern Europe, Russia, North, West and East Africa and North, East and South East Asia (Cockrill, 1974). Regular vaccination has enabled effective control of the disease. However, epidemiological studies have indicated the existence of carriers among healthy animals.

Diagnosis of HS is based on the signs, cultural examination of blood and mouse inoculation. The diagnostic tests used in various countries should be standardised with respect to (a) isolation, identification and serotyping of the organism, (b) use of ELISA for detection of HS antigen, (c) use of ELISA in epidemiological studies and (d) ribotyping for understanding the pathogenicity and epidemiology of *P. multocida*. The latter technique has been used for the differentiation of *P. multocida* isolates from reference vaccine strains in Turkeys (Kim and

Nagaraja, 1990). Selection of most antigenic strain of *P. multocida* is necessary for developing an effective vaccine. In most countries, where HS prevails vaccination is practised to control the disease. Wherever vaccination is not undertaken or is irregular, losses are high.

Three types of vaccines are commonly in use; broth bacterins, alum precipitated and oil adjuvant vaccines. The oil adjuvant vaccines provide longer immunity. However, these are not popular due to difficulties in mass production and poor acceptability by the farmers due to swelling at the site of inoculation. Vaccines based on thinner emulsions, including double emulsion oil adjuvant vaccine needs further evaluation for their efficacy. The use of avirulent mutants or serologically related avirulent strains that will multiply in the animal system and produce the relevant antibodies without causing disease will produce solid immunity which may not be attainable by conventional vaccines.

*Brucellosis (Bang's disease):* Brucellosis has world wide distribution wherever cattle and buffaloes are maintained. It is of zoonotic importance and is characterised by inflammation of foetal membranes, endometrium, foetal tissues and is associated with abortion or premature births, followed by infertility in aborted females. In males, Brucella organisms cause orchitis, which can lead to sterility.

Buffaloes and cattle are equally susceptible and incidence varies from herd to herd. Large herds tend to have a higher incidence of up to 80 per cent, whereas, in animals managed individually, the incidence is usually low, ranging between 2 and 5 per cent. Infected females are apparently normal and are recognised only when a serological test is performed or abortion takes place. Abortion takes place during the 2nd trimester of pregnancy following mild straining and is usually followed by retention of placenta.

All premature births should be suspected for brucellosis and confirmed by serological tests. Several serological tests such as Standard Tube Agglutination Test (STAT), Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT) are available, with marginal variations in specificity and sensitivity. For the large scale screening of dairy herds, MRT is applicable. For large surveys RBPT is the test of choice, whereas CFT is used for confirmation of brucellosis. Since CFT is more cumbersome than STAT, the latter is also used for confirmation. Recently an ELISA has been validated for differentiation of infected and vaccinated animals (Nielsen *et al.*, 1989). DNA probes and PCR are also being developed for diagnosis of brucellosis.

The affected animals should be suitably isolated, premises disinfected and the infected bulls should not be used for AI or natural service. Use of the *Brucella abortus* strain 19 vaccine in female calves between 3 to 6 months of age is the most common vaccination procedure. For adults, a reduced dose vaccination with *Brucella abortus* strain 19 is used in USA. The killed vaccine incorporating *Brucella abortus* strain 45/20, although available commercially, is not very popular in Asia as it requires annual revaccinations.

*Vibriosis:* Genital camphylobacteriosis is an important cause of infertility in cows and buffaloes and is caused by *Campylobacter fetus fetus*. The main clinical manifestations are early abortion, repeated oestrus and delayed conception. Repeated return of females to oestrus is an important sign of the disease in a herd. Cattle and buffaloes are equally susceptible. Vibriosis in buffaloes is a self limiting disease. Infected females should be given at least three months of sexual rest and semen should be properly treated with antibiotics. Positive bulls should not be used for breeding.

*Anthrax:* Buffalo like most other mammals is susceptible to infection by *Bacillus anthracis*. Although there is a decrease in the incidence following vaccination with spore vaccine, isolated cases of the disease continue to occur mainly due to economic factors such as (i) failure to recognize and report the disease, (ii) tendency to slaughter the sick animals and utilise for human consumption, (iii) use of hides, (iv) improper burial of carcasses and (v) use of brushes made from unsterilized sheep bristles.

*Tuberculosis:* Tethered buffaloes maintained for milk production are quite susceptible to tuberculosis. Like cattle, infection can be generalised or localised in the lungs, intestine, udder and brain in buffaloes. Surveys in India have reported higher incidence in buffaloes than in cattle (Lall, 1969). Most cultures of tubercle bacilli from buffaloes are *Mycobacterium bovis*. In Australia, of 11,909 buffaloes examined during slaughter, 16.4 per cent were found to be positive for tuberculosis of which 2.3 per cent had generalised lesions.

Diagnosis is based on delayed type hypersensitivity reaction following intradermal inoculation of PPD, the presence of granuloma and detection of acid fast bacilli. Simple serological tests are unsatisfactory because of low levels of antibodies, which also cross react with non-tuberculous Mycobacteria and Nocardia species (Daniel and Janicke, 1978). ELISA using *M. bovis* protein extracts lack desired specificity and sensitivity to be used in an eradication campaign (Radford *et al.*, 1988). Development of two stage *in vitro* blood cultures and gamma interferon enzyme immunoassay in which the whole blood from suspected animals is reacted with bovine tuberculin PPD derived from *M. bovis* ANS strain, to stimulate production of gamma interferon from the circulating T-cells already sensitized by prior exposure to *M. bovis* in the first stage; gamma interferon is then measured in an enzyme immunoassay with plates coated with antibodies against recombinant gamma interferon in the second stage. It is 93.6 per cent sensitive and being non-invasive can be repeated many times in a herd (Rothel *et al.*, 1993). PCR and DNA probes are being used to detect antigen in blood and tissues of carcasses (Ward *et al.*, 1995).

*Johne's disease:* It is a highly fatal chronic disease of cattle, buffalo, sheep and goat characterised by recurrent diarrhoea, emaciation, cachexia and debility associated with thickening and corrugation of intestinal walls. All breeds and types of buffaloes can get infected with Johne's disease. The incidence in an organised buffalo herd may be very high resulting in 12-15 per cent annual mortality. Generally the infection takes place at an early age but symptoms start to appear only after the 2nd or 3rd calving. The first symptom is a dry coat without fever, progressive emaciation followed by severe intermittent diarrhoea. The faeces is fluid, dark coloured with mucus which soils the hind quarters, perineum and tail.

It is difficult to eliminate the disease from a farm. Major steps include regular testing with Johnin and removal of positive reactors and clinically positive buffaloes, immediate weaning of calves and feeding pasteurised milk and the removal of doubtful reactors, which may act as shedders. Recently it has been demonstrated that *Mycobacterium paratuberculosis* gets generalized in the body and is also present in peripheral macrophages much before clinical symptoms appear, which can be demonstrated by PCR using specific IS gene as target. The test can detect the disease much before shedding of organisms starts and is very useful in eliminating future shedders (Van der Giessen, 1993). Vaccination may reduce the incidence of clinical disease but not the infection rate (Larsen *et al.*, 1978).

## References


Anon (1995a) The Office Internationale des Epizooties (OIE), *Pashudhan*, **10**, pp 1-8.

Anon (1995b) Annual Report of Biotechnology Laboratory, National Dairy Development Board, Anand, India, 1994-95, pp 16.

Barrett, T., Amarel-Deol, Kitching, R.P. and Gusev, A. (1993) Use of the polymerase chain reaction in differentiating rinderpest field virus and vaccine virus in the same animal. *Rev. Sci Tech. Off. Int. Epi.* **12,** 865-872.

Carter, G.R. (1961) A new serological type of *P. multocida* from Central Africa. *Vet. Rec.* **73**, 1052.

Cockrill, W.R. (1974) *The Husbandry and Health of the Domestic Buffalo.* In: Food and Agricultural Organisation, Rome.

Cockrill, W.R. (1994) Present and future of buffalo production in World. *Fourth World Buffalo Congress*, 27-30 June 1994, Sao Paulo, Brazil, Vol-III, pp 2-21.

Copland, J.W., Gleeson, L.J., and Chamnan, P. C. (1993) Diagnosis and epidemiology of foot and mouth disease in South East Asia. In: *Proceedings of International Workshop*, 6-9 Sept. 1993. Lapang, Thailand.

Daniel, T.M. and Janicke, B.W. (1978) Mycobacterial antigens: a review of their isolation, chemistry and immunological properties. *Microbiol. Reviews* **41**, 84-113.

Diallo, A., Barrett, T., Barbron, M., Shaila, M.S. and Taylor, W.P. (1989) Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones. *J. Virol. Methods* **23**, 127-136.

Donaldson, A.I. (1993) Epidemiology of foot and mouth disease; The current situation and new prospectives. In: *Proceedings of International Workshop on FMD*, 6-9 Sept. 1993, Lampang, Thailand. pp.9-15.

Donn, A. (1993) The pathogenesis of persistance of foot and mouth disease virus in experimentally infected cattle and in a model cell system. Ph.D thesis, University of Hertfordshire.

Khattar, S. and Pande, R. (1988) Epizootiology of rotavirus infection in buffalo calves as revealed by some antibody mediated tests and electrophoresis of genome-RNA. *Second World Buffalo Congress*, 12-16 December, 1988, India. Vol.IV, pp 21-26.

Kim, C.J. and Nagaraja, K.V. (1990) DNA finger printing in differentiation of field isolates from reference vaccine strains of *Pasteurella multocida* in Turkeys. *Am. J. vet. Res.* **51**, 207-210.

Lall, J.M. (1969) Tuberculosis among animals in India. *Vet. Bull.* **39**, 385-390.

Larsen, A.B., Moyle, I.A. and Himes, E.M. (1978) Experimental vaccination of cattle against paratuberculosis (Johne's Disease) with killed bacterial vaccines, a controlled field study. *Am. J. vet. Res.* **39**, 65-69.

Lau, H.D. (1994) Important economic diseases in buffaloes. In: *Fourth World Buffalo Congress*, 27-30, June 1994, Sao Paulo, Brazil, Vol.I, pp 209-220.

McNulty, M.S. and Logan, E.F. (1983) Longitudinal survey of rota virus infection in calves. *Vet. Rec.* **113**, 333-335.

Mohanty, P.K. and Rai, A. (1988) Immunogenicity of cell culture vaccines against buffalo pox. In: *Second World Buffalo Congress* 12-16 December 1988, India, Vol.IV, pp 1-4.

Nielsen, K.H., Cherwonogrodzky, J., Duncan, J.R. and Bundle, D.R. (1989) Enzyme immunoassay for differentiation of the antibody response of *Brucella abortus* infected or strain - 19 vaccinated cattle. *Am. J. vet. Res.* **50**, 5.

Pandit, K.K. (1995) Now what is new. *Asian Livestock* Vol. 9. FAO Regional Office, Bangkok, Thailand, pp 107-108.

Radford, A.J., Duffield, B.T. and Plackett, P. (1988) Cloning of a species specific antigen of *Mycobacterium bovis*. *Infect. Immun.* **56**, 921-925.

Rothel, J.S., Corner, L.A. and Wood, P.R. (1993) Bovine tuberculosis: immunodiagnosis. In: *Australian Standard Diagnostic Techniques for Animal Diseases*. Corner, L.A. and Baguest, T.J. (Eds.) The standard committee on Agriculture and Resource Management. East Melbourne.

Sanyal, P.K. (1995) Kinetic disposition of triclabendazole in buffalo compared to cattle. *J. Vet. Pharm.Toxicology.* **18**, 370-374.

Van der Giessen, J.W.B. (1993) A molecular approach to the diagnosis and control of bovine paratuberculosis. Ph.D Thesis, University of Utrecht.

Ward, B.T., Collins, D.M. and de Lisle, G.W. (1995) Detection of *Mycobacterium bovis* in tissues by polymerase chain reaction. *Vet. Microbiol.* **43**, 227-240.

Yilma, T., David, H.S.U., Johnes. L., Owens, S., Grubman, M., Mebus, C., Yamanka, M. and Dale, B. (1988) Protection of cattle against rinderpest with infectious vaccinia virus recombinants expressing the HA or F gene. *Science* **242**,1058-61.

NA - 143 - 371

# FURTHER STUDIES ON THE EPIDEMIOLOGY AND IMMUNOLOGY OF HAEMORRHAGIC SEPTICAEMIA IN BUFFALOES

M.C.L. de Alwis[1], N.U. Horadagoda[2], T.G. Wijewardana[1]
P. Abeynayake[2], A.A. Vipulasiri[1] and S.A. Thalagoda[1]

[1]*Veterinary Research Institute, Gannoruwa, Peradeniya,*
[2]*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

**Abstract:** Three separate studies were conducted to investigate some aspects of the epidemiology and immunology of haemorrhagic septicaemia (HS) in buffaloes. The existence of 'Latent' and 'Active' carriers in HS, and the intermittent conversion of latent carriers into an active state have been demonstrated in earlier studies. Further it was found that the carrier state could not be cleared by treatment using antibiotics to which the organism was sensitive *in vitro*. In the present study immunohistochemical methods were used to determine the exact site of localisation of the Pasteurellae within the tonsils of HS carriers. Attempts were made to determine the role of immunosuppression induced by corticosteriods in converting 'Latent' carriers to 'Active' carriers. The immunological aspects investigated in the present study were focused on the immune response of buffaloes to the current oil adjuvant vaccine.

The carrier state was established in 13 buffaloes by exposure to a marked strain of *Pasteurella multocida*. Five buffaloes were slaughtered and the tonsils collected and fixed in 10% buffered saline and subjected to immunohistochemical studies using the peroxidase anti-peroxidase test, to visualise the organisms in tissue sections. The immune status and carrier status of the balance eight animals were monitored. When these animals were in a latent phase, they were subjected to heavy dosing with steroids alone or in combination with Neostigmin. In the vaccination trial, 38 out of 47 antibody free calves were vaccinated with HS oil adjuvant vaccine. The serological responses of these and 9 unvaccinated controls were monitored monthly by the indirect haemagglutination test, passive mouse protection test and the ELISA. Animals from each group were challenged at 9 m and 12 m by subcutaneous inoculation of a field isolate of *P. multocida* type 6:B ($10^8$ cfu). The results indicated that the pasteurellae in HS carriers were localised exclusivley in the crypts of the tonsils and that treatment of latent carriers with steroids and Neostigmin failed to trigger the conversion of "latent carrier" to an "active carrier". The protection produced by the oil adjuvant vaccine to direct challenge at 9 m and 12 m was 50% and 67%, respectively. There was however no correlation between the response to direct challenge and antibody titres.

**Keywords**: Haemorrhagic septicaemia, buffalo, carrier state, tonsil, vaccines

## INTRODUCTION

Haemorrhagic septicaemia (HS) is an acute, fatal septicaemic disease of buffaloes and cattle caused by specific serotypes of *Pasteurella multocida*. The disease causes serious economic losses in Asia through morbidity and mortality, and it has been established that buffaloes are more susceptible than cattle.

Several workers (Singh, 1948; Gupta, 1962; Wijewantha and Karunaratne, 1968) have shown that the causative organism persists in a small percentage of carrier animals and that in these animals the organism is detectable in the nasopharynx. Other studies (Gupta, 1962; Hiramune and De Alwis, 1982) revealed that the presence of the organism in the nasopharynx of carriers is related to the recent incidence of the disease. More recent studies by De Alwis *et al*. (1986; 1990) with free range and experimentally produced carrier animals have demonstrated that (a) following an outbreak of disease a large number of clinically unaffected animals became carriers, (b) after a brief period the carriers became 'latent' with the organism disappearing from the nasopharynx, (c) the organism intermittently reappeared in the nasopharynx and (d) during the period of latency, the organism persisted in the tonsils and remained unaffected by antibiotics to which it was sensitive *in vitro*. These findings led to the investigation of the factors which triggered the conversion of a latent carrier to an active carrier and the exact localisation of the organism in the tonsil which allowed the organism to evade the effects of antibiotics.

The immunological studies reported herein are focused on the evaluation of immunity provided by the oil adjuvant vaccine (OAV) currently used in Sri Lanka. The OAV was originally introduced to the country in 1956 but discontinued in 1963 and re-introduced in 1965. Studies carried out in the late 1970's demonstrated that the OAV protected animals for 6-9 months following a single injection (De Alwis, *et al.*, 1978). Since then several improvements have been made in the production technology which include the incorporation of a newly defined vaccine seed culture, a newly formulated growth medium and emulsification formula. The equipment used for bulk culture production has also been upgraded. These changes demanded an evaluation of the immunity induced by the improved vaccine in buffaloes.

## MATERIAL AND METHODS

### Localisation of *Pasteurella multocida* Type B:2 in the Tonsils

Five non-immune buffalo calves aged 7 to 9 months were experimentally infected intranasally with a streptomycin resistant strain of *Pasteurella multocida* serotype B:2 (1.12-2.4 x $10^8$ CFU). Following infection, the presence of the organism in the nasopharynx was monitored daily up to 7 days and every third day thereafter. Blood samples were collected at weekly intervals for assay of antibodies by the indirect haemagglutination (IHA) test (Wijewardana *et al.*, 1986).

Three animals were slaughtered 24 days and 2 animals 52 days after infection. Tonsils collected at slaughter were subjected to bacteriological examination and portions of tissue were fixed in 10% neutral buffered formalin for immunohistochemical studies. The organism in tissue sections was visualised by the peroxidase-anti-peroxidase (PAP) technique using a 1:5000 dilution of a rabbit hyperimmune antiserum to *Pasteurella multocida* serotype B:2 (Horadagoda and Belak, 1990). Tonsillar tissue collected from 2 non-infected buffalo calves and 6 bovine calves at the Uppsala slaughter house in Sweden were used as negative controls. Isolation and identification of the organism in swabs and tonsillar tissue were carried out as described by De Alwis, *et al.*, (1990).

## Investigation of Factors Responsible for the Conversion of Latent Carriers to Active Carriers

Eight male buffalo calves were used in this study. Carriers were produced following intranasal inoculation of a streptomycin resistant strain of *P. multocida*. The infected animals were maintained at the laboratory premises and nasopharyngeal swabs were collected daily up to 10 days and once a week thereafter. Antibodies were examined by the IHA technique (Wijewardana, *et al.*, 1986) in blood collected on the 10th day after infection as well as in samples collected at weekly intervals thereafter.

The conversion of a latent carrier to an active carrier was studied in a series of experiments in which three preparations were administered to carrier animals at different time points leaving a sufficient time between each experiment for drug excretion.

*Experiment I:* The carrier animals were given 40 mg of Dexamethasone (Dexona injection; Cadilla ) intramuscularly.

*Experiment II:* The animals were given 5 mg of Neostigmin (Pratigmin; Roche) intramusculary followed by 15mg of Dexamethasone by the same route.

*Experiment III:* Methylprednisolone acetate (Depo-Medrol:Upjohn) was given on the first day of the experiment followed by 40 mg of Dexamethasone given intramuscularly two days later and Neostigmine on the third day of the experiment. The Neostigmine was given both intramuscularly (5mg) and intravenously (15mg) at the same time.

All animals were bled before and after each drug administration and the IHA test was performed to detect any changes in the antibody titre. In addition, nasopharyngeal swabs were obtained at weekly intervals in order to detect the organism during each period of drug administration.

## Immune Response to the Oil Adjuvant Vaccine

*Animals:* Forty seven buffalo calves (4-6 m) were selected for this study based on the absence of antibodies to *P. multocida* serotype B:2 as demonstrated by the indirect haemagglutination test (IHA) and the passive mouse protection test (PMPT). All animals were maintained at a state farm throughout the experiment and transported to the veterinary research institute only for the purposes of the challenge.

*Vaccine:* The oil adjuvant vaccine produced at the veterinary vaccine laboratory, Peradeniya (Peiris and De Alwis, 1991) was supplied for this study by Dr. L.K.P.N. Peiris. Thirty eight animals were given 3.0 ml of vaccine intramuscularly, followed by a booster 3 months later by the same route (Log protection of the vaccine used on two occasions were 6.71 and 7.0 when tested in mice).

*Serum:* Blood was collected from all 47 animals at monthly intervals. The serum was inactivated ($56^0$ C for 30 min) and then stored at $-20^0$ C until antibody titres were assayed.

*Serological Tests:* Serum samples were tested for the presence of antibodies by the IHA test described by Carter (1955) and later modified by Wijewardana *et al.* (1986) using fresh sheep erythrocytes. The passive mouse protection test was also performed to determine the antibody status. Briefly, serum from each buffalo was inoculated into a group of 5 mice (except in the 9th month where only 3 mice were used) with each mouse receiving a 0.5 ml volume of serum subcutaneously. Twenty four hours later they were challenged with 0.5 ml of a 6 hour broth culture of a field isolate (VRI culture collection No. 8406) diluted $10^{-7}$ that gave approximately a 100 $LD^{50}$. As there was no protection indicated in pre-vaccination sera only a challenge control group was included for the test. The antibody titre was also assayed by the ELISA described by Horadagoda *et al.* (1994).

*Direct Challenge Test in Buffaloes:* Vaccinated and control buffaloes were challenged at 9 and 12 months post-immunisation. One millilitre of a diluted (1:10) 6 hour broth culture of a local field isolate (VRI culture No. 8406) was injected by the subcutaneous route. At 9 months, six vaccinated and 4 control animals selected at random were also subjected to direct challenge with $1.1 \times 10^8$ live organisms. Similarly, at 12 months, 6 vaccinated and 3 control animals selected at random were subjected to a direct challenge with an inoculum containing $1.6 \times 10^8$ live organisms. All challenged animals were observed over two weeks and deaths recorded.

## RESULTS

### Localisation of *Pasteurella multocida* in the Tonsils

*P. multocida* antigen was detected as a brown precipitate in formalin-fixed, paraffin embedded tissue sections by the PAP technique. In the tonsils of infected animals, immunostaining was confined to the lumen of the tonsillar crypts. The epithelium and lymphoid follicles were devoid of any immunoreactivity (Figure 1). In the crypts, diffuse DAB precipitate was present amidst desquamated epithelial cells and necrotic debris of the lumen (Figure 2), while intracellular staining was evident in the cytoplasm of the macrophages (Figure 3). Tonsil sections from control animals did not show immunoreactivity. Moreover immunostaining was not present when the primary antibody was replaced by normal rabbit serum.

All infected animals demonstrated very high antibody titres to HS when assayed by the indirect haemagglutination test (Table 1). Bacteriological examination of the nasopharynx demonstrated that the pasteurellae were present only during the first few days after infection (Table 2 ). However, in all the animals the organism was isolated from the tonsils after slaughter.

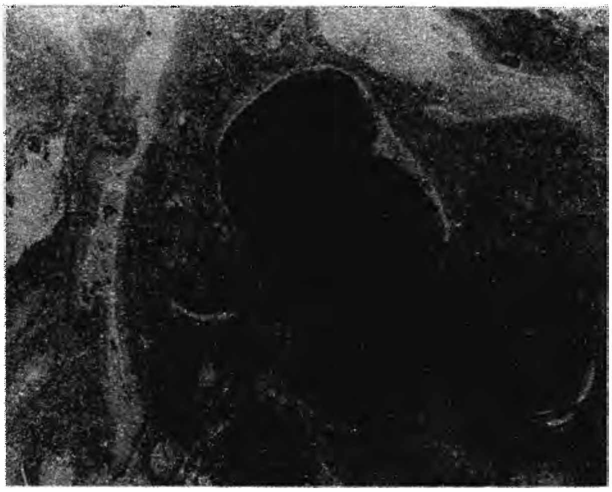


**Figure 1.** Tonsil of a HS carrier buffalo revealing immunoreactivity in the lumen of the tonsillar crypt. Immunoperoxidase/ haematoxylin x25.

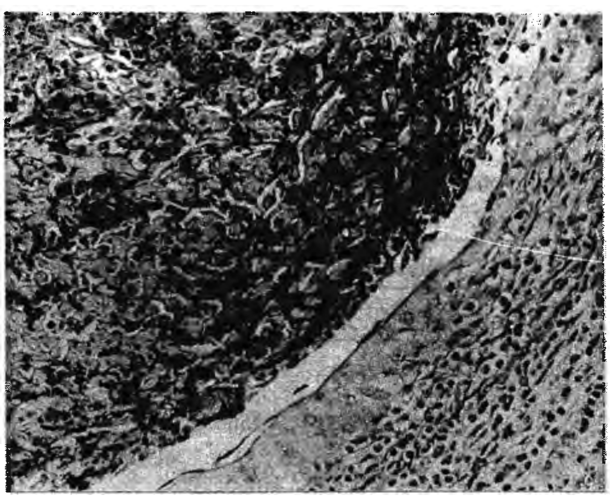


**Figure 2.** Diffuse immunostaining amidst the desquamated squamous epithelial cells and necrotic debri of the tonsillar crypt. Immunoperoxidase/haematoxylin x100.

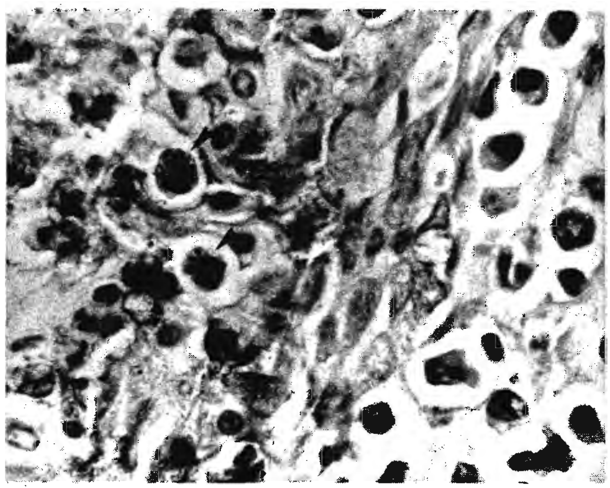


**Figure 3.** Intracytoplasmic immunostaining of macrophages (arrow) in the lumen of the tonsillar crypt. Immunoperoxidase /haematoxylin x250.

**Table 1.** Reciprocal antibody titres of HS - carriers after intranasal infection.

| Animal No. | Antibody titres in the post infection period (weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | - | - | 2560> | 2560> | | | |
| 2 | - | 40 | 2560> | 2560> | | | |
| 3 | - | - | 2560> | 2560> | | | |
| 4 | - | 1280 | 2560> | 2560> | 2560> | 1260> | 640 |
| 5 | - | 160 | 2560> | 2560> | 2560> | 2560> | 1260 |

**Table 2.** Isolation of *P. multocida* from tonsils and nasopharynx (0-7 days) of HS carriers after experimental infection (pi).

| Animal No. | Tonsil | Isolation of organism in the Nasopharynx pi (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | + | + | + | - | + | - | - | - |
| 2 | + | + | + | + | + | + | - | - |
| 3 | + | + | + | - | - | - | - | - |
| 4 | + | + | + | + | + | - | - | - |
| 5 | + | + | + | + | - | - | - | - |

Animals 1-3 - organism present in the tonsil 24 days pi
Animals 4-5 - organism present in the tonsil 52 days pi

## Investigation of the Factors Responsible for the Conversion of Latent to Active Carriers

*Experiment I:* Twenty four hours after the administration of dexamethasone there was profuse salivation and an elevation of body temperature in 4 of the 8 animals. The organism was not detected in the nasopharynx and there was no change in the antibody titre.

*Experiment II:* The response to Neostigmine was characterised by profuse nasal and salivary secretions. The clinical changes commenced thirty minutes after administration and persisted for two hours. In this experiment too, *P. multocida* was not isolated and there was no change in the antibody titre.

*Experiment III:* The clinical behaviour of animals following the administrations in this experiment was similar to that observed in experiments I and II. The organism was not isolated and there was no change in antibody titres.

## Immune Response to the HS Oil Adjuvant Vaccine

*Haemagglutinating antibodies:* Table 3 shows the IHA antibody response to the oil adjuvant vaccine. Antibodies were first detected two months after inoculation in two of the 26 animals. The percentage of animals showing IHA antibodies gradually increased reaching 100% by the 11th month, post-vaccination. In the control animals, haemagglutinating antibodies were observed during the course of the experiment although none of the animals showed titres at the time of vaccination.

**Table 3.** The number and the percentage of buffaloes showing IHA titres.

| Number of buffaloes | Proportion of animals showing IHA titres at months post-vaccination | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Vaccinated (38) | 0/38 | 2/26 | 15/26 | 26/37 | 32/37 | 32/38 | 26/28 | 30/33 | 21/31 | 23/33 | 23/27 | 26/26 | 11/27 |
| % | 0 | 8 | 58 | 70 | 86 | 84 | 93 | 90 | 68 | 70 | 83 | 100 | 40 |
| Control (09) | 9/9 | 0/5 | 0/4 | 4/8 | 3/9 | 3/3 | 7/9 | 5/8 | 4/9 | 3/9 | 1/5 | 3/5 | 0/5 |
| % | 0 | 0 | 0 | 50 | 33 | 100 | 77 | 63 | 44 | 77 | 20 | 60 | 0 |

*Passive Mouse Protection Test:* The results of the PMPT are shown in Table 4. The findings indicate a marked mouse protection during the 4th and 5th months followed by an uneven decline to 4% by the 12th month.

*Enzyme-Linked Immunosorbent Assay:* Table 5 shows the antibody titres when measured by the ELISA. In this assay, titres were observed only after 3 months from vaccination. The highest titres were observed between 6 and 12 months after vaccination (ELISA units up to 64), but there was no relationship between the ELISA titre and protection after direct challenge.

**Table 4.** The number and the percentage of buffaloes indicating mouse protection.

| Number of buffaloes | Proportion of buffaloes indicating mouse protection at months post-vaccination | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Vaccinated (38) | 0/38 | 25/38 | 3/36 | 13/38 | 20/36 | 24/37 | 7/27 | 7/35 | 11/28 | 3/27 | 0/26 | 4/26 | 1/27 |
| % | 0 | 66 | 8 | 34 | 56 | 65 | 26 | 20 | 39 | 11 | 0 | 15 | 4 |
| Control (09) | 0/9 | 0/8 | 0/4 | 0/9 | 0/9 | 0/9 | 8/9 | 6/8 | 9/9 | 9/9 | 3/5 | 4/4 | 5/5 |
| % | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 75 | 100 | 100 | 60 | 100 | 100 |

**Table 5.** The number and the percentage of buffaloes indicating titres in ELISA (ELISA units).

| Number of buffaloes | Proportion of buffaloes indicating ELISA units at months post-vaccination | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Vaccinated (38) | 0/38 | 0/31 | 0/30 | 27/35 | 1/37 | 26/36 | 27/27 | 26/33 | 31/31 | 32/32 | 19/26 | 27/27 | 26/26 |
| % | 0 | 0 | 0 | 72 | 3 | 72 | 100 | 79 | 100 | 100 | 73 | 100 | 100 |
| Control (09) | 0/9 | 0/8 | 0/4 | 0/9 | 0/9 | 0/9 | 8/9 | 6/8 | 9/9 | 9/9 | 3/5 | 4/4 | 5/5 |
| % | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 75 | 100 | 100 | 60 | 100 | 100 |

The antibody levels detected by the three serologic test *viz.* IHA, PMPT and ELISA together with the animals that survived the direct challenge at 9 and 12 months, post vaccination are shown in Table 6 and Table 7, respectively.

**Table 6.** The antibody level detected by IHA, PMPT and ELISA and animal survival after direct challenge at 9 months post vaccination.

| | Animal No. | Reciprocal IHA | PMPT | ELISA units | Survived or died |
|---|---|---|---|---|---|
| Vaccinated | 1685 | -ve | 0 | 4 | Died |
| | 1696 | 40 | NT | 4 | Died |
| | 1697 | 20 | NT | 4 | Survived |
| | 1699 | 40 | 0 | 8 | Survived |
| | 1721 | -ve | 0 | 8 | Survived |
| | 1728 | -ve | 0 | 4 | Died |
| Control | 1751 | -ve | 20 | 4 | Died |
| | 1752 | 40 | 0 | 2 | Survived |
| | 1754 | -ve | 0 | 2 | Died |
| | 1755 | -ve | 0 | 4 | Survived |

NT = Not tested

**Table 7.** The anitibody level detected by IHA, PMPT and ELISA and animal survival after direct challenge at 12 months post vaccination.

| | Animal No. | Reciprocal IHA | PMPT | ELISA units | Survived or died |
|---|---|---|---|---|---|
| Vaccinated | 1695 | -ve | 0 | 16 | Survived |
| | 1705 | 160 | 20 | 32 | Died |
| | 1707 | -ve | 0 | 32 | Survived |
| | 1717 | -ve | 0 | 64 | Survived |
| | 1722 | 20 | 0 | 32 | Survived |
| | 1741 | 20 | 0 | 64 | Died |
| Control | 1746 | -ve | 0 | 32 | Died |
| | 1749 | -ve | 0 | 32 | Survived |
| | 1750 | -ve | 0 | 32 | Died |

# DISCUSSION

## Localisation of *Pasteurella multocida* in the Tonsils

The present investigation confirmed that pasteurellae in carrier animals persisted in the tonsil as reported earlier by De Alwis *et al.* (1990) and clearly showed that the tissue distribution of the organism within this organ is confined to the lumen of the tonsillar crypts. In addition, the study also provides evidence to support the development of an antibody response following exposure and confirmed the relationship between antibody response and carrier status (De Alwis, 1982; De Alwis and Sumanadasa, 1982).

The use of a immunohistochemical technique to detect the distribution of the organism within the tissue also provided an opportunity to simultaneously examine the histopathological changes of the tonsil. Despite the presence of the organism for a considerable length of time within the tonsil, there was no evidence for histological changes except for a few areas of epithelial desquamation in some parts of the tonsillar crypts. In an earlier report, Omar *et al.* (1962) described the presence of a chronic suppurative inflammation in the tonsil of an apparently healthy buffalo from which HS causing pasteurellae were isolated. *P. multocida* is not regarded as a pyogenic organism hence, the histological observation reported by Omar *et al.* (1962) may reflect a tissue response to a concurrent infection with suppurative organisms such as *Actinomyces pyogenes* which are also known to colonise in the tonsil of clinically normal bovines (Bock, 1980; Duncanson and Slater, 1986 ).

The streptomycin resistant stain of *P. multocida* used in the present study was developed from a field isolate and known to possess the same virulence as the field isolate. Previous experiments (De Alwis *et al.*,1990) using this strain have demonstrated that the virulence of the organism was retained despite a long term persistence in tonsils. The streptomycin resistant strain of the organism used in this study also served as a marker during the isolation and identification of the organism from tonsillar tissue and nasal swabs following the intranasal inoculation.

In the studies reported by De Alwis *et al.* (1990), it was argued that the persistence of the organism for over 200 days after initial exposure indicated that the tonsil is a reservoir for pasteurellae. At present, factors that contribute to the persistence of the organism in the tonsil and role of the defence mechanisms in eliminating it is unclear. The intense immunostaining in the cytoplasm of macrophages within the tonsillar crypt indicate the presence of phagocytosis within the lumen. However, the relatively normal appearance of lymphoid follicles in the tonsil together with the absence of significant numbers of leukocytes or mononuclear cells within the lumen or in the process of migration, suggests the presence of only a weak cell-mediated immune response by the host. These observations need to be further investigated to understand the interaction between the host and the organism which may be necessary for the bacterium to remain viable in the tonsil for a considerable length of time.

In the earlier study (De Alwis *et al.*, 1990) attempts to eliminate the organism from the tonsil following antibacterial therapy failed. The possibility of the organism remaining in a "dormant state" in the tonsil was suggested as a reason for the lack of response to antibiotics to which it was sensitive *in vitro*. The findings of the present study, clearly shows that the colonisation of pasteurellae in carrier animals is exclusively in the lumen of tonsillar crypts which in actual fact is outside the tissues of the host where antibiotic penetration is insufficient to reach adequate concentrations to destroy the organism, hence its ability to survive despite the administration of antibiotics parentally.

The experimental studies by De Alwis *et al.* (1990) have clearly demonstrated the tonsil as reservoir site for HS-causing pasteurellae. The findings of the present investigation whilst confirming these observations have also identified the tissue location of the organism. In the light of this knowledge, it appears that previous assessments of the carrier status of HS, based on the detection of pasteurellae in the nasopharynx alone is an underestimation of the actual situation. In this context, it will be worthwhile to evaluate the validity of these experimental findings in the natural disease in order to fully appreciate the role of the tonsils of carriers in the epidemiology of HS.

## Investigation of the Factors Responsible for the Conversion of Latent to Active Carriers

It is believed that "stress" has some bearing on activation of carriers to shedders of the HS-causing organism. The identified term "stress" could be attributable to the predisposing factors such as overwork, extreme weather conditions, prolonged transportation, low plane of nutrition or a combination of any of these factors. Field observations appear to associate such stress factors with outbreaks of HS. The mechanisms whereby "stress" promote outbreaks is not established.

There is however, a clear indication for activation of neuro-humoral mechanisms during adverse environmental situations in domestic animals (Stephens, 1980). The intermediate physiological response to adverse situations is characterised by activation of the autonomic nervous system. Based on this understanding attempts were made to chemically stimulate the activation of carriers using drugs with known mechanisms of action.

The single administration of dexamethasone has provided the baseline information for future experiments in terms of dosage. Methylprednisolone acetate, the long acting preparation of corticosteroid provides long term suppression of immunity. Neostigmine which has a cholinergic effect increased the nasal and salivary secretions along with dilation of the tonsillar sinus. It was believed that these chemicals administered to carriers stimulate secretions of the organism lying in tonsillar crypts. In the present study however, there was no change in antibody levels as reflected by IHA titres. The immunosuppressive dose of methylprednisolone acetate used in buffaloes in this work failed to activate "latent carriers".

## Immune Response to the HS Oil Adjuvant Vaccine

One of the main objectives of the present study was to evaluate the level and duration of immunity against *P. multocida* serotype B:2 following vaccination against HS. Immunity is assessed by the level of serum antibodies as determined by serological tests. In this study, the conventional serological tests which included the IHA and the PMPT were employed together with the ELISA described by Horadagoda *et al.* (1994). It is apparent from the results that the primary OAV elicited a poor antibody response as indicated by IHA and ELISA titres. Chandrasekeran *et al.* (1992) have reported that most immunised buffalo sera did not contain IHA titres, indicating a poor immune response to *P. multocida* type B capsular antigen. However, the present study indicated that after a booster dose at 3 months, the IHA and ELISA titre gradually increased. The observation that the titres as well as the number of animals showing titres continued to increase steadily even up to the end of the experimental period of 12 months is noteworthy (Table 1). This observation appears to indicate that factors other than the vaccine have influenced the immune response as detected by IHA and ELISA. This was further borne out by the fact that unvaccinated control animals also developed titres. Similar observations have been made by De Alwis *et al.* (1978). It was postulated that sub-clinical HS infections or exposure to related serotypes may account for this phenomenon.

The results of PMPT are not consistent (Table 2). In the first month post-vaccination, PMPT indicated a 66% protection, which remained up to 5 months and declined to 4% by 12 months. Under natural conditions the most appropriate test for assessing immunity is the direct challenge test. As shown in Tables 6 and 7, at 9 months 50% of the vaccinated animals survived while at 12 months 67% survived the direct challenge of the virulent organism. Survival of unvaccinated control animals to direct challenge cannot be fully understood. Thomas *et al.* (1969) made similar observations and attributed this to individual resistance which they considered to be different to "naturally acquired immunity". If these animals were exposed to the infection and acquired natural immunity one would expect very high antibody titres detectable by IHA (De Alwis, 1982; De Alwis and Sumanadasa, 1982). Antibodies to the HS serotype has been detected even in countries such as Australia and USA where HS is not endemic (Bain *et al.*, 1982). Natural immunity in such situations may be due to the presence of serologically related non-HS pasteurellae and other bacteria.

The other objective of this study was to assess the performances of the serological tests used, i.e. IHA, PMPT and ELISA in relation to the results of the direct challenge test. Chandersekeran *et al.* (1993) reported a relationship between ELISA titres and the protection levels. Johnson *et al.* (1989) have stated that an apparent relationship between ELISA titres and the results of PMPT can be observed. However, in the present study, no such relationship was observed. The ELISA was more sensitive in detecting antibodies in that when both IHA and PMPT were negative the ELISA gave positive results. Nagy and Penn (1976) have reported a correlation between the IHA, PMPT and direct challenge test in cattle which however was not consistent. Conversely, Chandrasekeran *et al.* (1993) observed a lack of relationship between PMPT and direct challenge in buffaloes. It is further stated that immune sera produced in mice afforded 100% protection, while sera from immune buffaloes failed to give 100% protection. The results of the present study too indicate that PMPT is not an appropriate test to assess protection against HS in buffaloes. Mukkur and Nilakantan (1969) have also observed a lack of relationship between IHA, PMPT and direct challenge test. The results indicate that there is no correlation between serological tests and protection levels as determined by the direct challenge test.

Our findings provide further evidence towards the absence of correlation between the currently used serological test and direct challenge. It is important to note that the method of challenge itself, used by all workers is not the natural route, which is believed to be oral or intra-nasal. It is our view that firstly, specific protective immunogens must be identified and secondly, a technique to detect protective antibodies must be developed. A method of natural challenge has also to be developed. It is then that the most appropriate tests to determine protective immunity can be stipulated.

## General Discussion and Conclusions

The results of this study have clearly demonstarted that *Pasteurella multocida* that colonise the tonsil of latent HS carriers are located exclusively in the tonsillar crypts. This localisation has enhanced the survival by evading the defence mechanism and the effects of antibiotics to which it is sensitive *in vitro*. Epidemiological studies on HS have suggested that outbreaks of the disease are due to the spread of the infection by latent carriers and that some triggering mechanism has transformed them to become active carriers or shedders in which the organism is present in nasal secretions. In this regard the findings of the present study imply that it would not be possible to eliminate the organism in the latent carriers through antibiotic therapy, as a means of controlling outbreaks of the disease.

This study also attempted to investigate the effect of steroid-induced immunosuppression on transformation of latent carriers to an active state. In the dose-range used no effect was demonstrable. The factors responsible for this transformation remain an area for research in order to completely understand this segment of the epidemiological cycle.

The vaccination experiment with the OAV has demonstrated the need for a booster in order to achieve titres to last a 12 month period. The results of the vaccine experiment have demonstrated a lack of correlation between the different serological tests used to assess immunity as against the direct challenge test, which is the ultimate proof of protection afforded by the vaccine. The findings of this experiment have indicated several areas of basic research for the future which have direct implications on the evaluation of vaccines used to control the disease. The areas suggested include the need to (a) identify specific protective immunogens of the organism, (b) develop a test that will precisely detect and quantitate protective antibodies and (c) establish a better challenge model to evaluate immunity produced by vaccines.

## Acknowledgements

We wish to acknowledge with thanks the research grant awarded by the Swedish Agency for Research Cooperation with Developing Countries and the NARESA for their assistance in administering the grant.

## References

Bain, R.V.S., De Alwis, M.C.L., Carter, G.R. and Gupta, B.K. (1982) Haemorrhagic septicaemia. *Animal Production and Health Paper* No. 33 Rome, FAO.

Bock, R. (1980) Detection of *Corynebacterium pyogenes* in tonsils and lymph nodes of healthy calves and young cattle and its significance in the occurrence of summer mastitis. *Vet. Bull.* **52,** 178; Abstract No. 1540.

Carter, G.R. (1955) Studies on *Pasteurella multocida*. I. A haemagglutination test for the identification of serological types. *Am. J. vet. Res.* **16,** 481-484.

Chandrasekaran, S., Kennett, L., Yeap, P.C., Munianady, N., Rani, B. and Mukkur, T.K.S. (1993) Relationship between active protection in buffalo vaccinated against Haemorrhagic septicaemia and passive mouse protection and serological tests. In: *Proceedings of an International Workshop on Pasteurellosis in Production Animals.* ACAIR Proceedings No: 43 pp. 165-169.

De Alwis, M.C.L. (1982) The immune status of buffalo calves exposed to natural infection with Haemorrhagic septicaemia. *Trop. Anim. Hlth. Prod.* **14,** 29-30.

De Alwis, M.C.L., Gunatillake, A.A.P. and Wickramasinghe, W.A.T. (1978) Duration of immunity to Haemorrhagic septicaemia in cattle following immunisation with alum precipitated and oil adjuvant vaccines. *Ceylon vet. J.* **26,** 35-41.

De Alwis, M.C.L. and Sumanadasa, M.A. (1982) Naturally acquired immunity to Haemorrhagic septicaemia among cattle and buffaloes in Sri Lanka. *Trop. Anim. Hlth. Prod.* **14,** 27-28.

De Alwis, M.C.L., Wijewardana, T.G., Gomis, A.I.U. and Vipulasiri, A.A. (1990) Persistence of the carrier status in Haemorrhagic septicaemia (*Pasteurella multocida* serotype 6:B infection) in buffaloes. *Trop. Anim. Hlth. Prod.* **22,** 185-194.

De Alwis, M.C.L., Wijewardana, T.G., Sivaram, A. and Vipulasiri, A.A. (1986) The carrier and antibody status of cattle and buffaloes exposed to Haemorrhagic septicaemia: Investigations on survivors following natural outbreaks. *Sri Lanka vet. J.* **34,** 33-42.

Duncanson, G.R. and Slater, C.A. (1986) Post mortem health inspection of bovine tonsils. *Vet. Rec.* **118,** 459.

Gupta, B.K. (1962) Studies on the carrier problem in Haemorrhagic septicaemia. *Ph.D Thesis Punjab University Chandigar, India.*

Hiramune, T. and De Alwis, M.C.L. (1982) Carrier status of cattle and buffaloes in Sri Lanka. *Trop. Anim. Hlth. Prod.* **14,** 91-92.

Horadagoda, N.U. and Belak, K. (1990) Demonstration of *Pasteurella multocida* type 6:B (B:2) in formalin-fixed paraffin-embedded tissues of buffaloes by the peroxidase anti-peroxidase (PAP) techniques. *Acta Vet. Scand.* **31,** 493-495.

Horadagoda, N.U., Wijewardana, T.G., Mulleriyawa, I.S., Ramya Kumari, H.M.R. and Vipulasiri, A.A. (1994) Development of an enzyme-linked immunosorbent assay (ELISA) for detection of serum antibodies to Haemorrhagic septicaemia. In: *Strenghtening Research on Animal Reproduction and Disease diagnosis in Asia through the Application of Immunoassay Techniques.* IAEA publication No: IAEA-TECDOC-736. pp 185-193.

Johnson, R.B., Dawkins, H.J.S., Spencer, T.L., Saharee, A.A., Bahaman, A.R. Ramdani and Patten, B.E. (1989) Evaluation of bovine antibody responses to Haemorrhagic septicaemia vaccine. *Res. Vet. Sci.* **47,** 277-279.

Mukkur, T.K.S. and Nilakantan, P.R. (1969) The relationship of haemagglutinating antibody with protection in cattle immunised against Haemorrhagic septicaemia. *Cornell vet.* **59,** 643 -649.

Nagy, L.K. and Penn, C.W. (1976) Protection of cattle against experimental Haemorrhagic septicaemia by the capsular antigens of *Pasteurella multocida* types B and E. *Res. Vet. Sci.* **20,** 249-253.

Omar, A.R., Cheah, P.P. and Shanta, C.S. (1962) The isolation of a virulent strain of Pasteurella. *British vet. J.* **118,** 71-73.

Peiris, L.K.P.N. and De Alwis, M.C.L. (1991) Simplified techniques for Haemorrhagic septicaemia vaccine production. In: *Proceedings of the International Workshop on Haemorrhagic Septicaemia* Ed. De Alwis, M.C.L. and Wijewardena, T.G. FAO/APHCA Publication No: 1991/13 pp117-120.

Singh, M. (1948) Nasal carriers in bovine pasteurellosis. *Indian J. vet. Sci.* **18,** 77-80.

Stephens, D.B. (1980) Stress and its measurement in domestic animals: A review of behavioural and physiological studies under field and laboratory situations. *Adv. Vet. Sci. Comp. Med.* **24,** 179-210.

Thomas, J., Omar, A.R., Fadzil, M., Mustaffa-Babjee, A. and Vendargon, X.A. (1969) Studies on Haemorrhagic septicaemia oil-adjuvant vaccine. II. Field and laboratory trials. *Kajian Veterinaire, Malaysia-Singapore* **2**, 4-12.

Wijewantha, E.A. and Karunaratne, T.G. (1968) Studies on the occurrence of *Pasteurella multocida* in the nasopharynx of healthy cattle. *Cornell vet.* **58**, 462-465.

Wijewardana, T.G., De Alwis, M.C.L. and Bastiansz, H.L.G. (1986) Cultural, biochemical, serological and pathogenicity studies of *Pasteurella multocida* isolated from carrier animals and outbreaks of Haemorrhagic septicaemia. *Sri Lanka vet. J.* **34**, 43-57.

NA - 143 - 393

# CHARACTERIZATION OF STRAINS OF BUFFALO CALF ROTAVIRUS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

U. Ariyaratne and S. Mahalingam

*Department of Veterinary ParaClinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** The objective of the present study was to detect non-group A ('atypical') rotaviruses as well as to study the genome profiles of strains of buffalo group A rotaviruses by polyacrylamide gel electrophoresis (PAGE).

Faecal samples collected from 175 buffalo calves 1 to 150 days old were examined by the Enzyme Linked Immunosorbent Assay (ELISA) test for group A rotaviruses antigen. These samples were also examined by PAGE. Of the 175 calves 139, (79·4%) were diarrhoeic at the time of sampling whilst the rest were non-diarrhoeic, but in contact with them.

Fourteen of the 175 (8%) samples were positive for rotavirus by ELISA, and were of group A, sub-group 1 specificity. By PAGE only 9 of these samples showed banding pattern typical of group A rotavirus, although one of them was indistinct. None of the ELISA negative samples gave any banding pattern by PAGE similar to the atypical rotavirus. Six of these strains were different from each other. These strains were circulating in farms, 60-100 km distant from each other, but in the same region. Variations in the electropherograms of rotaviruses detected in the same farm but at different times of sampling were also observed. The Sri Lankan strains of buffalo calf rotavirus differed from the bovine strain in the migratory pattern of segment 5, whereas the Indian strains differed in the migratory pattern of segment 10.

PAGE is a useful technique to distinguish isolates and outbreaks of disease, and in detecting "atypical" rotaviruses where typing antisera are hard to come by. However, electropherotyping of strains of rotaviruses may not indicate variation in antigenicity of these strains.

**Keywords:** Buffalo, rotavirus, diarrhoea, PAGE, calf.

## INTRODUCTION

Group A rotaviruses are the most important agents causing non-bacterial gastroenteritis in humans and animals (Bishop *et al.*, 1973; Flewett *et al.*, 1974). In Sri Lanka, 36.6% of diarrhoeas in buffalo calves, aged 10 to 51 days are rotavirus associated (Sunil Chandra and Mahalingam, 1994a). The genome of these RNA viruses consist of 11 gene segments, which code for different viral proteins. These segments have different molecular weights and hence varying mobilities, when the extracted viral RNA is subjected to polyacrylamide gel electrophoresis (PAGE), they band themselves into a 4 - 2 - 3 - 2 configuration. This is known as the electropherogram for that particular virus group. These segments can be made visible by silver staining of the gels (Herring *et al.*,1982). Variations of this basic electrophoretic pattern may occur amongst different isolates within the group A rotaviruses in different farms within the country, in different outbreaks within the same farm, and amongst isolates from different countries (Rodger and Holmes, 1979).

Very few studies have been made on the electropherograms of group A strains of buffalo rotavirus. Muniappa *et al.* (1987) have reported that their Bulgarian buffalo strains differed from the U.S. and Japanese bovine strains, Lincoln and 11-2, respectively. Singh *et al.* (1993) showed that their Indian buffalo strains too differed from cattle strains in the migratory pattern of the $10^{th}$ segment.

The PAGE technique has also been exploited to detect "novel" or atypical rotaviruses in the faeces of humans and farm animals having diarrhoea. On the basis of their electropherograms, these atypical rotaviruses have been classified into five distinct groups B to F (Bridger, 1984).

The objectives of this work were to detect the occurrence of atypical rotaviruses associated with buffalo calf diarrhoea, and study the variabilities in the electropherograms of strains of group A buffalo rotaviruses detected in Sri Lanka.

## MATERIALS AND METHODS

*Faecal samples:* Samples were collected from 175 diarrhoeic and non-diarrhoeic calves in three organized farms, 139 of whom were diarrhoeic, and 36 non-diarrhoeic but in contact with them. These animals were one day to five months of age.

*Enzyme linked immunosorbent assay (ELISA):* Faecal samples were examined for rotavirus group A antigen by the ELISA, using a commercial kit (Dakopatts, K 349, Denmark). The positives were confirmed by the blocking ELISA using reagents obtained from the W.H.O. (Sunil Chandra and Mahalingam, 1994b) and subgrouped (Sunil Chandra, 1988).

*PAGE:* Samples were also examined for their rotavirus genome profiles by PAGE (Sevensson *et al.*, 1986). Briefly, a 10% faecal suspension in phosphate buffered saline (PBS) was incubated with extraction buffer, pH 8.0 in the ratio 5:1 for 30 minutes at $37^0$ C (10mmol/l Tris hydrochloride, 10 mmol/l EDTA, 0.5% sodium dodecyl sulphate (SDS) and 10μl of 1% Proteinase K ). An equal volume of phenol, chloroform, isoamyl mixture in the ratio 24:24:1 was added, vortex mixed and centrifuged at 10,000g for 5 minutes. To the supernatant, sample buffer was added in the ratio 3:1 (0.5 mmol/l Tris hydrochloride, 5% SDS, 0.005% EDTA, 40% glycerol, 5% mercapto ethanol and 0.001% phenol red) and vortex mixed. Twenty five microlitres of this preparation was then loaded into a well of the stacking gel (4.5%) and electrophorosed at 100v for 15 min, after which the voltage was increased to 160v for 70 minutes (10% separating gel of size 8 x 7cm) using Bio Rad Mini Protean Cell 11 (Biorad, U.S.A). At the end of the run, the separating gel was removed, fixed for 15 min in a mixture containing 10% methanol-0.5% acetic acid with gentle shaking in an orbital shaker, washed with distilled water, stained with silver for 15 min, dried on cellophane membranes for 3 days and bands examined. Samples which gave similar banding patterns were co-electrophorosed.

## RESULTS

Fourteen of the 175 samples examined were positive for rotavirus, and were all of group A, subgroup I specificity. Thirteen of the samples were from diarrhoeic calves and one from a non-diarrhoeic calf. These calves were 1 to 2 months of age. By PAGE, only 9 of these samples showed banding pattern typical of group A rotaviruses. None of the ELISA negative samples gave a banding pattern similar to atypical rotaviruses.

The electropherogram of the 9 buffalo rotavirus strains, together with those of a simian SA II and a Swedish bovine strain are shown in Figures 1 and 2. Strains D2, 4850, 4851 and 823 were similar, whereas the other four strains, 514, 1898/1, 613 and 530 were dissimilar. The bands of strain 477 were indistinct.

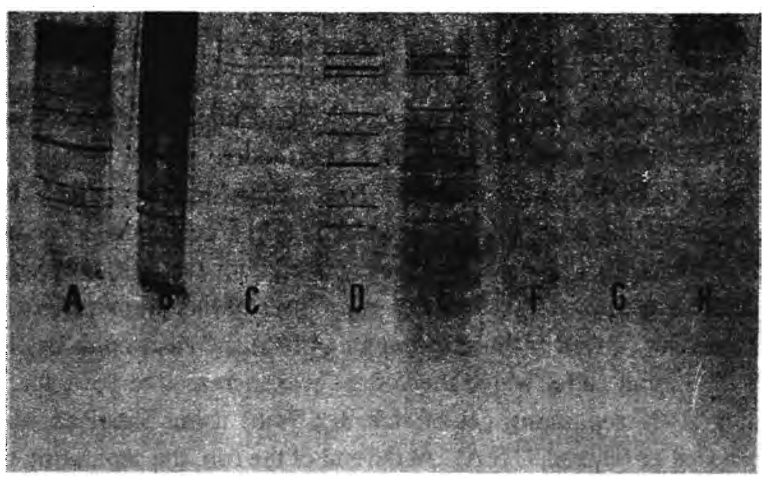



**Figure 1.** Electropherograms of five different strains of buffalo rotaviruses and those of a reference simian and bovine group A rotavirus. Lanes: A, SA11; B, Bovine; C, 514; D, 1898/1; E, D2; F, 613; G, 4850; H, 4851.

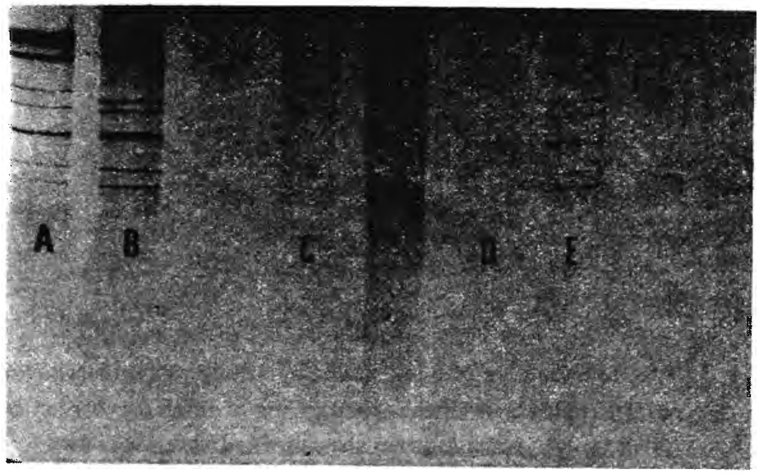



**Figure 2.** Electropherograms of a further three different strains of buffalo rotavirus, and a reference simian and bovine group A rotavirus. Lanes: A, SA11; B, Bovine; C, 477; D, 530; E, 823.

Co-electrophoresis of the similar strains showed that two of them (4850 and 4851) were similar, whereas strains D2 and 823 were different from each other (Figures 3 and 4). These variations amongst strains appeared to be minor. When buffalo strains were compared with the Swedish bovine strains, the only difference noted was in segment 5 (Figures 3 and 4), whilst there were only minor differences in segment 10. This bovine strain gave identical banding pattern as with the U.K. bovine strain, as well as to cattle calf strains detected in Sri Lanka.

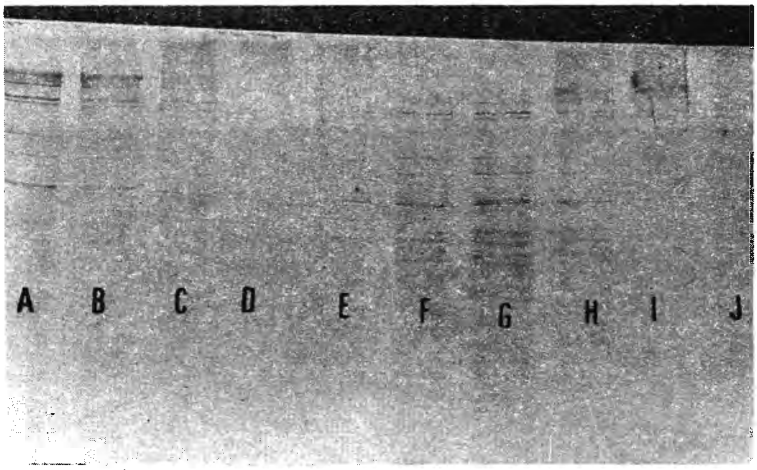



**Figure 3.** Electropherogram of three strains of buffalo rotavirus following co-electrophoresis. Electropherograms of reference simian and bovine group A rotavirus are also shown. Lanes: A, SA11; B, SA11+Bovine; C, Bovine; D, 4851+4850; E, 4850; F, 4850+D2; G, D2; H, D2+4851; I, 4851; J, 4851+Bovine.

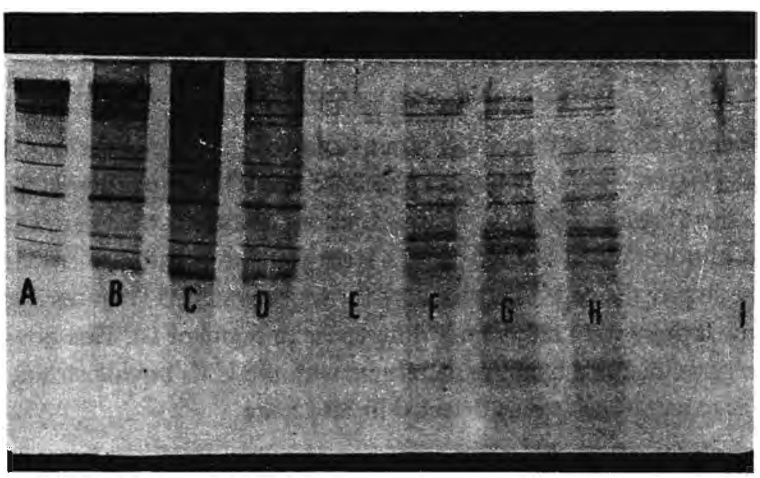



**Figure 4.** Electropherograms of three other strains of buffalo rotaviruses following co-electrophoresis. Electropherograms of reference simian and bovine group A rotavirus are also shown. Lanes: A, SA11; B, SA11+Bovine; C, Bovine; D, Bovine+823; E, 823; F, 823+D2; G, D2; H, D2+530; I, Bovine+530.

## DISCUSSION

In the present study all strains of rotavirus detected both by ELISA and PAGE were group A, subtype I rotaviruses. None of the faecal samples examined by PAGE gave any genome profiles representative of the atypical rotaviruses. This is not surprising since atypical rotaviruses are believed to be comparatively uncommon. For instance, Snodgrass *et al.* (1984) examined 222 samples of calf faeces containing rotaviruses and only two atypical specimens were detected. The electropherograms of these two cases were those of group B. Although detection rates have been low, seroprevalence studies have shown that these atypical rotaviruses are widely distributed among animals. In a sero survey for prevalence of antibodies to Group B and C rotaviruses in pigs, Bridger and Brown (1985) found that 86% of the sera tested had antibodies to group B and 77% antibodies to group C rotaviruses. In another study, Brown *et al.* (1987) found nearly 71% of cattle sera examined had antibodies to group B rotaviruses, whereas the prevelence of antibodies to this group in humans was very low (4-10%). Although the association of atypical rotaviruses with diarrhoeas in cattle and other animals have not being studied to the same extent as group A rotaviruses, their pathogenicity is not in doubt. Group B rotaviruses have been associated with two large outbreaks in humans in China, involving mainly adults over 15 years of age and not infants and young children under three years of age. Group C rotaviruses too have been associated with an outbreak of diarrhoea in adult cattle (Tsunemitsu *et al.*, 1980). Considerable variations were observed amongst the buffalo rotaviruses detected in our study. Six of them (514, 530, 823, D2, 613, 1898/1) were different from each other. These strains were circulating in different farms, 60-100 km from each other, but in the same region. One of these strains (514) was from a non-diarrhoeic calf (Figure 1). Six months later when diarrhoeic and non-diarrhoeic animals were sampled from this same farm, the electropherogram of the virus detected (1898/1) was different from that of 514 (Figure 1). In another farm in the same region (Polonnaruwa), rotavirus was detected on three separate occasions, all from diarrhoeic animals. The electropherograms of these three strains (530, 823 and 4850) were all different from each other. Similar results have been found by others as well. Singh and Pandy (1988) in their study of rotavirus diarrhoeas in a buffalo farm in India found that the electropherogram of the rotaviruses detected at the second sampling 4 months later was different to that detected at first sampling. De Costa Mendes *et al.* (1993) in their studies on rotavirus diarrhoeas in 6 weeks old Afrikander calves maintained in two farms, found that the electropherograms of the rotavirus strains detected at first sampling in the second farm under study was different to the rotavirus strains detected at the second sampling two months later, and at the third sampling eight months later, in one of the farms. Although electropherotyping of rotavirus strains does not give an indication of their antigenicity, it is still a useful technique for the study of variability of these strains within farms, and for the detection of sources of outbreaks.

## References

Bishop, R.F., Davison, G.P., Holmes, I.H. and Ruck, B.T. (1973) Viral particles in epithelial cells of duodenal mucosa from children with adult non bacterial gastroenteritis. *Lancet* **ii,** 1281-1282.

Bridger, J.C. (1984) Novel rotaviruses in animals and man. In: *Novel Diarrhoea Viruses*. CIBA foundation symposuim 128, John Wiley and Sons, Chichester, U.K pp. 5-33.

Bridger, J.C. and Brown, J.F. (1985) Prevalence of antibody to typical and atypical rotaviruses in pigs. *Vet. Rec.* **116,** 50.

Brown, D.W.G., Beards, G.M., Guang-mu C. and Flewett, J.H. (1987) Prevalence of antibody to group B (atypical) rotavirus in humans and animals. *J. Clin. Microbiol.* **25,** 316-319.

De Costa Mendes, V.M., De Beer, M., Peenze, I. and Steele, A.D. (1993) Molecular epidemiology and subgroup analysis of bovine group A rotavirus associated with diarrhoea in South African calves. *J. Clin. Microbiol.* **31,** 3333-3335.

Flewett, T.H., Bryden, A.S., Davies, H.A., Woode, G.N., Bridger, J.C. and Derick, J.M. (1974) Relation between viruses from acute gastroenteritis of children and newborn calves. *Lancet* **ii**, 61-63.

Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R. and Menzies, J.D. (1982) Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. *J. Clin. Microbiol.* **16,** 473-477.

Muniappa, L., Georgine, K.G., Dimitrov, D., Mitow, K.B. and Haralambiv, E.H. (1987) Isolation of rotaviruses from buffalo calves. *Vet. Rec.* **3**, 23.

Rodger, S.M. and Holmes, J.H. (1979) Comparison of the genomes of simian, bovine and human rotaviruses by gel electrophoresis and detection of genomic variations among bovine isolates. *J. Virol.* **30**, 839-846.

Sevensson, L., Uhnoo, J., Grandien, M. and Wadell, G. (1986) Molecular epidemiology of rotavirus infection in Uppsala, Sweden 1981: Dissappearance of predominant electropherotypes. *J. Med. Virol.* **18,** 101-111.

Singh, A. and Pandey, R. (1988) Analysis of electropherotypes of rotavirus from diarrhoeic faeces of neonatal calves in India. *Acta Virol.* **32,** 156-159.

Singh, A., Kapoor, S. and Pandey, R. (1993) Molecular epidemiology of bovine rotavirus during 1987 as determined by electrophoresis of genomic RNA. *Indian J. Virol.* **9,** 1-8.

Snodgrass, D.R., Heering, A.J., Campbell, I., Inglis, J.M. and Hargreaves, F.D. (1984) Comparison of atypical rotaviruses in calves, piglets, lambs and man. *J. Gen. Virol.* **65,** 909-914.

Sunil Chandra, N.P. (1988) Studies on rotavirus infection of buffalo calves in Sri Lanka. *M. Phil Thesis,* University of Peradeniya, Sri Lanka.

Sunil Chandra, N.P. and Mahalingam, S. (1994a) Rotavirus associated diarrhoea in buffalo calves in Sri Lanka. *Res. Vet. Sci.* **56,** 393-396.

Sunil Chandra, N.P. and Mahalingam, S. (1994b) Application of ELISA in the diagnosis of rotavirus infection in buffalo calves. *Buffalo J.* **3,** 237-248.

Tsunemitsu, H., Saif, L.J., Tiang, B., Shimizu, M., Hiro, M., Yamaguchi, H., Ishiyama, T. and Hirai, J. (1980) Isolation, characterization and serial propagation of a bovine group C rotavirus in a monkey kidney cell line (MA104). *J. Clin. Microbiol.* **29,** 2609-2613

NA - 143 - 403

# MARKERS OF INFLAMMATION IN BUFFALO MILK

Silva, I.D.[1], Silva, K.F.S.T.[2], Ambagala, A.P.N.[1] and Cooray, R.[3]

[1] *Department of Veterinary Clinical Studies,* [2] *Department of Animal Science, University of Peradeniya, SRI LANKA,*
[3]*National Veterinary Institute, SWEDEN*

**Abstract:** Immunity in milk against invading pathogens is asserted by phagocytes and serum factors. The somatic cell count (SCC) in buffalo milk ranged from 50,000 to 375,000/ml, of which 56% (range 22-88) were neutrophils which are the most active phagocytes in the body. The lymphocytes constituted 28% (range 10-54) of the SCC, and the rest consisted of macrophages (8%), epithelial cells (5%) and eosinophils (1%). The presence of a small percentage (2%) of cells undergoing necrosis was a unique observation. The pH of the buffalo milk was 6.5 (range 6.1-7.0).

In inflammation, the vascular permeability in the gland increases permitting leukocytes and serum factors in blood to enter the lacteal secretion. Experimentally induced aseptic subclinical mastitis elevated the SCC from 50,000/ml to $75\times10^6$/ml, and 90% of these were neutrophils. The presence of serum factors in normal milk was detected by the electrical conductivity (EC) and chloride ion percentage (Cl%) which were 3.86 milli Siemens/cm and 0.11%, respectively. The entry of serum factors into milk preceeded the entry of cells, and a raise in EC to 4.87 mSiemens/cm (or above the mean plus twice the standard deviation, 4.46 milli Siemens/cm) and a rise in Cl% to 0.14% were observed in milk following induction of subclinical mastitis. Significant alterations were not detected in acidity percentage and pH. The CMT negative milk had $5\times10^{-10}$ moles/ml of adenosine 5'-triphosphate (ATP) and the values elevated with increasing SCC. A significant increase to $33\times10^{-10}$ moles/ml was obtained for milk with CMT scores above 2 which had an average SCC of $4.25\times10^6$/ml. Streptococci were the major bacteria isolated from subclinical mastitic samples while 44% of the samples did not yield any bacteria on culture. Therefore SCC, EC, Cl% and ATP are markers of subclinical mastitis in the buffalo.

**Keywords:** Somatic cell count, conductivity, chloride, buffalo, milk

## INTRODUCTION

Mastitis is the greatest single cause of loss of milk in the dairy industry. Bacteriological testing of milk from all cows in a herd is too cumbersome and expensive for regular testing, especially in large herds (Guterbock and Blackmer, 1984). Therefore, it has become a standard practice in developed countries to monitor the somatic cell counts (SCC) in milk, as an indicator of mastitis in cattle (Schultz, 1977). This is because, in response to an insult to the mammary gland, the increase in capillary permeability, hyperalgesia and haemodynamic derangement results in a breakdown of the blood-milk barrier (Robbin, *et al.*, 1984; Zia, *et al.*, 1987; Schuster, *et al.*, 1993 ). This in turn leads to marked oedema, swelling and tenderness of the gland, and transudation of serum proteins and recruitment of blood leukocytes, mainly neutrophils, into the lacteal secretion (Jain, 1976; Kerhli and Schuster, 1994). These responses are critical to the cow's ability to survive an infection and to return to normal production.

The serum factors and cells act together to remove the invading pathogens. Opsonization of the invading pathogens by immunoglobulins and complement components assist the leukocytes to eliminate the pathogens (Niemialtowski, *et al.*, 1988). The widely accepted California Mastitis Test (CMT) is a cowside test which gives an estimate of the total cell count in milk (Schalm and Noorlander, 1957). Measurement of the adenosine triphosphate (ATP) generated by the SCC indirectly estimates the degree of inflammation. The NaCl and bicarbonate ions enter the milk due to the alteration of the permeability, thus elevating the electrical conductivity (EC), chloride ion levels (Cl%), pH and acidity ( Schalm, *et al.*, 1971; Linzell and Peaker, 1975).

The incidence of subclinical mastitis in the buffalo is apparently lower than that in cattle (Kalorey, *et al.*, 1983; Khalaf, 1983). Therefore, it is possible that the quantity and/or quality of cells and plasma factors in buffalo milk during a mammary infection is different to those of cattle. However, udder infections can present a serious problem in buffalo herds due to poor hygiene (Chamberlain, 1993; Uppal, *et al.*, 1994). The present study describes the cytology and the serum factors in normal and mastitic buffalo milk, and their relationship with the SCC. This information will assist veterinarians and farmers in the early detection of subclinical mastitis.

## MATERIALS AND METHODS

*Animals:* A total of 100 milking buffaloes used in this study were from five large organized farms in the north western, central and north central provinces, and those reared individually or in groups in small farming households in Sri Lanka. The breeds were Lankan, Murrah, Surti, Nili Ravi and their crosses. Twelve cattle from a large farm in the central province were used for comparison, wherever indicated.

*Milk samples:* The CMT was performed on all samples according to the method described by Schalm and Noorlander (1957). The pH of the milk samples were also recorded. The number of quarter milk samples used for the different tests are indicated under each test.

*Normal buffalo milk:* A composite sample of 250 to 400 ml of milk from each of the four quarters from each cow was collected. Milk was collected separately and aseptically into covered sterile beakers and was transported in ice to the laboratory, to carry out the tests. Samples from the north central and north western provinces were tested on location. Milk was identified as non-mastitic by using the CMT as an indicator.

*Mastitic buffalo milk:* Aseptic inflammation of the udder (EAM) was induced in 51 quarters of 9 Lankan and 5 Surti buffaloes as described earlier (Silva, 1993a). Additionally twenty two quarter milk samples from 9 buffaloes from a farm (FM) which gave CMT scores of between trace and 3 were also collected as aseptically as possible.

*Cattle milk samples:* Twenty one quarter milk samples from 12 neat cattle were also collected aseptically and subjected to the same tests, for purposes of comparison.

*Somatic cell counts (SCC):* The SCC of each quarter milk sample was measured microscopically using a haemocytometer as described for blood leukocyte counting (Silva, 1993a).

*Differential cell counting (DC):* CMT negative quarter milk samples (n=148) from 37 buffaloes were used. Milk cells were isolated by filtering the milk through 4 layers of gauze to remove flakes, if any, and centrifuging at 300 RCF for 10 minutes. The sediments were resuspended in 2 ml of sterile phosphate buffered saline, pH 7.2 (PBS) and 2% bovine serum albumin (BSA) was added to prevent cell damage and loss during smear preparation and staining. The DC was done on a maximum of 200 cells in Leishman stained smears.

*Measurement of electrical conductivity (EC):* The EC of quarter milk samples were measured by using a conductometer ("Analis K520", Belgium). The electrode was immersed in the milk sample and the measurement (L) was taken at a reference temperature of $20^0$C, after the digital reading stabilized itself. The specific conductance was calculated by using the following formula, K = L.k/1 + a(t-20) in which, K = specific conductance related to $20^0$C in Siemens/cm; L = conductivity at $t^0$C in Siemens; a = sample temperature coefficient in 2%/K; t = sample temperature in $^0$C; k = cell constant in 1/cm.

*Measurement of chloride (Cl%):* The Cl% was measured as described by Marth (1978). One millilitre of 10% potassium dichromate (indicator) and 9 ml of milk were titrated with 0.1N silver nitrate ($AgNO_3$) to the first perceivable pale red-brown colour, lasting 30 seconds. The Cl% was calculated using the following formula = (ml of $AgNO_3$ x N of $AgNO_3$ x 3.55)/weight of sample, where factor 3.55 = (35.46 (m.w.) x 100)/1000.

*Measurement of acidity:* The acidity was measured as described by Marth (1978). Five hundred millilitres of 1% phenolphthalein and 9 g of milk was titrated with 0.1N NaOH to the first permanent colour change from white to pink. Acidity was expressed as lactic acid where 1 ml of 0.1N NaOH = 0.009 g of lactic acid. Percent acidity was calculated by using the following formula = ml of 0.1N NaOH x 0.009 x 100 /weight of the sample.

*ATP analysis:* This was carried out at the National Veterinary Institute in Sweden. Milk samples (n=88) were transported to Sweden using one "Mastistrip"$^R$ cassette for each cow. Each cassette contained 8 paper discs fixed to 4 plastic strips (2 discs per quarter) which were used for bacteriological examination and for ATP measurement. These cassettes allowed milk to remain uncontaminated up to 3-4 days and silica gel ensured rapid drying of the discs after sampling.

Nine hundred microlitres of 0.1 M Tris/2mM EDTA buffer and one paper disc was allowed to interact in a 4 ml plastic cuvette by shaking for 12 minutes. Then 100µl AMR reagent (1243-200 ATP monitoring reagent) was added and the cuvette was kept in a luminometer 1250 (LKB/Wallac). The light emission (in mV) for 60 seconds was measured using a 1-channel/recorder (LKB\Wallac 2210). The light emission for 30 seconds was measured after adding 10µl of ATP standard. The ATP moles/ml = (Sample (mV)-Blank (mV))/ (Standard (mV) - Sample (mV)) x 50 x$10^{-10}$.

*Bacteriological analysis:* The second paper disc on the "Mastistrips" was mixed with 0.5ml RPMI for 60 seconds and 50µl of this solution was incubated for 24 or 48 hours on blood agar.

*Statistical analyses:* The following tests were used: Paired t-test at 5% significance level for values between normal and subclinical samples; pooled t-test at 5% significance levels for the effect of breed; Tukey's multiple comparison at ($P<0.05$) level for the ATP values (Daniel, 1987).

## RESULTS

The experimental induction of inflammation in the mammary glands of the cows resulted in subclinical mastitis, with higher SCC and CMT scores in milk and no evidence of clinical mastitis. The mean and the range for the concentrations for the various cells in the SCC were as follows: Neutrophils 56% (22-88), lymphocytes 28% (10-54), macrophages 8% (0-28), epithelial cells 5% (0-15), eosinophils 2% (0-6). The nuclear morphology of 2% of cells showed pyknosis, karyorrhexis or karyolysis. The CMT scores of the EAM milk were between trace - 2, and between 1-3 for the 22 FM milk samples. Milk from the 21 cattle had CMT scores ranging from trace to 3 although signs of inflammation of the udder were not evident at the time of sampling, suggesting that those cows were affected with subclinical mastitis.

The values obtained for SCC, Cl%, EC, acidity and pH for all the normal and mastitic milk from buffalo and cattle are given in Table 1.

**Table 1.** Mean values (S.E.M.) and the range for somatic cell count (SCC), chloride% (Cl%), electrical conductivity, acidity%, and pH for normal and mastitic buffalo and cattle milk.

| | SCC/ml | Cl% | Conductivity (mS/cm) | Acidity (%) | pH |
|---|---|---|---|---|---|
| Normal | 74,800 | 0.11 | 3.86 | 0.15 | 6.5 |
| buffalo | (8,400) | (0.002) | (0.04) | (0.003) | (0.02) |
| milk | 0-375,000 | 0.08-0.21 | 3.40-4.75 | 0.08-0.22 | 6.1-7.0 |
| n | 216 | 105 | 57 | 78 | 83 |
| Normal | NT | 0.13 | 4.58 | 0.16 | 6.3 |
| cattle | | (0.005) | (0.12) | (0.007) | (0.02) |
| milk | | 0.10-0.16 | 3.74-5.13 | 0.12-0.20 | 6.2-6.4 |
| n=10 | | | | | |
| Mastitic buffalo milk – Milk from experimental induced subclinical mastitis (EAM) | | | | | |
| | $14 \times 10^6$ | 0.14 | 4.87 | 0.14 | 6.4 |
| | $(2 \times 10^6)$ | (0.004) | (0.08) | (0.003) | (0.03) |
| | $0.45\text{-}75 \times 10^6$ | 0.11-0.21 | 3.85-5.67 | 0.10-0.19 | 6.2-6.8 |
| n | 39 | 38 | 32 | 37 | 39 |
| - Milk from Mastitic quarters from farm buffaloes (FM) | | | | | |
| | $5.5 \times 10^6$ | 0.13 | 4.31 | 0.14 | 6.4 |
| | $(1.7 \times 10^6)$ | (0.006) | (0.14) | (0.007) | (0.04) |
| | $0.15\text{-}24 \times 10^6$ | 0.09-0.22 | 3.32-5.52 | 0.08-0.18 | 6.2-6.8 |
| n | 14 | 22 | 22 | 22 | 22 |
| Mastitic | NT | 0.18 | 5.43 | 0.15 | 6.5 |
| cattle | | (0.01) | (0.16) | (0.02) | (0.05) |
| milk | | 0.10-0.35 | 4.03-7.25 | 0.06-0.60 | 6.0-7.2 |
| n=21 | | | | | |

n- number of quarter samples; mS/cm-milli siemens/centimeter; NT-not tested

Significant effect of the breed on these parameters were not observed in normal milk except for a higher EC in Surti milk (4.43 mS/cm) when compared with that of Lankan buffaloes (3.89 mS/cm). The changes in these parameters before and after induction of subclinical mastitis are given in Table 2. The ATP values increased significantly between CMT 2, 1 scores (Table 3). There was no difference between the ATP values for zero, trace and 1 CMT scores.

**Table 2.** Mean (SEM) values for somatic cell counts (SCC), chloride% (Cl%), electrical conductivity (EC), acidity % and pH of milk before and after induction of subclinical mastitis in buffaloes.

| Parameter | n | Pre-induction milk | Post-induction milk |
|---|---|---|---|
| SCC/ml | 37 | 47,800 (10,900) | $14x10^{6*}$ ($2.2x10^{6}$) |
| Cl % | 36 | 0.108 (0.002) | $0.142^{*}$ (0.004) |
| EC (mS/cm) | 33 | 3.78 (0.04) | $4.87^{*}$ (0.08) |
| Acidity % | 34 | 0.145 (0.08) | 0.142 (0.004) |
| pH | 39 | 6.37 (0.02) | 6.42 (0.03) |

* - mean value was significantly different than the preinduction value ($P<0.05$)
n - number of quarters tested, mS/cm - milli siemens/centimeter

**Table 3.** Mean ATP values observed for the different somatic cell counts (SCC) and corresponding California Mastitis Test (CMT) scores.

| CMT score | Mean SCC/ml | ATP ($10^{-10}$ moles/ml) |
|---|---|---|
| Negative | $72,000^{d}$ | $5.0^{a}$ |
| Trace | $300,000^{e}$ | $10.4^{b}$ |
| 1 | $600,000^{f}$ | $10.0^{c}$ |
| 2 | $4,250,000^{d,e,f}$ | $32.7^{a,b,c}$ |

$^{a\text{-}f}$ Means with the same letter were significantly different ($P<0.05$).

Of the samples cultured for bacterial growth, 31% had CMT scores varying from trace to 3, suggesting the presence of a subclinical infection. The bacteria isolated from these milk samples from subclinical quarters are as follows: 22% coagulase negative staphylococci, 15% *Streptococcus uberis*, 11% *Streptococcus agalactiae*, 7% other streptococci. About 44% of the samples did not yield any bacterial growth.

## DISCUSSION

The SCC is a valuable tool for identifying major udder infections, and it is now recommended as an indicator trait to achieve genetic improvement for mastitis resistance (Kerhli and Schuster, 1994; Shook and Schutz, 1994). The mean SCC in normal milk of *Bubalus bubalis* was 140,000/ml and ranged from 50,000 to 375,000/ml. These values are similar to that is normal cattle milk (Jasper, 1963). Individual cow variations in the SCC could be attributed to the level of management in the different agro-climatic zones. The cells in buffalo milk were similar to those in cattle milk in that the neutrophil was the predominant cell, although its concentration was higher (22-88%) in buffaloes than in cattle (26-29%) (Miller, *et al.,* 1990). The lymphocyte was the second predominent cell (10-54%).

The SCC in EAM and FM samples ranged from 0.45-75x$10^6$/ml, and 0.15-24x$10^6$/ml, respectively (Table 1), and 90% of those were neutrophils. The lower count of 450,000 SCC/ml obtained for EAM samples was a result of cell dilution, as these low counts were obtained from high milk producers. The presence of necrotic cells (2%) in normal milk, with pyknosis, karyorrhexis or karyolysis of the nuclei, was not reported in cattle. This may be because the buffalo leukocytes are functionally more active and therefore, may undergo the process of cell necrosis faster than cattle cells (Silva, 1993b). Furthermore, in contrast to cattle, the functional activity of milk neutrophils during an acute onset of udder inflammation in the buffalo is similar to that of blood neutrophils

(Dulin, *et al.*, 1988; Silva, 1993a). Therefore, although the SCC in buffalo is similar to cattle, the concentration and functional efficiency of milk phagocytes is superior in buffaloes.

The ATP appears in milk as a consequence of milk somatic cells, and its concentration is elevated with increasing SCC (Table 3). An ATP concentration of $33 \times 10^{-10}$ moles/ml was observed for a SCC of $4.25 \times 10^{6}$/ml with a corresponding CMT score of 2. However, the measurement of SCC and ATP require laboratory facilities which may not be practical under local situations. The present trend towards larger herds producing quality milk, automation and higher profits from the milk industry necessitates a rapid and easy measurement of mastitis at or around milking time. Therefore, other inflammatory markers can be used for this purpose.

In the normal udder, the major ions responsible for EC in milk are sodium (Na), potassium (K) and chloride (Cl) (Linzell and Peaker, 1975). During inflammation, the increase in vascular permeability precedes the entry of leukocytes thereby permitting partial equilibration of milk with extracellular fluid, which is rich in Na, Cl and low in K, by raising the salt concentration of milk (Linzell and Peaker, 1975; Schalm *et al.*, 1971). Therefore, measurement of the EC of milk is an accurate, rapid and an inexpensive method of detecting mammary infections, especially subclinical inflammation, before other clinical signs become evident (Linzell and Peaker, 1975). Only the SCC, Cl% and EC levels elevated significantly following induction of subclinical mastitis in buffaloes, whereas, the acidity and pH changes were insignificant. In cattle, high correlations have been observed between the EC, infection status, and SCC (Fernando, *et al.*, 1982; Jasper, 1963). An elevation in EC to above the mean plus twice the standard deviation ($3.87+0.3 \times 2=4.47$ mS/cm) of the normal milk, when measured individually for each buffalo, can be used as an indicator of subclinical mastitis. A relatively higher EC of 4.43 mS/cm was observed for Surti buffaloes than for the Lankan buffaloes (3.89 mS/cm) which could be attributed to the breed differences and to the low milk yield of the Lankan buffaloes (Greatrix *et al.*,1968). The elevation in the EC and Cl% were similar while the SCC elevation in FM samples were relatively lower than that of EAM samples, which reiterates the fact that elevations in EC and Cl% precede that of the SCC.

About 44% of the buffalo quarters with subclinical inflammation did not yield bacteria on culture. This may imply the cause of the elevated SCC as of non-bacterial origin, such as irritation due to improper milking practices, leading to aseptic mastitis. Streptococci and staphylococci were the major bacterial species isolated from 26% and 22% of the subclinical quarters, respectively.

The findings presented reveal that SCC, ATP, EC, Cl% are markers or indicators of subclinical mastitis in the buffalo. Further studies are needed to observe correlations between infections, day-to-day variations, yield and the lactation number on these parameters in the buffalo.

## Acknowledgements

This study was supported in part by funds from the Swedish Agency for Research Cooperation with Developing Countries (SAREC) awarded through the Natural Resources, Energy and Science Authority of Sri Lanka (NARESA), and by a research grant from the University of Peradeniya, Sri Lanka. The authors wish to thank N. Weerakkody, L.U.S.S. de Silva, K.R.J.K. Amararatne, K.A.M.J. Silva, S.S. Withanage and I.N.K. Iriyagolla for assisting in sample collection and for technical assistance.

## References

Chamberlain, A. (1993) *Milk Production in the Tropics*. Longman Scientific and Technical, UK.

Daniel, W.W. (1987) *Biostatistics: A Foundation for Analysis in the Health Sciences* 5th Ed. John Wiley and Sons, Inc., USA.

Dulin, A.M., Paape, M.J. and Nickerson, S.C. (1988) Comparison of phagocytosis and chemiluminescence by blood and mammary gland neutrophils from multiparous and nulliparous cows. *Am. J. Vet. Res.* **49,** 172-177.

Fernando, R.S., Rindsig, R.B. and Spahr, S.R. (1982) Electrical conductivity of milk for detection of mastitis. *J. Dairy Sci.* **65,** 659-664.

Greatrix, G.R., Quayle, J.C. and Coombe, R.A. (1968) The detection of abnormal milk by electrical means. *J. Dairy Sci.* **35,** 213-221.

Guterbock, W.M. and Blackmer, P.E. (1984) Veterinary interpretation of bulk-tank milk. *Veterinary Clinics of North America* **6,** 257-268.

Jain, N.C. (1976) Neutrophil leukocytes and inflammation of the bovine mammary gland. *Theriogenology* **6,**153-173.

Jasper, D.E. (1963) A summary of screening methods for leukocytes and their significance in milk quality. *The California Veterinarian* **July-August**: 37-40.

Kalorey, D.R., Purohit, J.H. and Dholakia, P.M. (1983) Studies on the incidence of subclinical mastitis, its aetiology and *in vitro* sensitivity of isolates. *Indian J. Anim. Sci.* **53,** 961-963.

Khalaf, A.M. (1983) Studies on mastitis in buffaloes in Iraq with particular reference to prevalence rates, aetiology and diagnosis. *Third International Symposium of the World Association of Veterinary Laboratory Diagnosticians*. June 13-15, Iowa, **2,** 591.

Kerhli, M.E. Jr. and Schuster, D.E. (1994) Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *J. Dairy Sci.* **77,** 619-627.

Linzell, J.L. and Peaker, M. (1975) Efficacy of the measurement of the electrical conductivity of milk for the detection of subclinical mastitis in cows: Detection of infected cows at a single visit. *British vet. J.* **131,** 447-451.

Marth, E.H. (1978) *Standard Methods for the Examination of Dairy Products*. 14th edition. American Public Health Association, Washington DC, USA.

Miller, R.H., Paape, M.J. and Link, S. (1990) Detection of changes in percent neutrophils (PMN%) in milk by flow cytometry. (Abstract) *J. Dairy Sci.* **73** (Suppl.1), 211.

Niemialtowski, M., Nonnecke, B.J. and Targowski, S.P. (1988) Phagocytic activity of milk leukocytes during chronic staphylococcal mastitis. *J. Dairy Sci.* **71**, 780-787.

Robbin, S.L., Cotran, R.S. and Kumar, V. (1984) Inflammation and repair. In: *Pathological Basis of Diseases*. 3rd ed. W.B. Saunders Company, Philadelphia. 40-80.

Schalm, O.W., Carroll E.J. and Jain N.C. (1971) *Bovine Mastitis*. Lea and Febiger, Philadelphia, USA.

Schalm, O.W. and Noorlander, D.O. (1957) Experiments and observations leading to development of the California Mastitis Test. *J. Am.Vet. Med. Assoc.* **130**,199-204.

Schultz, L.H. (1977) Somatic cell counting of milk in production testing programs as a mastitis control technique. *J. Am.Vet. Med. Assoc.* **170**, 1244-1246.

Schuster, D.E., Kerhli Jr., M.E. and Stevens M.G.(1993) Cytokine production during endotoxin-induced mastitis in lactating dairy cows. *Am. J. vet. Res.* **54**,80-83.

Shook, G.E. and Schutz, M.M. (1994) Selection on somatic cell score to improve resistance to mastitis in the United States. *J. Dairy Sci.* **77**, 648-658.

Silva, I.D. (1993a) Phagocytic efficiency of Buffalo (*Bubalus bubalis*) blood neutrophils for common mammary pathogens. *Buffalo J.* **2**, 181-185.

Silva, I.D. (1993b) A comparison of the phagocytic efficiency of buffalo (*Bubalus bubalis*) blood and milk neutrophils. *Sri Lanka vet. J.* **40**, 7-14.

Uppal, S.K., Singh, K.B., Roy K.S., Nauriyal, D.C. and Bansal, B.K. (1994) Natural defense mechanisms against mastitis: a comparative histomorphology of buffalo and cow teat canal. *Buffalo J.* **2**, 125-131.

Zia, S., Giri, S.N., Cullor, J., Emau, P., Osburn, B.I. and Bushnell, R.B. (1987) Role of eicosanoids, histamine and serotonin in the pathogenesis of Klebsielle pneumoniae-induced bovine mastitis. *Am. J. vet. Res.* **48**, 1617-1625.

NA-143-415

# PREVALENCE OF LEPTOSPIRAL ANTIBODIES IN BUFFALOES IN SRI LANKA

T. G. Wijewardana, B.D.R. Wijewardana, W.N.D.G.S. Appuhamy and K.R.V.P.M. Premaratna

*Veterinary Research Institute,*
*Gannoruwa, Peradeniya,*
*SRI LANKA*

**Abstract:** Infections among humans, domesticated and wild animals caused by *Leptospira interrogans* occur worldwide. In Sri Lanka the prevalence of leptospirosis among domesticated animals has not been determined. The present sero-epidemiological study was to determine the prevalence of leptospirosis among buffaloes in Sri Lanka. Buffaloes are closely associated with humans as they are reared for both draught and dairy purposes. The infection is a zoonosis. The data generated from the study would be useful in the formulation of future control programmes.

Based on the prevalence of leptospirosis among humans, the districts of Anuradhapura, Badulla, Colombo, Galle, Gampaha, Kandy, Kegalle, Kalutara, Kurunegala, Matara, Polonnaruwa and Ratnapura were selected for the survey. From each Divisional Secretariat division within a district, serum was collected from 0.4 % of the buffalo population, giving a total of 1500 serum samples. Each sample was examined by the microscopic agglutination test against a panel of 10 serovars of Leptospira that are representative of sero groups present in Sri Lanka. Those showing an agglutination at a dilution of 1:50 or above were considered positive. The overall prevalence was 41.93 %. The highest prevalence was in Badulla district (72.91 %) while the lowest was in the Kegalle district (11.84 %). The most common serovar was *weerasingha* (30.2%) followed by *pomona* (26.55 %) and *hardjo* (24 %). The least common serovar was *ceylonica* (0.7 %). These serovars maintained in other hosts have become adapted to buffaloes.

**Keywords:** Leptospirosis, buffaloes, antibodies, MAT, prevalence.

## INTRODUCTION

The term leptospirosis refers to a range of disease syndromes in man and animals associated with infection by spirochaetes of the genus Leptospira. There are two species within this genus, *L. biflexa* which is considered non-pathogenic and *L. interrogans*, which is pathogenic. *L. interrogans* comprises 25 serogroups with over 200 serovars within the serogroups reported to date and, has a world wide distribution (Faine, 1993). The serovars exhibit a host predilection and they differ from one another on the basis of agglutinating surface antigens (Faine, 1993). The infection occurs through direct contact with infected tissues, body fluids, urine or virulent laboratory cultures or indirectly, through contaminated food, water or urine (Kingscote, 1986). Skin and mucous membranes are the commonest portals of entry through cuts and abrasions and the organism can also be transmitted by inhalation (Mazzonelli, 1984). After an incubation period of 4-10 days, the organism disseminates itself throughout the body in the blood circulation. This phase, could last from a few hours to 7 days and is followed by a clearance phase from the blood and most of the tissues. Leptospiras are retained in the proximal renal tubules and in the female reproductive tract leading to a carrier stage, which are the foci of infection for animals and man (Ellis, 1984).

Leptospirosis is an economically important disease as losses occur through abortions, stillbirths, loss of milk production, deaths and infertility (Ellis, 1984). It is an occupational disease in man, the risk groups being agricultural workers, workers in mines, abattoirs, laboratories, wool workers and animal handlers. In the tropics, infections initiated by wild mammals such as rodents and carnivores are more prevalent and many serovars are present. The microclimatic conditions in the tropics with warm wet conditions and a pH closer to neutral, especially in paddy fields favour the survival of the spirochaetes outside the host, thus enhancing the danger to animals and man in these surroundings (Faine, 1993).

In Sri Lanka only a few studies on leptospirosis in cattle (Peiris and Wettimuny, 1972) and in dogs and goats (Babudieri and Jagels,

1962) have been carried out. Human leptospirosis in Sri Lanka has also been investigated (Babudieri and Jagels, 1962; Rajasuriya *et al.*, 1964; Walloopillai *et al.*, 1966). In this country, buffaloes constitute an integral part of the agricultural sector. The importance of the buffalo lies in the fact that they perform a dual role of a draught animal and that of a milk producer. Leptospirosis in buffaloes has been reported in many countries (Ciceroni *et al.*, 1995). Despite the close association between buffaloes and the agricultural community in Sri Lanka, no studies on the prevalence of leptospirosis in buffaloes have been undertaken yet. Therefore, a study was designed to gather information on leptospirosis in buffaloes in Sri Lanka and it's importance. This communication describes the findings of a sero-epidemiological survey, which was the initial step in achieving the objective.

## MATERIALS AND METHODS

*Method of sampling:* Multistage sampling technique was employed. Data pertaining to prevalence of human leptospirosis were extracted from records maintained at district and base hospitals in each of the districts, except in districts of the north and east. Colombo district had the highest prevalence of human leptospirosis followed by Gampaha, Kandy, Matara, Kegalle, Kurunegala, Anuradhapura, Badulla and Polonnaruwa. Table 1 shows the mean of the number of cases of human leptospirosis for the 4 year period from 1989 to 1992. Data on buffalo populations in the Divisional Secretaries Divisions (DS divisions) within each district under study were obtained from each DS division and 0.4 % of the buffalo population was sampled. A total of 1500 samples of sera were included in the study (Table 2).

*Collection of samples:* Blood samples were collected by jugular puncture. The blood was allowed to clot and the serum was separated at the Veterinary Research Institute by centrifugation at 1000 rpm for 10 minutes. The sera were stored at -20° C until required. Each animal sampled was given an identification number using ear tags. Wherever possible, details of age, sex, history of abortions/stillbirths or reproductive failures and the purpose for which the buffaloes were reared were also recorded. The samples and relevant data were collected over a period of 18 months from December 1993 to May 1995.

**Table 1.** Average number of cases of human leptospirosis during the four year period from 1989 to 1992.

| District | Average No. of cases |
|---|---|
| Colombo | 150 |
| Gampaha | 63 |
| Kandy | 40 |
| Kalutara | 39 |
| Galle | 21 |
| Matara | 21 |
| Kegalle | 21 |
| Kurunegala | 15 |
| Anuradhapura | 15 |
| Ratnapura | 12 |
| Badulla | 11 |
| Polonnaruwa | 8 |

*Microscopic Agglutination Test (MAT):* The test was performed as per recommendations of the WHO/FAO Leptospirosis Reference Centre in Brisbane, Australia. Table 3 indicates the sero groups, serovars and the panel of reference strains of the antigens used in the test (Kmety and Dikken, 1988). The WHO/FAO Reference Centre for Leptospirosis selected the panel of antigens, which were representative of serogroups prevalent in Sri Lanka. The antigens and the standard positive hyperimmune sera raised in rabbits against the 10 individual strains were supplied by the Reference Centre.

**Table 2.** The composition of the samples and the prevalence in each district.

| District | No. of samples expected to be collected from each DS division | No. actually collected | No. positive | % positive |
|---|---|---|---|---|
| Badulla | 55 | 48 | 35 | 72.91 |
| Kandy | 93 | 91 | 54 | 59.34 |
| Polonnaruwa | 180 | 193 | 114 | 59.06 |
| Colombo | 17 | 17 | 10 | 58.82 |
| Gampaha | 66 | 63 | 31 | 49.20 |
| Ratnapura | 43 | 44 | 20 | 45.45 |
| Anuradhapura | 207 | 190 | 78 | 41.05 |
| Kurunegala | 675 | 686 | 256 | 37.31 |
| Galle | 22 | 23 | 06 | 26.08 |
| Kalutara | 54 | 44 | 11 | 25.00 |
| Matara | 24 | 25 | 05 | 20.00 |
| Kegalle | 79 | 76 | 09 | 11.85 |

The reference antigen strains were maintained at 30°C in EMJH (Difco) medium in screw capped tubes and used in the test at a concentration of approximately $10^8$, 4-7 days old live leptospire organisms per ml. The test was performed in 96 well, flat bottom microtitre plates. Each test serum sample was diluted 1:25 in phosphate buffered saline, pH 7.5, and screened against the 10 serovars. Any degree of agglutination with any of the serovars was recorded and, each test compared with the readings against the pooled standard positive sera. Serum samples showing agglutination were serially diluted, using 2 fold dilutions from 1:25 to 1:12,800 in phosphate buffered saline, pH 7.5, and equal volumes of the corresponding serovar antigen suspension added, mixed well by agitation and incubated at 30°C for 90 minutes. The plates were viewed under darkfield microscopy using a magnification of x100.

The end point was taken as the highest dilution showing 50 % agglutination. A test was considered positive where agglutination occurred at a dilution of 1:50 or above.

**Table 3.** The panel of antigens indicated in the MAT (supplied by the WHO/FAO Leptospirosis Reference Laboratory in Australia).

| Serogroup | Serovar | Strain |
|---|---|---|
| Canicola | canicola | Hond Utrecht IV |
| Tarassovi | trassovi | Perepelitsin |
| Australis | australis | Ballico |
| Pyrogenes | pyrogenes | Salinem |
| Pomona | pomona | Pomona |
| Sejroe | hardjo | Hardjoprajitno |
| Javanica | ceylonica | Piyasena |
| Icterohaemorrhagia | gem | Simon |
| Autumnalis | weerasingha | Weerasingha |
| Grippotyphosa | ratnapura | Wimalasena |

## RESULTS

Of the 1500 serum samples collected, 629 were found to be serologically positive indicating a prevalence of 41.93 % leptospirosis in buffaloes in Sri Lanka. Of these, 41 serum samples reacted with another serovar at a similar titre, indicating mixed infections. In instances where serum samples reacted with 2 or more serovars, the serovar indicating the highest titre was considered as the one responsible for the infection. The titres ranged from the lowest value of 1:50 to 1:3200. Table 2 indicates the targetted number of animals to be sampled, the number actually sampled and the percentage of prevalence of leptospirosis in each district under study. Only animals aged 6 months and over were sampled. As shown in Table 2, the highest prevalence (72.91%), was observed in the Badulla district while the lowest was in Kegalle district (11.85%). As given in Table 4 serovar ***weerasingha*** in the sero group *Autumnalis* had the highest prevalence at 30.2 %, followed by ***pomona*** (sero group *Pomona*) and ***hardjo*** (sero group *Sejroe*), while serovar ***ceylonica*** had the lowest prevalance at 0.7 %. Table 5 illustrates the predominant serovars in each district.

**Table 4.** Numbers of animals giving positive reactions to different serovars at litres ranging from 1:50 to 1:3200.

| Serovar tested | 50 | 100 | 200 | 400 | 800 | 1600 | 3200 | Total | % |
|---|---|---|---|---|---|---|---|---|---|
| Canicola | 3 | 10 | 7 | 3 | 6 | 3 | 1 | 33 | 5.25 |
| Tarassovi | 6 | 2 | 4 | 6 | | | | 18 | 2.86 |
| Australis | 1 | 1 | 2 | | 1 | | 1 | 6 | 0.96 |
| Pyrogenes | 17 | 24 | 16 | 11 | 4 | | 1 | 93 | 11.28 |
| Pomona | 23 | 31 | 36 | 25 | 31 | 15 | 6 | 167 | 26.55 |
| Hardjo | 30 | 40 | 21 | 28 | 23 | 5 | | 152 | 24 |
| Ceylonica | | 1 | | 2 | 2 | | | 5 | 0.79 |
| Gem | 1 | 5 | 4 | 1 | 1 | 1 | 1 | 14 | 2.23 |
| Weerasingha | 21 | 59 | 56 | 26 | 17 | 9 | 2 | 190 | 30.2 |
| Ratnapura | 2 | 2 | 2 | 4 | 1 | | 1 | 12 | 1.75 |
| Total | 104 | 180 | 148 | 106 | 86 | 33 | 13 | 670 | |
| % | 17 | 28.3 | 24 | 16.9 | 13.7 | 5.74 | 1.74 | | |

**Table 5.** Percentage distribution of serovars among seropositive animals in the different districts.

| *Serovars* | RTP | GMH | CMB | KDY | POL | ANR | KNG | BDA | KEG | KAL | GAL | MTR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Canicola | 5 | 6.45 | 30 | 18.5 | 13.15 | | 0.39 | 2.85 | | | | |
| Tarrasovi | | | | 3.7 | 3.5 | 10.25 | 1.17 | 2.85 | | | | |
| Australis | 5 | | | | 1.75 | 2.56 | | 2.85 | | | | |
| Pyrogenes | | | 20 | 25.92 | 4.38 | 7.69 | 15.62 | 8.57 | 11.1 | 9 | 16.6 | 20 |
| Pomona | | 41.93 | | 18.5 | 29.82 | 29.48 | 27.73 | 22.85 | 11.1 | 36.3 | 33.3 | 20 |
| Hardjo | 60 | 16.12 | 40 | 20.37 | 36.84 | 17.94 | 18.36 | 37.14 | 22.2 | 9 | 16.6 | |
| Oeylonica | | | | | 0.87 | 3.84 | 0.39 | | | | | |
| Gem | 5 | | | | 3.5 | 1.28 | 1.56 | 2.85 | 22.2 | 9 | | |
| Ratnapura | 10 | | | 5.55 | 1.75 | 1.28 | 0.78 | | | | | 40 |
| Weerasingha | 20 | 38.7 | 20 | 14.8 | 16.66 | 29.48 | 37.5 | 42.85 | 33.3 | 36.3 | 33.3 | 40 |

RTP - Ratnapura GMH - Gampaha CMB - Colombo KDY - Kandy POL - Polonnaruwa ANR - Anuradhapura
KNG - Kurunegala BDA - Badulla KEG - Kegalle KAL - Kalutara GAL - Galle MTR - Matara

## DISCUSSION

The objective of the present study was to ascertain the prevalence of leptospirosis in buffaloes in Sri Lanka in relation to dominant serovars and age, sex and the use (milk/draught/both) of the animal. The survey revealed an overall prevalence of 41.96 % of leptospirosis in buffaloes in the country. According to Mazzonelli (1984), a serological survey correctly performed and interpreted would reflect the true situation of leptospirosis in the area. The sampling method, sample size, the performance of the serological test (MAT) and the selection of the reference strains of the antigens, which were representative of serogroups prevalent in Sri Lanka were in line with the recommendations of the WHO/FAO Reference Centre for Leptospirosis, Brisbane, Australia. As such, assuming that the results of the MAT as absolute, the positive animals could be considered as having been exposed to the bacterium, while the negatives are to be considered as susceptibles. As the kinetics of antibodies involve rising and falling antibody titres, the true prevalence could be higher than found in this survey (Mazzonelli, 1984).

The most predominant serovar was *weerasingha* of the *Autumnalis* sero group (30.2 %). Among the seropositives, it was the commonest in Badulla (42.86%), followed by Matara (40%). Kurunegala (37.15%), Kalutara (36.36%), Galle (33.33%), Kegalle (33.33%) and Anuradhapura (29.48%). Other significant serovars were *pomona* (26.56 %), *hardjo* (24 %), *pyrogenes* (11.28 %) and *canicola* (5.2 %). Each serovar has a specific maintenance host and when other animal species are exposed they could become infected. Favourable conditions for the leptospires to survive outside the animal host include warm wet conditions and a pH closer to neutral (Faine, 1993) commonly found in paddy fields, water holes and reservoirs with inter connecting irrigation canals. These could become contaminated by the leptospires through wild mammals, such as rodents and carnivores, or other domesticated mammals. Serovars *pomona*, *hardjo* and *canicola* are maintained in pigs, cattle and dogs respectively, while sero group *Autumnalis* represented by serovar *weerasingha* is associated with small rodents. Hence, it appears that these serovars which are maintained in other animal hosts have caused incidental infections in buffaloes and through prolonged contact, they have become adapted to buffaloes.

All the sero groups included in the survey are represented in Polonnaruwa district, while Anuradapura and Kurunegala districts each have one less and Badulla district two less. The one factor common to these four districts is the large scale of cultivation of paddy with an abundance of sprawling paddy fields and irrigation reservoirs with interconnecting canals. The reason for the wider representation of serovars could be the favourable environment and the large population of buffaloes which have close interaction with a large variety of domestic and wild mammals. Of the animals tested, 42.2 % of the females and an equal number (42.35%) of males were found to be sero-positive for leptospirosis. It was not possible to determine the relationship between prevalence and age, as the age given by the farmers could not be substantiated.

In most of the areas the animal performed a dual role as a draught and dairy animal. As a draught animal it was mainly used in the paddy fields for ploughing and threshing, while in some areas it was used to tread clay prior to making of bricks. In all those pursuits, the buffaloes come into very close contact with humans.

Economically, leptospirosis is an important disease causing enormous losses through abortions, still births, infertility and loss of production. It had been estimated that prenatal losses which occur due to serovar *hardjo* amount to 2.2 %. If countries of South and Central America with a cattle population of 220 million are considered, the enormity of the annual calf losses and the gravity of the problem could be envisaged (Ellis, 1984). The condition is a zoonosis. In Sri Lanka agricultural farmers working in paddy fields, gem miners, laboratory workers handling infective material, and livestock farmers handling buffaloes have been found to be infected with leptospirosis.

Although serological surveillance provides an indication of the extent of exposure to the infection, it can be supplemented with microbiological and clinical investigations. Clinical signs are neither specific nor pathognomonic for leptospirosis. Therefore, a microbiological investigation should be initiated which would elucidate the carrier status and its importance as a zoonosis.

## Acknowledgements

The survey was supported through funds provided by the Swedish Agency for Research Cooperation with Developing Countries chanelled through NARESA. The role played by WHO/FAO Leptospirosis Reference Centre, Brisbane, Australia headed by Mr. L. Smythe and the staff is gratefully acknowledged. Thanks are due to Dr. R.J. Chappel, VIAS, Melbourne, Australia. We are also appreciative of the assistance extended by the medical officers of district and base hospitals and the Epidemiologist of the Health Department. Finally we wish to express our gratitude to the field veterinarians and their staff for the cooperation.

## References

Babudieri, B. and Jagels, G. (1962) Serological research on the presence of leptospirosis in Ceylon. *Ceylon Med. J.* **7**, 213 - 214.

Ciceroni, L., Aniello, P.D., Russo, N., Picarella, D., Nese, D., Lauria, F., Pinto, A. and Cacciapuoti, B. (1995) Prevelence of leptospire infections in buffalo herds in Italy. *Vet. Rec.* **137**, 192 -193.

Ellis, W.A. (1984) Bovine leptospirosis in the tropics: Prevalence, pathogenesis and control. *Prev. Vet. Med.* **2**, 411 - 421

Faine, S. (1993) *Leptospira and leptospirosis*. CRC Press BocaRaton, Florida Kingscote, B.F. (1986) Leptospirosis: An occupational hazard to Veterinarians. *Can. Vet. J.* **27**, 78 - 81.

Kmety, E. and Dikken, H. (1988) *Revised List of* "Leptospira" *serovars. I. Alphabetical order*. University Press. Groningen, Netherlands.

Mazzonelli, J. (1984) Advances in bovine leptopirosis. *Res. Sci. Tech. Office Int. Epizooties*. **3**, 775 - 808

Peiris, G.S., and Wettimuny, S.G.de.S. (1972) Presence of leptospiral antibodies in sera of cattle. *Ceylon vet. J.* **20,** 64 - 66.

Rajasuriya, K., Munasinghe, D.R., Vitarane, U.T., Wijesinghe, C.P.de.S., Ratnaike, V.T. and Peiris, M.D. (1964) Leptospirosis in Ceylon: A Clinical Study. *Ceylon Med. J.* **9,** 136 - 153.

Walloopillai, N.J., Markus, H.K.N.I. and Nityananda, K. (1966) Leptospirosis in Ceylon. *Ceylon Med. J.* **11,** 50 - 58.

NA - 143 - 427

# OBSERVATIONS ON *Schistosoma nasale* RAO, 1933 INFECTIONS IN THE VERTEBRATE AND SNAIL HOSTS

D.J. Weilgama

*Department of Parasitology, Faculty of Medicine,*
*University of Peradeniya*
*SRI LANKA*

**Abstract:** Carcasses of cattle and buffaloes were examined for *S. nasale* infections during the period August 1992 to November 1993 at the Colombo abattoir, Government Livestock Farms and in the field. Infections were detected in 5.9% cattle and in 23.9% buffaloes. In contrast, the infection rate at the Narangalla Buffalo Research Station was 91%. Monthly examination of nasal secretions of these infected buffaloes showed *S. nasale* eggs persisting up to 8 months. The only clinical sign observed was a thick nasal discharge.

Snails belonging to six species were examined although in the natural disease only *Indoplanorbis exustus* is infected. The infection rate, however, was low (0.55%). Laboratory bred snails (F gen) of *I. exustus* from Narammala, Peradeniya, Nikaweratiya and Girandurukotte were tested and only snails from Peradeniya and Girandurukotte were susceptible. Miracidial doses of 1, 4, 6 and 10 per snail produced infections, and infection rates of up to 50% were obtained. Higher doses (20/snail) caused heavy snail mortality. The prepatent period varied from 30 to 59 days and cercarial emergence peaked in the morning hours (0800 -1130 hr). Attempts to infect snails of *Bithynia sp.* and *Bulimus sp.* failed. The cercaria were observed to migrate through skins of rats, mice and guinea pigs but schistosomulae were recovered only from lungs of mice, 5 to 7 days post exposure. Attempts to infect calves in the laboratory were not successful.

Circumoval Precipitin Test (COPT) was developed and was found to be useful in detecting *S. nasale* infections in cattle and buffaloes. COPT produced clear precipitin reactions around schistosomulae with sera of infected cattle and buffaloes.

**Keywords:** *Schistosoma nasale, Indoplanorbis exustus*, Schistosomiasis, buffalo, circumoval precipitin test.

## INTRODUCTION

*Schistosoma nasale* Rao, 1933 which causes nasal schistosomiasis in cattle and buffaloes is widespread in India (Dutt and Srivastava, 1968) and in the adjacent countries (Islam, 1975). It develops in the veins of the nasal mucosa and causes a marked rhinitis and granulomatous growths. *S. nasale* and *S. spindale*, a closely related parasite, have been reported from Sri Lanka (Crawford, 1935; Dissanayake and Jayasuriya, 1959). Recently De Bont *et al.* (1989), reported a prevalence rate of 12.6% for *S. nasale* in cattle. Much higher prevalence rates have been reported from India (Muraleedharan *et al.*, 1976a) and from Bangladesh (Islam, 1975). Very few attempts, however, have been made to study the development of *S. nasale* in animals.

Snails of the species *Indoplanorbis exustus* have been incriminated as the intermediate hosts in India (Dutt and Srivastava, 1968) and in Sri Lanka (De Bont *et al.*, 1991). Diagnosis of *S. nasale* infection in cattle and buffaloes is usually based on clinical signs and detection of eggs in nasal secretions as reliable serological tests are not available.

This paper reports on the studies made on the prevalence of *S. nasale* in buffaloes and in cattle and its development in the vertebrate and invertebrate hosts. Attempts made in the development of a serological test for diagnosis is also reported.

## MATERIALS AND METHODS

### Examination of Animals

Carcasses of cattle and buffaloes were examined for *S. nasale* infection at the Colombo abattoir, in Government farms and in the field during the period August 1992 to November 1993. The cattle were of Zebu breeds whilst the buffaloes were of the indigenous type. During the same period buffaloes stationed at the Buffalo Research Farm, Narangalla were also examined. Simple scrapings of the nasal mucosae were made using either a metal scoop (dead animals) or cotton wool swabs (live animals). The scrapings were immediately washed into about 40 ml of water in bottles and examined under a stereomicroscope.

## Examination of Snails

Snails collected from different geographical areas namely, Hasalaka, Girandurukotte, Dehiattakandiya (dry zone), Nikaweratiya (intermediate zone), Narammala (low country wet zone) and Peradeniya (mid country wet zone) were examined for natural *S. nasale* infections. The snails were kept individually in vials with water and observed daily for cercarial shedding.

## Colonisation of Snails

*I. exustus* snails were maintained in fish tanks (30x15x16 cm). The water was aerated continuously and snails were fed with fish pellets and dried cabbage leaves. Eggs laid were transferred periodically from main tanks into sub tanks to obtain snails of different age groups for infection studies.

## Experimental Infection of Laboratory Snails

Snails were exposed to *S. nasale* miracidia either singly or in groups. Single infections were carried out in a 24-well plate (Linbro R) whilst group infections were carried out in beakers with a sufficient quantity of water. Miracidia were added with a pasteur pipette and left undisturbed for 6 hours. The snails were kept individually from 20 days post-infection (PI) onwards and examined daily for cercarial shedding. On two occasions snails belonging to *Bithynia sp.* and *Bulimus sp.*, collected from the field, were also exposed to miracidia using the same procedure.

## Experimental Infection of Animals

Laboratory rats, mice and guinea pigs were exposed to *S. nasale* cercariae obtained from experimental infections. The animals were anaesthetized and 50 or 100 cercaria per animal were introduced on to shaved areas of the skin using a pasteur pipette. Impression smears were prepared from skin nips from rats and guinea pigs at 10, 15, 20 and 30 mins PI. They were stained with neutral red and examined under oil immersion. The mice were sacrificed on days 5, 7, 10, 15 and 90 PI and examined for developing and/or adult stages. Lung schistosomulae recovery assays were performed following the methods described by Janecharut *et al.* (1987).

Two crossbred calves, aged 5 and 7 months, were also exposed to *S. nasale* cercariae obtained from laboratory infections. About 500 cercariae were instilled into the nostrils of each calf using a pipette. Nasal swabs were examined from 30th day onwards for eggs.

*Sero-diagnostic tests:* The circumoval precipitin test (COPT) was performed in small screw capped vials and involved exposing *S. nasale* cercaria to known positive and negative cattle and buffalo sera. Freshly emerging cercariae were collected, concentrated by centrifugation in MEM and a known volume (500µl) put into each vial. An equal volume of serum was added into each vial and was incubated overnight at $37^0$ C. Penicillin at 100 iu/ml and streptomycin at 10 iu/ml were added to each vial to suppress bacterial growth. Foetal calf serum and PBS were used as controls.

# RESULTS

## Prevalence in Cattle and Buffaloes

A total of 336 cattle and 142 buffalo carcasses were examined during the study period and *S. nasale* was detected in 5.9% cattle and in 23.9% buffaloes. At the Buffalo Research Farm, Narangalla, a total of

134 buffaloes were examined of which 113 were indigenous adult buffaloes, 5-8 years of age, whilst 21 were Murrah crosses, aged 2-3 years. The *S. nasale* infection rate in Murrah crosses was 100% while in indigenous adults it was 89.4%; the overall infection rate was 91%.

Twenty seven of these adult buffaloes were sampled on 8 occasions at monthly intervals. *S. nasale* eggs were seen in all animals on each sampling day. The only clinical sign noticed was a thick nasal discharge which was unrelated to the status of infection. In nearly 45% of buffaloes there was egg excretion with no noticeable nasal discharge.

## Prevalence in Snails

A total of 796 snails belonging to 6 species *viz*, *Indoplanorbis exustus* (363), *Gyraulus sp.* (39), *Bulimus sp.* (184), *Bithynia sp.* (82), *Melanoides sp.* (42) and *Lymnoea luteola* (86) were examined. One each of *I. exustus* from Nikaweratiya and Girandurukotte produced *S. nasale* giving an infection rate of 0.55%.

## Laboratory Infection of Snails

Snails were exposed to miracidia both at Narangalla and at the Colombo abattoir. At the latter location the miracidia were from animals from different geographical areas whilst at Narangalla they were from a single strain of *S. nasale* (Polonnaruwa strain). Only snails from the Peradeniya colony produced cercaria both in single and in group infections with miracidia from different areas (Table 1). These also developed infections readily with the Polonnaruwa strain of *S. nasale*. The infection rate, however, was seen to decrease with age of snails (Table 2). Of the other snails tested only one snail from Girandurukotte developed an infection whilst all others were refractory.

**Table 1**. Susceptibility of *Indoplanorbis exustus* from different localities to *Schistosoma nasale* collected from different sources.

| Locality | Age | No. Exp | Min P.P. (d) | P.D.R. (%) | I.R. (%) | D.I. (d) |
|---|---|---|---|---|---|---|
| * Peradeniya | 3m | 55 | 40 | 76.4 | 15.4 | 2 |
| * Girandurukotte | 3m | 33 | | 97.0 | 00 | 0 |
| * Peradeniya | 4m | 29 | 40 | 51.7 | 7.1 | 3 |
| * Girandurukotte | 4m | 15 | | 46.7 | 00 | 0 |
| + Peradeniya | 4m | 09 | 40 | 66.7 | 33.3 | 4 |
| + Nikeweratiya | 4m | 08 | | 87.5 | 00 | 0 |

No. Exp. = Number exposed, Min P.P.(d) = Min prepatent period (days), P.D.R. = prepatent death rate, I.R. = Infection rate, D.I.(d) = Duration of infection (days), * Group infection - 06 miracidia/snail, + Single infection - 06 miracidia/snail

**Table 2**. Susceptibility of *Indoplanoribis exustus* from different localities to *Schistosoma nasale* (Polonnaruwa strain).

| Locality | Age | No. Exp | Min P.P. (d) | P.D.R. (%) | I.R. (%) | D.I. (d) |
|---|---|---|---|---|---|---|
| + Peradeniya | 2m | 50 | 35 (35-59) | 64 | 50 | 2-22 |
| + Narammala | 2m | 24 | | 00 | 00 | |
| + Peradeniya | 4m | 20 | 41 (41-47) | 15 | 47 | 6-29 |
| + Peradeniya | 5m | 24 | 36 | 50 | 25 | 2 |
| + Narammala | 5m | 24 | | 41.7 | 00 | |
| * Peradeniya | 5m | 25 | 40 | 64 | 11.1 | 1 |
| * Narammala | 5m | 25 | | 28 | 00 | |
| * Girandurukotte | 5m | 24 | 35 | 66.7 | 12.5 | 1 |

No. Exp. = Number exposed, Min P.P.D. = Min prepatent period (days) P.D.R. = prepatent death rate, I.R. = Infection rate, D.I.(d) = Duration of infection (days), + Single infection - 06 miracidia/snail, * Group infection - 06 miracidia/snail

Miracidial dose was seen to influence the infection and large numbers caused heavy snail mortality (Table 3). The maximum number of cercaria produced per snail was 1845 whilst the highest daily cercarial production was 537. Peak emergence of cercariae was between 0800 hr to 1130 hrs. *S. nasale* did not develop in the *Bithynia sp.* and *Bulimus sp.* of snails.

**Table 3**. Susceptibility of *Indoplanorbis exustus* after exposure to different numbers of miracidia of *Schistosoma nasale* (Polonnaruwa strain).

| Locality | No. Exp | No. M/S | P.D.R. % | Min P.P. (d) | I.R. % | D.I. (d) |
|---|---|---|---|---|---|---|
| Peradeniya | 14 | 01 | 85.7 | 30 | 50 | 08 |
| | 14 | 10 | 85.7 | 41 | 50 | 14 |
| | 14 | 20 | 100 | | 00 | |
| Narammala | 14 | 10 | 50 | | 00 | |
| Girandurukotte | 12 | 04 | 25 | | 00 | |

No. Exp. = Number exposed, No. M/S. = No of miracidia/snail, P.D.R. = Prepatent death rate, Min P.P.(d) = Min prepatent period (days), I.R. = Infection rate, D.I.(d) = Duration of infection (days)

## Development in Animals

In rats and guinea pigs the cercariae began to penetrate through the skin after 20 min and schistosomulae were seen in smears made at 20 and 30 min post exposure. Rats that were exposed to cercariae repeatedly, developed hypersensitive reactions that manifested in violent scratching after exposure. No schistosomulae were detected in the skin impression smears made from such rats. Schistosomulae were recovered from lungs of 3 mice on day 5 (8/100, 5/100, 3/100) and from 2 mice on day 7 (2/100, 1/100) post exposure. No schistosomulae were recovered thereafter. *S. nasale* did not develop into adults in mice.

The two calves which were exposed to *S. nasale* cercariae were sampled by nasal swabbing up to 150 days post exposure but no eggs were recovered from them. All known infected buffalo and cattle sera gave positive results. Precipitin reactions were seen in COPT at the anterior and posterior ends of schistosomulae (Figure 1). No precipitin reactions were seen with negative sera or foetal calf serum.

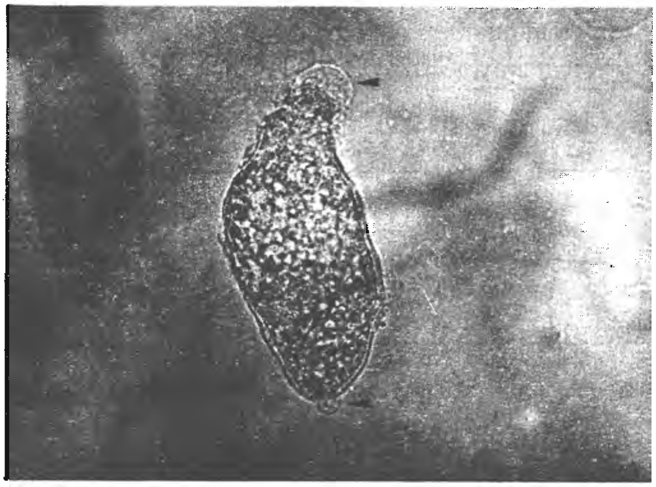


**Figure 1.** Schistosomulum showing circumoval precipitin reaction at the anterior and posterior ends (arrows).

## DISCUSSION

*S. nasale* is known to cause a more serious condition in cattle than in buffaloes (Dutt and Srivastava, 1968; De Bont *et al.*, 1989). These authors reported granulomatous growths that caused snoring in affected cattle. In the present study, the only clinical sign noted in buffaloes was a thick nasal discharge. Dutt and Srivastava (1968) made similar observations and was of the opinion that buffaloes possessed an innate resistance to *S. nasale*.

De Bont *et al.* (1989) in a survey done over a 12m period at the abattoir in Kandy, found a prevalence rate of 12.6% in cattle. A lower prevalence rate was detected in cattle in the present study but the prevalance rate in buffaloes was high. At the Narangalla farm where buffaloes obtained from different areas were stationed, the rate was much higher (91%). Such variations in prevalence have been reported by many workers (Dutt and Srivastava, 1968; Muraleedharan *et al.*, 1976 b; Islam, 1975).

These variations may be due to the susceptibility patterns of animals and to the presence of the intermediate hosts. Islam (1975) and De Bont *et al.* (1989) found higher infection rates in older cattle than in the young. In buffaloes, in the present study, the rates were similar in both young and old; a factor which could be related to the wallowing habits of the buffalo.

This study has reconfirmed the observations of De Bont *et al.* (1991) that *I. exustus* is the only intermediate host of *S. nasale* in Sri Lanka. Infection rate (IR) in nature however was low. Muraleedharan *et al.* (1976b) too reported low infections in Karnataka but Dutt and Srivastava (1968) observed a high IR (7%) in Uttar Pradesh.

In experimental studies, however, IRs of up to 50 % were obtained and are in agreement with those reported by previous authors. High IRs have also been reported with other schistosomes (Arfaa *et al.*, 1989), and Richards (1976) was of opinion that such a change in infectivity was due to a shift in gene frequencies.

Only snails from Peradeniya and Girandurukotte became infected whilst snails from Narammala and Nikaweratiya were refractory. Refractoriness has been noted with other snails and schistosome combinations (Sulaiman and Ibrahim, 1985; Arfaa *et al.*, 1989). Susceptibility to infection in snails decreased with age. Further, large numbers of miracidia caused heavy snail mortality. Such an inverse relationship between snail survival and miracidial dose has been reported with *S. mansoni* and *Biomphalaria pfeiiferi* snails (De Kock, 1992).

Cercarial shedding was seen to peak in the morning hours. This phenomenon would certainly facilitate transmission as it coincides with the wallowing times of the buffaloes. Theron (1984) observed two shedding patterns for *S. mansoni* that coincided with transmission patterns to man (early) and to rats (crepuscular). Failure in this study to infect calves is probably due to the low numbers of cercariae used. Dutt and Srivastava (1968) obtained infections only when very large numbers were used (700-21000). Though no adults were recovered from mice schistosomulae were found from 5th to 7th day PI. Similar findings have been reported for other schistosomes too (Janecharut *et al.*, 1987; Uswattanakul *et al.*, 1982).

Effective serological tests have not been reported for animal schistosomes though many are available for human schistosomiasis (Janitschke *et al.*, 1987; el-Ganayani *et al.*, 1988). COPT used in the present study was found to be effective in diagnosing *S. nasale* infections in cattle and buffaloes. A disadvantage of this test, however, was that an ongoing infection in snails was necessary to conduct the test on a routine basis.

## Acknowledgements

I am grateful to Swedish Agency for Research Cooperation with Developing Countries for the financial assistance provided through NARESA for this project. I am thankful to Dr. Vipula Dharmawardena for the facilities provided at the abattoir, Colombo and to Mr. Rex Fernando for the support extended at the Buffalo Research Station, Narangalla. The technical assistance of Mr. Gamini Perera is much appreciated.

## References

Arfaa, F., Mahboubi, E., and Al Jeffri, M. (1989) The potential role of various species of intermediate and host of *Schistosoma haematobium* Saudi Arabia. *Trans. R. Soc. Trop. Med. Hyg.* **83**, 216-218.

Crawford, M. (1935) Report of the Government Veterinary Surgeon, Ceylon Admin. Rep. 1934, IV, 46-47.

De Bont, J., Van Aken, D., Vercruysse, J., Fransen, J., Southgate, V.R. and Rollinson, D. (1989) The prevalence and pathology of *Schistosoma nasale* Rao, 1933 in cattle in Sri Lanka. *Parasitology* **98**, 197-202.

De Bont, J., Vercruysse, J., Van Aken, D., Southgate V.R. and Rollinson, D. (1991) Studies of the relationships between *Schistosoma nasale* and *S. spindale* and their snail host *Indoplanorbis exustus*. *J. Helminthol.* **65**, 1-7.

De Kock, K.N. (1992) The effect of exposure to selected numbers of *Schistosoma mansoni* miracidia on survival of cohorts of *Biomphalaria pfeifferi*. *Ann. Trop. Med. Parasitol.* **86,** 557-562.

Dissanayake, A.S. and Jayasuriya, D.J.C. (1959) Some parasites hitherto unreported from pigs and cattle in Ceylon. *Ceylon Vet. J.* **7,** 30-33.

Dutt, S.C. and Srivastava, H.D. (1968) Studies on *Schistosoma nasale* Rao,1933. II Molluscan and mammalian hosts of the blood-fluke. *Indian J. Vet. Sci.* **38,** 210-216.

el-Ganayni, G.A., el-Shazly, A.M., Youssef, M.C. and Abdel-Mageid, S.A. (1988) ELISA and COPT in serodiagnosis of *Schistosomiasis mansoni* in children. *Journal of Egypt Society of Parasitology* **18,** 165-171.

Islam, K.S. (1975) Schistosomiasis in domestic ruminants in Bangladesh. *Trop. Anim. Hlth. Prod.* **7,** 244.

Janecharut, T., Usawattanakul, W., Sornmani, S. and Kitikoon, V. (1987) Lung-migration patterns of *Schistosoma mekongi* and *S. spindale* in mouse. *Southeast Asian J. Trop. Med. Public Health* **18** (4): 484-87.

Janitschke, K., Reinhold, A. and Bode, L.(1987) Nitrocellulose dot-ELISA for serodiagnosis of schistosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* **81,** 956-958.

Muraleedharan, K., Kumar, S.P., Hedge, K.S. and Alwar, V.S. (1976a) Studies on the epizootiology of nasal schistosomiasis of bovines. 1. Prevalence and incidence of infection. *Mysore J. Agric. Sci.* **10,** 105-117.

Muraleedharan, K., Prasanna Kumar, S.,Alwar, V.S. and Hegde, K.S. (1976b) A note on the intermediate host of *Schistosoma nasale* Rao, 1933 and on the seasonal prevalence of its cercaria. *Indian Vet. J.* **53,** 819-820.

Richards, S.R. (1976) Variations of infectivity for *Biomphalaria glabarata* in strains of *Schistosoma mansoni* from the same geographical area. *Bull. World Health Organ.* **54,** 706-707.

Sulaiman, S.M. and Ibrahim, S.M. (1985) *Schistosoma mansoni* (Gezira) infection in *Biomphalaria pfeifferi* snails from Gezira and Jebel Marra. *J. Trop. Med. Hyg.* **88,** 91-93.

Theron, A. (1984) Early and late shedding patterns of *Schistosoma mansoni* cercariae: ecological significance in transmission to human and murine hosts. *J. Parasitol.* **70,** (5): 652-655.

Usawattanakul, W., Kamijo, T. and Kojima, S. (1982) Comparison of recovery of schistosomula of *Schistosoma japonicum* from lungs of mice and rats. *J. Parasitol.* **68**, 783.

# HAEMATOLOGICAL AND BIOCHEMICAL PROFILES OF ADULT FEMALE LANKA BUFFALOES (*BUBALUS BUBALIS*)

N.U. Horadagoda, I.S. Gunawardena, A.P.N. Ambagala and D.M.S. Munasinghe

*Department of Veterinary ParaClinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya Peradeniya,*
*SRI LANKA*

**Abstract:** The objective of this study was to establish reference values for haematological parameters and serum biochemical constituents of the Lanka buffalo in order to interpret results from experimental and clinical investigations. Jugular blood collected from 80 apparently healthy female buffaloes (2-7 years) at the Narangalla buffalo research farm were used to determine the haematological parameters whilst serum biochemical constituents were assayed in 120 animals from both Narangalla and Mawela buffalo research farms. The haematological parameters were measured using standard methods while serum analytes were assayed using biochemical kits (Randox Laboratories, UK)

Irrespective of age, the reference values (mean±one standard deviation) for haematological parameters were: red blood cells, 5.34±1.23 ($10^{12}/1$); haemoglobin, 11.88±1.69 (g/dl); packed cell volume, 0.31±0.05 (1/1); erythrocyte sedimentation rate, 31.17±22.2 (mm/hr); plasma protein, 76±11.5 (g/l); Fibrinogen, 6.8±2.7 (g/l); icterus index, 5.66±2.23; white blood cells, 11,642±3991/µ1; lymphocytes, 54.05±11.31(%) or 6171±3540/µl; neutrophils, 36.23±11.04 (%) or 3797±1928/µl; monocytes 3.48±2.26 (%) or 682±580/µl; eosinophils, 6.79±5.51 (%) or 388±357/µl and basophils 0.38±0.3 (%) or 39±24/µl. The reference values (mean±one standard deviation) for biochemical constituents were: albumin, 31.64±4.54 g/l; aspartate aminotransferase (AST), 105±36.56 U/l; alanine aminotransferase (ALT), 36.2±11.8 U/l; creatine phosphokinase, 66.75±35.5 U/l; alkaline phosphate (ALP), 118.5±64.3 U/l; gamma glutamyl transferase, 18.8±16.03 U/l; glucose, 3.22±0.87 mmol/l; urea, 4.82±1.62 mmol/l; creatinine, 130.27±27.25 mmol/l and bilirubin 4.9±4.4 (total), 2.05 ± 1.7 (direct) µmol/l.

The results of this study indicate that the haematological parameters of the Lanka buffalo are comparable to those reported for both swamp and river type buffaloes in Asia, characterised by a low icterus index, a high ESR and a predominance of lymphocytes over neutrophils. The biochemical values, in general, correspond to values reported for the swamp buffalo in Australia and are similar to the values described in cattle, except for AST, ALP and ALT which are higher in the buffalo. In contrast to other domesticated ruminants, the serum ALT concentration in the buffalo is high, and may reflect a species characteristic.

**Keywords:** Buffalo, reference values, blood constituents, haematology, serum biochemistry

## INTRODUCTION

Haemograms and measurements of serum biochemical constituents are widely used in veterinary medicine for disease diagnosis and to understand the pathophysiological events of diseases during clinical and experimental investigations. The interpretation of the haematological and biochemical data of animals is dependant on the availability of reference or baseline values of the parameters as distinct species differences have been reported (Jain, 1986; Kaneko, 1989). For example, in the haemogram of the dog, cat, horse and pig, neutrophils are the most predominant leucocyte in circulation while in ruminants, laboratory rodents and chicken, lymphocytes are more frequent than neutrophils. Similarly, biochemical constituents have also shown species variation. Alanine aminotransaminase (ALT), an enzyme primarily of hepatic origin is present in relatively high concentrations in the serum of the dog and cat and the levels increase considerably with hepatic diseases/disorders therefore, ALT is a useful indicator of liver injury in these species. In contrast, the serum ALT levels in the cow and horse are very low and are not significantly increased with hepatic injury. In addition to such species characteristics, other factors such as age, breed, sex, climate, nutrition and physiological status are also known to influence the haematological and biochemical profiles.

Information on the haematology and serum biochemistry of the buffalo is scanty compared to other domesticated animals. The current literature, is to a large extent based on studies conducted in the river type buffalo in India (Jain, 1986). These studies have examined the haemogram in relation to breed (Patel *et al.*, 1969; Rao and Rao, 1977), age (Sharma *et al.*, 1973), management (Banga *et al.*, 1980), physiological status (Jain *et al.*, 1982) and climate (Patel *et al.*, 1971; Hassan *et al.*,1981). In contrast to the river type buffalo which prefer clear running water to wallow, the Lanka buffalo wallows in stagnant water and has phenotypic characteristics of the swamp buffalo present in South East Asia. It is therefore classified as a swamp buffalo although it has 50 chromosomes. In comparison to the river type buffalo only a very few studies have examined the haematology of the swamp buffalo. Sulong *et al.* (1980) studied the haematology of the Malaysian swamp buffalo while Canfield *et al.* (1984) reported on the heamatological and biochemical profiles of swamp buffalo in the Northern Territory of Australia. These studies have demonstrated that the red blood cells, packed cell volume and haemoglobin are higher in the swamp buffalo than that observed in river type buffaloes. In addition, the Malaysian study (Sulong *et al.*, 1980) showed that the leucocyte counts are also high in the swamp buffalo. This study was undertaken to establish reference values for haematological and biochemical constituents in order to provide a basis for interpretation of data from clinical and experimental investigations on the Lanka buffalo.

## MATERIALS AND METHODS

*Locations of study :* The blood analysed in this study was collected from adult Lanka buffaloes (2-7 years) managed at the SAREC/NARESA Buffalo Research farms in Peradeniya and Narangalla. All animals tested were free from disease, fed only on natural pasture and had been on the two farms for at least one year prior to bleeding.

*Blood collection*: The animals were restrained with great care to avoid undue excitement. Five millilitres of jugular blood collected into a vacutainer tube (Becton Dickinson, USA) containing EDTA (2mg/ml) were used for haematological studies while serum prepared from 10 ml of blood collected into a second vacutainer tube without anticoagulant was used for measurement of biochemical constituents. A further 2 ml collected into fluoride oxalate tubes were used for glucose estimation. All blood collections were made between 7.00 and 9.00 a.m. and the samples were transported to the laboratory within four hours of collection, in a polystyrene box maintained at 4°C.

## Haematological Analysis

All haematological estimations, except the differential counts were performed within 24 hours of collection. The methods used in this study were selected from procedures described by Jain (1986); a brief description of these procedures is given below.

*Erythrocyte enumeration*: The red blood cells were counted in an improved Neubaur haemocytometer after diluting (1:200) the cells with Gower's solution in standard blood diluting pipettes.

*Leucocyte enumeration*: The white blood cells were also counted in an improved Neubaur haemocytometer after diluting cells in standard blood diluting pipettes. Turk's solution was used to dilute (1:50) the white blood cells.

*Haemoglobin estimation*: The haemoglobin concentration was measured by the cyanmethhaemoglobin method. The absorbance of samples as calculated using a standard curve prepared with 4 serial dilutions of aquaglobin, a haemoglobin standard (30mg/dl) from Sigma Chemical Co. (Poole, UK).

*Packed cell volume*: The packed cell volume (PCV) was determined by using a microhaematocrit centrifuge. Two capillary tubes (75 x 1 mm) were filled with blood, centrifuged for 5 minutes at 5000g and the height of the packed cells was determined using a reader designed for this purpose. The mean of two readings was used as the PCV for the animal.

*Erythrocyte indices*: The red cell indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated using standard formulae.

*Erythrocyte sedimentation rate*: The erythrocyte sedimentation rate (ESR) was measured after standing the blood for one hour in Westergren tubes.

*Differential counting*: Blood smears were prepared immediately after collection, fixed in acetone and later stained with Giemsa. Both cell size and appearance were assessed in areas of the smear showing little or no overlapping of erythrocytes. The distribution of leucocytes in each animal was based on the differentiation of 200 cells.

*Plasma protein and fibrinogen concentrations*: Total plasma protein and fibrinogen concentrations were determined with a refractometer (Golberg). For this purpose, two microhaematocrit capillary tubes were filled with blood and centrifuged in a microhaematocrit centrifuge (5000 rpm) for five minutes and then one of the tubes was heated in a water bath at 56°C for three minutes and recentrifuged to sediment the heat-precipitated fibrinogen. Protein concentration of fluid in both capillary tubes were measured with the refractometer. The value of the unheated plasma represented the total plasma protein concentration while the difference between the readings of unheated and heated plasma tubes reflected the fibrinogen level.

## Biochemical Assays

Serum concentrations of albumin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), bilirubin, creatine phosphokinase (CPK), creatinine, glucose, gamma glutamyl transferase (GGT) and urea were measured using biochemical kits from Randox (RANDOX Laboratories Ltd. 55, Diamond Road, County Antrim, Northern Ireland BT29 4QY, UK). Total proteins were assayed by the biuret method (WHO Clinical Chemistry and Haematology Manual,1986).

## RESULTS

The values for haematological parameters of 80 clinically healthy, adult Lanka buffaloes are presented in Table 1 as means with one standard deviation.

**Table 1.** Haematological parameters for the adult female Lanka buffalo.

| Parameters | Mean±SD (n = 80) |
|---|---|
| Red blood cells ($x10^{12}/1$) | 5.34 ± 1.23 |
| Haemoglobin (g/dl) | 11.88 ±1.69 |
| Packed cell volume(1/l) | 0.31± 0.05 |
| Mean corpuscular haemoglobin (pg) | 22.76± 7.08 |
| Mean corpuscular volume (fl) | 59.1± 19.22 |
| Mean corpuscular haemoglobin concentration (g/dl) | 38.21±14.8 |
| White blood cells (number/ l) | 11,642± 3, 991 |
| Plasma protein (g/1) | 76 ±11.5 |
| Fibrinogen (g/1) | 6.8± 2.7 |
| Erythrocyte sedimentation rate (mm/hr) | 31.2± 22.2 |
| Icterus index (units) | 5.66± 2.23 |

The prominent features of the haemogram of the Lanka buffalo include the average size (MCV) of erythrocytes, similar to that in cattle, low icterus index, conspicuous ESR and absence of reticulocytes. The morphological features of red blood cells are comparable to those observed for cattle cells. Erythrocytes exhibit a varying degree of rouleaux formation, slight rouleaux formation, slight anisocytosis and uniform staining. Individual red cells, however, appeared as slightly biconcave discs with an indistinct central pallor. Polychromasia was absent. The distribution of the leucocytes are given in Table 2. The results indicate a predominance of lymphocytes over neutrophils, in common with other domesticated ruminants. Table 3 shows the results of 11 serum biochemical constituents assayed in 120 clinically healthy adult female buffaloes.

**Table 2.** Differential leukocyte count of the Lanka buffalo.

| Parameter | Percentage | Absolute number (number of cells/$\mu$l) |
|---|---|---|
| Lymphocytes | 54.05±11.3 | 6171±3540 |
| Neutrophils | 36.2±11.04 | 3797±1928 |
| Eosinophils | 6.8±5.51 | 388±357 |
| Monocytes | 3.48±2.26 | 682±580 |
| Basophils | 0.38±0.3 | 39±24 |

**Table 3.** Serum biochemical constituents of adult female Lanka buffaloes.

| Serum constituent | Mean± SD (n=120) |
|---|---|
| Albumin (g/1) | 31.6±4.5 |
| Alanine aminotransferase (U/1) | 36.2±11.8 |
| Aspartate aminotransferase (U/1) | 105±36.56 |
| Creatine phosphokinase (U/1) | 66.8±35.5 |
| Alkaline phosphatase (U/1) | 118.6±64.3 |
| Gamma glutamyl transferase (U/1) | 18.8±16.03 |
| Glucose (mmol/1) | 3.2±0.87 |
| Urea (mmol/1) | 4.82±1.62 |
| Bilirubin (total; µmol/1) | 4.9±4.4 |
| Bilirubin (direct; µmol/1) | 2.05±1.7 |
| Creatinine (mmol/l) | 130.27±27.25 |

## DISCUSSION

Reference values for blood parameters and serum biochemical constituents form a useful basis not only for the interpretation of results from clinical and experimental investigations but also to establish physiological trends and species characteristics. Most of the work on buffalo haematology and serum biochemistry is based on studies conducted on the river type breeds such as Murrah and Surti found in India. These studies have examined the influence of breed, age, sex, management and a range of other animal and environmental factors on the haemogram (Jain, 1986). One of the first systematic studies on the haemogram of the swamp buffalo was reported by Sulong *et al.* (1980) in Malaysia. These workers sampled 50 clinically healthy Malaysian swamp buffaloes between the ages of 2 and 4 years and reported the existence of differences in the blood profiles of the river and swamp type buffaloes. In another study, Canfield *et al.* (1984) sampled 99 mature and 55 immature feral swamp buffaloes which were domesticated and managed at an experimental unit in the Northern Terrority of Australia. The haematology profiles in both the Malaysian and Australian studies indicated that the RBC count, haemoglobin concentration and packed cell volume in the swamp buffaloes were higher than the values reported for the river type buffaloes (Simon and Jacob, 1961; Patel *et al.*, 1969; Jain *et al.*, 1982). However, the results of the present study does not lend support to this observation as the RBC count, Hb concentration and PCV in the Lanka buffalo are comparable to values reported for the river type buffalo. Despite these differences in the red cell parameters the values for red cell indices, MCHC, MCH and MCV tended to overlap between the two types of buffalo. The morphology of red blood cells in the Lanka buffalo was very similar to that of the Murrah (Jain *et al.*, 1982) and had some features such as a slight anisocytois, absence of polychromasia which are characters frequently observed in cattle.

In the present study, the total WBC counts tended to have a large range which made it difficult to make comparisons with other studies in the buffalo (Sulong *et al.*, 1980; Jain *et al.*, 1982; Canfield *et al.*, 1984) or to comment on the observation made by Sulong *et al.* (1980) that the

WBC count was higher in the swamp buffalo compared to the river type animals. The percentage distribution of the various leucocytes, particularly the lymphocytes and neutrophils were remarkably similar in our study compared to that reported for both the river type (Simon and Jacob, 1961; Moustafa, *et al.*, 1964; Jain *et al.*, 1982) and swamp type buffalo (Sulong *et al.*, 1980; Canfield *et al.*, 1984). Jain *et al.* (1982) also noted that in some animals the neutrophil percentage exceeded the lymphocytes and they attributed this phenomenon to a redistribution of neutrophils in the vascular pool either due to handling of the animals, or to subclinical infection. Similar observations were made in the present study among a few of the animals tested. The distribution of monocytes, eosinophils and basophils in our study were in general, comparable to that reported by other workers. The morphological features of the leucocytes in the Lanka buffalo were essentially similar to those described in detail by Jain *et al.* (1982) for the Murrah buffalo.

The increased ESR in the buffalo is indeed, a clear discriminating feature in the heamatology when compared to other domesticated ruminants such as cattle, sheep and goats. Purushotham and Mahendar (1963) in a comparative study, reported that the buffalo has a higher ESR even when compared to the horse and dog. The precise reason for the increased ESR in the buffalo is not known. In the horse, the rapid ESR is attributed to rouleax formation but rouleax formation is not a feature.in buffalo blood and it was not observed to a large extent in the smears examined in the present study. Several workers have attempted to determine the relationship between the ESR and other blood parameters of the buffalo. In this connection, Raghavan and Gaffar (1960) reported an inverse relationship between the PCV and ESR. It was observed that buffalo calves with a high PCV had a slow ESR compared to adults animals that had low PCV. They also noted that the ESR increased with age. In a subsequent study, Raghavan *et al.* (1961) demonstrated a direct relationship between the specific gravity of blood and PCV. The findings described in the latter two reports (Raghavan and Gaffar, 1960; Raghavan *et al.*, 1961), when taken together, suggest that the ESR of the buffalo is related to the specific gravity of blood. Accordingly, the low ESR present in calves is attributed to the high specific gravity and PCV whereas the high ESR observed in adults is due to the low PCV and specific gravity. In a separate study, Escudero and Resoso (1968) reported that both ESR and PCV in the buffalo were high in the females compared to that in males but the authors failed to demonstrate these differences statistically. Unlike in other species, the ESR in the buffalo has not been exploited as yet, as a clinical aid in disease diagnosis. Future research need to focus on the ESR of the buffalo in order to explore the possibilities of using it as a routine diagnostic test in heamatology as in man and other domestic animals such as horse, dog and cat.

Most biochemical data available for the buffalo has been derived from studies in the Indian and Egyptian buffalo. However, Sulong *et al.* (1980) and Canfield *et al.* (1984) reported on the plasma protein concentrations of the swamp buffalo in Malayasia and Australia, respectively. These values are essentially similar to that reported in the present study and those recorded for the Murrah buffalo (Jain *et al.*, 1982) and the Egyptian buffalo (Hafeez and Anwar, 1956). In addition, the fibrinogen concentration reported herein is comparable to that reported for the Murrah buffalo by Jain *et al.* (1982). The values for albumin, gamma glutamyl transferase and urea in the Lanka buffalo were essentially similar to those reported for the swamp buffalo in Australia and in general, they were within the reference range for cattle. The creatine phosphokinase values tended to overlap in our study with that of the Australian work although the mean values were different in the two studies. The blood glucose concentration reported in this study was similar to that recorded by Canfield *et al.* (1984) as well as by other workers in India (Kehar and Murty, 1951) and Egypt (Hafeez and Anwar, 1956). The levels of ALP and AST recorded in this study and that by Canfield *et al.* (1984) were higher than values reported for cattle. The enzyme ALT is generally low in ruminants but the present study has demonstrated relatively high concentrations of this enzyme in the buffalo which may very well reflect a species characteristic.

## Acknowledgements

The authors wish to thank the Swedish Agency for Research Co-operation with Developing Countries (SAREC) for providing the financial support and the Natural Resources, Energy & Science Authority of Sri Lanka (NARESA) for operating the grant.

## References

Banga, G.S., Gangwar, P.C., Srivastar, R.K. and Dinga, D.P. (1980) Effect of spray cooling and wallowing on blood composition in buffaloes during summer. *Indian J. Dairy Sci.* **33,** 294-298.

Canfield, P.J., Best, F.G., Fairburn, A.J., Purdie, J. and Gilham, M. (1984) Normal haematological and biochemical values for the swamp buffalo (*Bubalus bubalis*) in Australia. *Aust.vet. J.* **61,** 89-93.

Escudero, S.H. and Resoso, B.B. (1968) The erythrocyte sedimentation rate and packed cell volume of blood of Philippine carabaos. *Philippine J. vet. Med.* **7,** 57-68.

Hafez, E.S.E. and Anwar, A. (1956) Cytological, physiological and chemical studies of Egyptian buffalo blood. *Indian J. vet. Sci.* **25,** 125-130.

Hassan, A., Samak, M. and Badaway, A. (1981) Seasonal variation in lactational performance and blood haematological characteristics of cross-bred (Egyptian x Holstein) and buffalo (*Bubalus bubalis*) cows under sub tropical conditions. *World Review of Animal Production* **17,** 65-72.

Jain, N.C. (1986) *Schalm's Veterinary Haematology.* 4th Edition. Lea Febiger USA.

Jain, N.C., Vegad, J.L., Jain, N.K. and Shrivastava, A.B. (1982) Haematological studies on normal lactating Indian water buffaloes. *Res. Vet. Sci.* **32,** 52-56.

Kaneko, J.J. (1989) *Clinical Biochemistry of Domestic Animals.* 4th Edition. Academic Press, USA.

Kehar, N.D. and Murty, V.N. (1951) Physiological studies on the blood of domestic animals. II. Male buffaloes. *Indian J. vet. Sci.* **21,** 13-16.

Moustafa, I.H., Soliman, M.K. and Nasr, H. (1964) Some pecularities of buffalo blood. *Vet. Med. J.* **9,** 263-269.

Patel, B.M., Vaidya, M.B., Patel, P.M. and Patel, C.A. (1969) Blood picture of the Surti buffalo. *Indian vet. J.* **46,** 860-865.

Patel, B.M., Vaidya, M.B., Thakore, V.R. and Shukla, P.C. (1971) Seasonal variation in certain biochemical and haematological constituents in blood of Surti buffaloes. *Indian J. Anim. Sci.* **41,** 537-541.

Purushotham, N.P. and Mahendar, M. (1963) A note on the comparative study of the blood picture in domestic animals. *Indian vet. J.* **40,** 553-558.

Raghavan, R.S. and Gaffar, A.A. (1960) Observations on the blood sedimentation rate and packed cell volume of buffaloes. *Indian vet. J.* **37,** 583-586.

Raghavan, R.S., Mahendar, M. and Gaffar, A.A. (1961) Specific gravity of blood and serum in relation to packed cell volume of buffaloes. *Indian vet. J.* **38,** 349-352.

Rao, N.M. and Rao, R.P. (1977) A note on the haematological picture of Murrah breeding bulls. *Indian vet. J.* **54,** 940-942.

Sharma, D.P, Malik, P.D. and Sapra, K.L. (1973) Age-wise and species-wise haematological studies in farm animals. *Indian J. Anim. Sci.* **43,** 289-295.

Simon, K.J. and Jacob, A. (1961) Studies on the constituents of buffalo blood. Part I. Cellular elements and haemoglobin. *Indian vet. J.* **38,** 183-187.

Sulong, A., Hilmi, M. and Jainudeen, M.R. (1980) Haematology of the Malaysian swamp buffalo. *Pertanika* **3,** 66-70.

World Health Organisation (WHO) *Methods Recommended for Essential Clinical Chemistry and Heamatological Tests for Intermediate Hospital Laboratories (1986)* WHO, Geneva, Switzerland.

# "DOMOSEDAN" AS A SEDATIVE ANALGESIC IN INDIGENOUS BUFFALO

D.D.N. De Silva

*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya SRI LANKA*

**Abstract**: Detomidine HCl (Domosedan, Farmos, Finland) is an Imidazole derivative which acts upon $\alpha$-2 adrenoreceptors. Its sedative/analgesic effect in horses is well documented. In Sri Lanka, the limited availability of an effective sedative/analgesic drug for buffaloes for such purposes as immobilization, preoperative medication, analgesia and restraint of intractable animals for handling and transportation prompted the present study to evaluate the effectiveness of Detomidine HCl as a sedative analgesic.

Four dosages 0.1, 0·2, 0·4 and 0·8 ml/100 kg of 1% detomidine HCl solution were tested in 32 male and female buffaloes (8 months to 8 years). Rectal temperature, pulse rate and respiratory rate were measured prior to and at 1, 5, 10, 15, 20, 30, 45, 90 and 120 minutes after intramuscular administration of the drug into the gluteal region. Qualitative observations such as ataxia, drowsiness, salivation, relaxation of the jaw, recumbency and response to painful stimuli were also made at the aforementioned time intervals after injection. The results indicated that the dosage of 0·1 ml/100 kg caused only a mild sedation with limited practical use, whereas dosages of 0·2 and 0·4 ml/100 kg produced desirable sedative/analgesic effects for clinical examination, preoperative medication, minor surgical procedures and transportation. The highest dosage level of 0·8 ml/100 kg produced complete immobilization characterised by recumbency, very deep sedation and marked analgesia. In general, detomidine caused bradycardia, reduced pulse pressure and salivation while with higher dosages it produced regurgitation. The degree of sedation and analgesia were dose dependent.

**Keywords**: Buffalo, Detomidine hydrochloride, sedation, analgesia, immobilization.

## INTRODUCTION

Indigenous buffaloes are reared mainly for draft purposes in Sri Lanka. They are considered to be a cheaper alternative to tractors and other farming machinery. By nature, these animals are aggressive and often need to be restrained by physical methods or by chemical means. In some instances, the physical methods of restraint cause serious injuries to the handlers therefore, chemical restraint is preferred. Morever in the use of the Lanka buffalo and their crosses for milk production, the need for taming and handling these "semi wild" animals poses a challenge. In order to produce superior crosses between Lankan and Indian breeds, numerous research programmes are under way and implementation of management procedures demand safe handling of the animals with an effective sedative/analgesic drug. In Sri Lanka, the availability of an effective sedative/analgesic drug is limited, therefore it was felt worthwhile to investigate the use of an alternative drug. The present study was undertaken to evaluate the sedative/ analgesic effects of the alfa 2 agonist, Detomidine hydrochloride ("Domosedan", Farmos, Finland) on the Lanka buffalo.

## MATERIALS AND METHODS

Thirty two buffaloes (18 males and 14 females) aged between 8 months to 8 years were randomly divided into 4 groups. The estimated body weights of these animals ranged from 100 to 550 kg. Four dosage levels of Domosedan (10 mg/ml) suggested by De Silva *et al.* (1990), namely 0.1ml/100kg (10µg/kg), 0.2ml/100kg (20µg/kg), 0.4ml/100kg (40µg/kg) and 0.8ml/100kg (80µg/kg) were administered intramuscularly into the gluteal region. Rectal temperature, respiratory rate, pulse rate and heart rate were measured prior to and, at 1, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after administration of Domosedan. The qualitative observations such as ptosis, head lowering, relaxation of the jaw, penis and muscles of the flank, ataxia, recumbency, salivation, urination, defecation, lacrimation, regurgitation, expiratory grunt, reaction to pin pricks at the base of the horn, interdigital space, coronet and flank, reaction to handling of teats and vagina in females and the penis and the scrotum in males were carried out at the aforementioned time intervals after administration of the drug.

## RESULTS

*Rectal temperature:* No significant changes in the rectal temperature were observed with any of the dosages.

*Respiratory rate:* There were no significant changes in respiratory rates with the dosages of 0.1, 0.2 and 0.4 ml/100 kg. However, the animals given 0.8 ml/100 kg showed a significantly ($P<0.05$) increased respiratory rate above the pre-injection value from 15 to 90 min. and then returned to almost normal rates at 120 minutes.

*Heart rate and pulse rate:* At all four dosage levels, heart rate and pulse rate were decreased significantly ($P<0.005$) from the pre-injection values, 1 minute after the injection. With the last two dosages (0.4 ml/100 kg and 0.8 ml/100 kg) pulse could not be palpated in the coccygeal artery from 5 to 20 minutes and 30 minutes respectively, after the injection. The pulse rate did not return to normal values even at 120 minutes with any of the dosages.

### Sedation and Analgesia

*0.1 ml/100 kg:* There was slight ataxia after 10 minutes. Animals became slightly sedated but could be aroused easily. Reactions to painful stimuli remained intact. Relaxation of the jaw was seen in 3 young animals and salivation was evident in all eight animals after 15 minutes.

*0.2 ml/100 kg:* There was ptosis, lowering of the head with relaxation of the jaw and salivation 10 minutes after the injection. There was no reaction to painful stimuli from minute 20 onwards and examination of the vulva and udder in all the females and the scrotum and penis in about

75% of the males was possible. Voluntary ear flapping was present in all the animals throughout the procedure with this dosage.

*0.4 ml/100 kg:* Animals showed signs of sedation (ptosis, head lowering, respiratory grunt) in 5 to 10 minutes after the injection. There was relaxation of the jaw, muscles of the flank and marked ataxia after 10 minutes. Half the animals became recumbent. There was profuse salivation and in 2 animals (25%) there was regurgitation of ruminal contents after recumbency. The recumbent animals were resting their muzzles on the ground from 30 to 60 minutes and raised their heads intermittently but remained recumbent until 90 to 115 minutes after injection. They stood up when forced and remained standing but sedated until about 180 minutes. No reactions to pin pricks at the base of the horn, interdigital space, coronet and flank were shown and also examination of the vulva and udder in females and the penis and scrotum in males were not resented. Two pregnant females (2 - 3 months of gestation) included in this group did not show any adverse changes during and after the experiment.

*0.8ml/100kg:* There was a marked sedation in 1 to 5 minutes. Ptosis, head lowering, marked ataxia and salivation was evident and recumbency s was observed in all the animal within 10 to 20 minutes. A marked relaxation of the muscles of the flank and lack of reaction to needle pricks was observed and their muzzles were rested on the ground or on the flank (milk fever position). There was a prominent expiratory grunt, regurgitation and the animals remained recumbent for about 180 minutes.

## DISCUSSION

The results showed that a dosage of 0.1 ml/100 kg had limited value for practical use. The time of onset of sedative effect with dosages of 0.2ml/100 kg and 0.4 ml/100 kg was comparable with the findings reported by a previous worker for cattle following intramuscular injection (Vainio, 1985). The dosage of 0.2 ml/100 kg is recommended for sedation for clinical examination, minor surgical interventions in standing animals in conjunction with local analgesia and also for transportation. The dosage of 0.4 ml/100kg was suitable for clinical examination and for major surgical interventions with local analgesia and for immobilization of animals. No adverse effects on pregnancy were evident with this dosage at least in the first trimester of pregnancy, which supports the findings of a previous worker (Alitalo, 1986). Further studies have to be done to draw a definitive conclusion on its safety in pregnant animals because, it has been shown that 40-60 μg/kg (0.4 - 0.6ml/100kg) caused contractions similar to that at parturition (Jedruch and Gajewski, 1986). The high dosage of 0.8 ml/100 kg was suitable for major surgical interventions and complete immobilization of animals.

The fact that there was no significant change in rectal temperature with any of the dosages used suggests that the thermoregulatory mechanism of this species is not affected by detomidine. The respiratory rates were also not significantly altered with the first three dosages probably because adequate ventilation was maintained. But, with 0.8 ml/100kg the tidal volume would have been low thus adequate ventilation had to be maintained by compensatory increase in the respiratory rate. However, without measuring the corresponding tidal volume this hypothesis cannot be proved. With all four dosages, the heart and the pulse rates were lowered together with the pulse pressure. Since the mean arterial blood pressure was not measured in this study it is not possible to comment on the significance of hypotension.

In general it can be concluded that the sedation and analgesia with Detomidine was dose dependent. For most clinical and practical purposes a dosage between 0.2 ml and 0.4 ml/100 kg is sufficient. Although a dosage at 0.8 ml/100 kg produced complete immobilization and pronounced muscle relaxation, it should be used cautiously as there is a risk of aspiration of regurgitated ruminal contents.

## References

Alitalo, I (1986) Clinical experience with Domosedan in horses and cattle. A Review. *Acta vet. Scand.* Suppl 82/1986, pp. 193-196

Jedruch, J. and Gajewski, Z. (1986) The effect of Detomidine hydrochloride (Domosedan) on uterine electrical activity of the uterus in cows. *Acta vet. Scand.* Suppl.82/1986, pp. 189-192.

De Silva, D.D.N., Dangolla, A. and De Silva, L.N.A. (1990) Preliminary studies on sedative and analgesic effects of Detomidine (Domosedan) in buffaloes. *Proceedings of the 43rd Annual Convention of Sri Lanka Veterinary Association.* August 1990.

Vainio, O. (1985) Detomidine, a new sedative and analgesic drug for veterinary use; *Pharmacological and Clinical Studies in Laboratory Animals, Horses and Cattle.* Academic dissertation. Department of Pharmacology and Toxicology, College of Veterinary Medicine, Helsinki, Finland.

NA - 143 - 457

# FUNCTIONAL EFFICIENCY OF BUFFALO NEUTROPHILS

I.D. Silva

*Department of Veterinary Clinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** The functional efficiency of neutrophils of the buffalo was studied by measuring their phagocytic and postphagocytic bactericidal activities. The observations were derived from simultaneous *in vitro* comparative evaluations using blood and milk neutrophils, and three common mastitis causing bacteria, namely, *Staphylococcus aureus, Streptococcus agalactiae* and *Eschesichia coli* in pure and mixed cultures.

The percent phagocytosis (PP) was optimum at 45 minutes and was highest for *Staph. aureus* (86%) followed by *Strep. agalactiae* (77%) and *E. coli* (74%), in pure cultures. The total phagocytic efficiency for pure or mixed bacterial cultures was similar, although with mixed cultures, a majority of neutrophils (40%) phagocytised both species of bacteria while others preferentially phagocytized one species. With mixed cultures of *E. coli* and *Staph. aureus* a significant preference was seen for *Staph. aureus* (approximately 40% PP) while with a mixed culture of *E. coli* and *Strep. agalactiae*, a significant preference was seen for *Strep. agalactiae* (approximately 32% PP). Thus, the affinity of phagocytising *E. coli* was much less in the presence of *Staph. aureus* or *Strep. agalactiae*. The postphagocytic bactericidal activity (PPBA) of blood neutrophils was optimum at 60 minutes. The degree of resistance to PPBA was similar with all three bacteria, but was relatively lower with *E. coli*.

Experimental induction of aseptic subclinical mastitis resulted in a high cell count of $5.6x10^6$/ml of milk after 18 hours. About 90% of those cells were neutrophils which were as efficient as blood neutrophils with the highest PP of 83% for *Staph. aureus* and similar PP for *Strep. agalactiae* and *E. coli* (65%). However, the phagocytic process was relatively slower for *Strep. agalactiae*.

**Keywords:** Phagocytosis, bactericidal activity, buffalo, neutrophils, bacterial mastitis.

## INTRODUCTION

Mastitis is a major disease causing economic losses in the dairy industry. Although buffaloes are relatively resistant to mastitis than cattle, udder infections can cause serious problems in large buffalo herds due to poor hygiene (Wanasinghe, 1985; Chamberlain, 1993; Uppal *et al.*, 1994). Most subclinically affected buffalo quarters do not yield bacteria on culture, while streptococci and staphylococci were the major bacteria isolated from the rest (Silva, *et al.*, 1995).

The efficiency of the defence mechanisms of the mammary gland, namely, the conformational changes in the streak canal, immunoglobulins (Ig), phagocytes and their products (lactoperoxidase/ thiocyanate/hydrogen peroxide complex, lysozymes, lactoferrin) are critical to the cow's ability to survive mastitis (Schalm, *et al.*, 1971; Jain, 1976; Paape, *et al.*, 1976; Paape, *et al.*, 1979; Niemialtowski, *et al.*, 1988). Neutrophils are the most prominent cells in buffalo milk (Silva and Silva, 1994). The invading bacteria will be opsonized by Ig in milk and phagocytised by these neutrophils (Watson, 1976; Niemialtowski, *et al.*, 1988). The phagocytic efficiency of cattle neutrophils vary for different pathogens, and their efficiency is relatively lower, when in the milk (Jain, 1976; Russell, *et al.*, 1977; Silva and Jain, 1988). Bacteria can resist phagocytosis and subsequent microbicidal mechanisms (Henricks, *et al.*, 1986). Impaired leukocytic microbicidal mechanisms will favour the bacteria resulting in an infection or progression of an existing infection (Nagahata, *et al.*, 1988; Kerhli, *et al.*, 1989; Kerhli and Schuster, 1994)

The objectives of the present study were to evaluate and compare the phagocytic efficiency of blood and milk neutrophils of the buffalo for pure and mixed cultures of *Escherichia coli, Staph. aureus* and *Strep. agalactiae,* and to assess the neutrophil microbicidal activity.

## MATERIALS AND METHODS

For each experiment, about 25 ml of blood was collected from each Lanka buffalo by jugular venepuncture into EDTA vacutainers (10 ml capacity). The number and the age of buffaloes used are mentioned for each experiment. Blood was also collected into two 10 ml vacutainers without anticoagulant to obtain fresh serum. Total and differential leukocyte counts were within the normal range of these parameters for the buffalo (Shukla, *et al.*, 1981).

*Isolation of neutrophils from blood:* In all experiments the neutrophil isolation procedure was similar to that for cattle blood (Silva and Jain, 1988). The isolated cell suspension consisted of 80 to 90% neutrophils and the viability varied from 94 to 100% (Jasper, *et al.*, 1966). A working suspension consisted of 5 x $10^3$ viable neutrophils/μl in sterile 0.8% NaCl in 0.0132M phosphate buffer, pH 7.2 (PBS) with 2% bovine serum albumin (BSA).

*Preparation of bacterial cultures:* Separate suspensions of *E. coli* (ATCC 25922), *Staph. aureus* (ATCC 29213) and *Strep. agalactiae* (bovine isolates) consisted of approximately 1 x $10^6$ organisms/μl in PBS (Silva and Jain, 1988). The three bacteria were opsonized separately by incubating at 37°C for 30 minutes with serum of the particular buffalo for each assay. In all assays the neutrophil:bacteria ratio was approximately 1:50 for optimum phagocytosis.

*Experiment 1:* To evaluate the phagocytic efficiency of blood neutrophils, blood was collected from 25 buffaloes between 2-10 years of age.

*Phagocytosis assay:* Four hundred microliters of the neutrophil suspension and opsonized bacteria were incubated at 37°C for 15 and 30 minutes. Assays were done separately for each bacteria. The tubes were gently shaken every 2-3 minutes and were centrifuged at 60 x g for 5 minutes at room temperature to concentrate neutrophils. The smears were made from the pellet for examination. The bacteria was substituted with PBS in the negative control.

*Staining procedures:* Thin, air-dried smears of leukocytes interacted separately with each bacteria were fixed in buffered methanol for 60 seconds followed by 2% methyl green (MG) for 1 minute and counterstained with Leishman stain (Silva and Jain, 1988).

*Microscopic evaluation.* Stained smears were examined microscopically under an oil immersion objective (x 1,250). The nuclei of leukocytes stained light green and the bacteria acquired different degrees of blue. The percentage of phagocytically active neutrophils (cells with ingested bacteria) was determined by counting 500 neutrophils. The neutrophils in the negative control tube (without bacteria) had no ingested particles.

*Statistical analysis:* This was done using the student's T-test at 0.05 level.

*Experiment 2*: To evaluate the phagocytic efficiency of milk neutrophils, six lactating Lankan buffaloes between 4 and 6 years of age were used. Milk and blood samples were collected from these buffaloes after inducing aseptic inflammation of the udder.

*Milk samples*: Aseptic mastitis was induced by infusing 50 ml of sterile distilled water, under aseptic conditions, through the teat canal after milking (Jain, *et al.*, 1967). Milk was collected, aseptically 18 hours postinfusion and transported in ice to the laboratory. These samples gave CMT values between 2 and 3 and consisted an average of 5,600 cells/μl.

*Isolation of neutrophils from milk*: Milk was filtered through 4 layers of gauze to remove flakes, if any, and centrifuged at 300 RCF for 10 minutes. The fat layer and the supernatant were discarded and the pellet was resuspended in sterile 0.8% NaCl in PBS and recentrifuged. The washed pellet was resuspended in 2 ml of PBS with 2% BSA and kept at $4^{0}C$ until used. This suspension consisted an average of 65,000 cells/μl and 90% were neutrophils with an average viability of 94%. A working suspension of $5 \times 10^{6}$ viable milk neutrophils/μl in sterile PBS with 2% BSA was used in the assay.

*Phagocytosis assay:* The assay was performed as described for Experiment 1, using the isolated milk neutrophils.

*Statistical analysis:* This was done using the student's T-test at 0.05 level.

*Experiment 3:* To evaluate the phagocytic activity of blood neutrophils for pure and mixed bacterial cultures, twelve buffaloes between 3 to 10 years of age were used.

*Phagocytosis assay*: Four hundred microlitres of opsonized *E. coli, Staph. aureus and Strep. agalactiae* were aliquoted separately into 3 glass tubes. The 3 tubes constituted the pure bacterial cultures. Then 200µl each of opsonized *E. coli and Staph. aureus*, and 200 microlitres each of opsonized *E. coli and Strep. agalactiae* were aliquoted separately into 2 glass tubes. These two tubes constituted the mixed bacterial cultures.

The five suspensions of microorganisms were incubated separately with 400 µl of the neutrophil suspension prepared from one buffalo. The phagocytosis assay was as described in Experiment 1.

*Microscopic evaluation*: Neutrophils incubated with pure cultures were counted as described in Experiment 1. Neutrophils incubated with mixed bacterial cultures were categorized into 3 groups - cells that had ingested only rods (*E. coli*), cells that had ingested only cocci (*Staph. aureus* or *Strep. agalactiae*), and cells that had ingested both rods and cocci (*E. coli* and *Staph. aureus* or *E. coli* and *Strep. agalactiae*). The neutrophils in the negative control tube (without bacteria) did not show ingested particles.

*Statistical analyses:* The means and standard errors of the means (SEM) were calculated for the PP of neutrophils in pure cultures. To analyze the phagocytic activity in mixed cultures of *E. coli* + *Staph. aureus* and *E. coli* + *Strep. agalactiae*, the 3 groups of cells were subjected to the analysis of variance and the least significant difference was used to compare the means.

*Experiment 4*: To observe the efficiency of postphagocytic bactericidal activity (PPBA), 9 buffaloes between 6-12 years were used. A working suspension consisted of $10^4$ viable neutrophils/µl. Four hundred microlitres of opsonized *E. coli*, *Staph. aureus* and *Strep. agalactiae* were aliquoted separately into three glass tubes.

*Preparation of Eosin B solution:* A 5% solution of Eosin B (C.I. 45400, Certistain BDH Chemicals Ltd., UK) in distilled water was used as a supra vital dye to differentiate the viable and nonviable bacteria intracellularly.

*Phagocytosis assay:* In a single experiment run, the three suspensions of microorganisms were incubated with 200 microlitres each of Eosin B and the neutrophil suspension prepared from one buffalo. The assay was carried out as described in Experiment 1.

*Staining and microscopic evaluation:* Thin air-dried smears were prepared after 30, 45 and 60 of incubation at 37°C. In the unstained smears, the intracellular dead bacteria stained pink with eosin and the live bacteria remained colourless but the intracellular organelles of the neutrophils could not be differentiated. Therefore the smears were stained with 2% MG, for 45 sec to 1 min, and were examined under the oil immersion objective (x 100) of a phase contrast microscope. The neutrophil nucleus and the cytoplasm stained green and pink, respectively. The intracellular dead bacteria stained green whereas, the intracellular live bacteria remained colourless. Those bacteria which were alive at the time of smear making remained colourless even after the smears were fixed and stained with MG. Most of the bacteria outside the neutrophils were colourless while a few stained green.

The PP was determined by counting a minimum of 200 neutrophils. Out of the neutrophils which had performed phagocytosis, the percentages of the following three categories were determined; (a) neutrophils which failed to kill any of the ingested bacteria, (b) neutrophils which had killed all the ingested bacteria, and (c) neutrophils which killed some but failed to kill all the ingested bacteria (with both live and dead intracellular bacteria). The neutrophils in the negative control (without bacteria) did not show ingested particles.

*Statistical analysis:* The means and the SEM were calculated for all the parameters measured and the two sample T-test `at 5% significance level was used for analysis.

## RESULTS

The percent phagocytosis (PP), or the number of neutrophils which performed active phagocytosis, for the three bacteria are presented in Table 1. Data obtained for PP of buffalo blood and milk neutrophils for the three bacteria, studied simultaneously are in Table 2. The PP with pure and mixed cultures of *E. coli* with *Staph. aureus* or *Strep. agalactiae* and the least significant differences used to compare the PP means in mixed cultures are shown in Table 3. Neutrophils exposed to mixed cultures did not always phagocytize both species of bacteria. The values obtained for PP and PPBA are given in Table 4.

**Table 1.** Mean (± 1 SD) of percent phagocytosis of buffalo blood neutrophils for mastitis causing bacteria.

| Bacteria | Time (min) | Percent phagocytosis |
|---|---|---|
| *Staphylococcus aureus* | 15 | 86.09 (± 7.09) |
| | 30 | 87.69 (± 7.31) |
| *Escherichia coli* | 15 | 73.50 (± 11.88) |
| | 30 | 78.03 (± 10.34) |
| *Streptococcus agalactiae* | 15 | 77.38 (± 17.75) |
| | 30 | 80.65 (± 17.91) |

**Table 2.** Mean (± SD) of percent phagocytosis of buffalo blood and milk neutrophils for the three bacteria.

| Bacteria | Neutrophils | Incubation Time | % Phagocytosis |
|---|---|---|---|
| *Staphylococcus aureus* | Milk | 15 | 81.6 ± 10.2 |
| | Blood | 15 | 77.2 ± 12.2 |
| | Milk | 30 | 84.2 ± 10.2 |
| | Blood | 30 | 82.7[ab] ± 11.0 |
| *Escherichia coli* | Milk | 15 | 69.4 ± 11.2 |
| | Blood | 15 | 56.0 ± 12.9 |
| | Milk | 30 | 74.8 ± 11.1 |
| | Blood | 30 | 65.5[b] ± 14.4 |
| *Streptococcus agalactiae* | Milk | 15 | 65.2[c] ± 09.8 |
| | Blood | 15 | 55.6[c] ± 13.9 |
| | Milk | 30 | 76.0 ± 10.6 |
| | Blood | 30 | 65.2[a] ± 08.4 |

a,b,c - mean values with same letter show a significant difference in the percent phagocytosis.

**Table 3.** Percent phagocytosis of buffalo neutrophils for *Escherichia coli, Staphylococcus aureus* and *Streptococcus agalactiae* in pure and mixed cultures.

| Organism | Phagocytosis Mean | S.E.M. |
|---|---|---|
| Pure cultures | | |
| *E. coli* | 76.2 | 1.9 |
| *Staph. aureus* | 90.8 | 0.9 |
| *Strep. agalactiae* | 85.4 | 2.0 |
| Mixed culture of *E. coli* and *Straph. aureus*[1] | | |
| Total | 87.1 | |
| *E. coli* only | 8.0[b] | |
| *Straph. aurues* only | 38.6[a] | |
| Both Bacteria | 40.5[a] | |
| Mixed culture of *E. coli* and *Strep. agalactiae*[2] | | |
| Total | 82.4 | |
| *E. coli* only | 10.8[d] | |
| *Strep. agalactiae* only | 31.5[c] | |
| Both bacteria | 40.1[c] | |

a -d - Means with common letters were not significantly different.

**Table 4.** Means (± SEM) of percent phagocytosis of buffalo neutrophils for the 3 bacteria and the means (± SEM) of percent neutrophils which killed all or some of the ingested bacteria, and those which failed to kill ingested bacteria.

| Bacteria | Time (min) | n | % phago-cytosis | % N with all killed bacteria | % N with all live bacteria | % N with live & killed bacteria |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 30 | 9 | $82^{A,B,1}$ (1.5) | $33.8^{G,H,7}$ (1.8) | $60.3^{N,P,8}$ (2.2) | 5.9 (1.1) |
| | 45 | 9 | $88^{A,2}$ (1.6) | $57.5^{G}$ (0.7) | $39.2^{N,O}$ (1.1) | 3.4 (0.7) |
| | 60 | 9 | $88^{B,3}$ (1.2) | $59.0^{H}$ (1.9) | $36.5^{O,P,9}$ (1.8) | 4.5 (1.5) |
| *Escherichia coli* | 30 | 9 | $77^{C,D,4}$ (1.5) | $33.2^{I,K}$ (1.7) | $63.1^{Q,R}$ (1.6) | $3.6^{U}$ (0.7) |
| | 45 | 9 | $84^{C,5}$ (1.5) | $56.8^{I,J}$ (3.0) | $36.2^{Q}$ (2.9) | $7.0^{U}$ (1.3) |
| | 60 | 9 | $86^{D,6}$ (1.3) | $64.0^{J,K}$ (1.6) | $30.8^{R,9}$ (1.5) | 5.1 (0.7) |
| *Streptococcus agalactiae* | 30 | 8 | $72^{E,F,1,4}$ (1.5) | $27.5^{L,M,7}$ (2.1) | $68.0^{S,T,8}$ (1.8) | 4.5 (1.1) |
| | 45 | 8 | $78^{E,2,5}$ (1.1) | $56.8^{L}$ (2.9) | $39.1^{S}$ (3.0) | 4.2 (1.0) |
| | 60 | 8 | $81^{F,3,6}$ (1.0) | $58.0^{M}$ (2.9) | $36.2^{T}$ (2.9) | 5.8 (1.5) |

$^{A-U}$ Comparison of means within the same organism; means with the same letter were significantly different (<0.05).
$^{1-9}$ Comparison of means between two organisms; means with the same number were significantly different (<0.05). N = neutrophils

## DISCUSSION

The results indicated that the phagocytic activity of buffalo neutrophils was different for mastitis causing bacteria and that individual variations exist among the buffaloes (Table 1). Simultaneous comparative evaluation of the functional activity revealed that the PP was highest for *Staph. aureus* (86.1%). The PP for *Staph. aureus* was statistically similar to that of *Strep. agalactiae* (77.4%) but was significantly higher than that for *E. coli* (73.5%). In comparison, the PP of cattle neutrophils for the same bacterial strains was high for *E. coli* and *Strep. agalactiae* (81.3% and 77%, respectively) and intermediate for *Staph. aureus* (63.6%) (Silva and Jain, 1988). Accordingly, the phagocytic activity of buffalo neutrophils was 22.5% higher for *Staph. aureus*, 7.8% lower for *E. coli* and similar for *Strep. agalactiae*. This finding explains the resistance of buffaloes to mastitis caused by *Staph. aureus* (Wanasinghe, 1985).

Lymphokines secreted by the primed lymphocytes in the cattle mammary mucosae attract blood leukocytes (90-95% of total milk cells), irrespective of the cause of the inflammation (Schalm, *et al.*, 1971; Jain, 1976; Sears, 1984). Similarly, an average of $5.6 \times 10^6$ cells/ml of buffalo milk was observed in subclinical mastitis and 90% of these cells were neutrophils. The 94-100% viability of these milk cells were consistent with that reported in cattle (Dulin, *et al.*, 1988). During acute subclinical mastitis, the phagocytic activity of neutrophils which appear in milk were as efficient as blood neutrophils, (Table 2) and the PP was highest for *Staph. aureus* (82.7%) and similar for *E. coli* (65.5%) and *Strep. agalactiae* (65.2%). An irregularity seen with *Strep. agalactiae* was that the PP reached the level of blood neutrophils only after 30 minutes of incubation. Incidentally, streptococcal species were the major mastitis causing bacteria isolated in clinically normal buffaloes in Sri Lanka (Silva *et al.*, 1995). However, staphylococci, streptococci and *E. coli* cause mastitis in buffaloes (Chander and Baxi, 1975; Fenizia, *et al.*, 1988; Kapur, *et al.*, 1988).

The phagocytosis of milk neutrophils was 17% higher for *Staph. aureus* further explaining the resistance observed in buffaloes to staphylococcal mastitis (Wanasinghe, 1985). In contrast, cattle milk neutrophils have reduced phagocytic activity for *Staph. aureus* and Klebsiella species than blood neutrophils and this had been ascribed to ingestion of fat globules, reduced glycogen content, and surface coating of casein which makes the cells less mobile and less active (Jain and Lasmanis, 1978; Dulin *et al.*, 1988). However, the neutrophils in the present study did not show ingested fat globules. This is attributed to the acute nature of the inflammation induced in these buffaloes. The extravasation of blood neutrophils begins soon after the initiation of an inflammatory process and are devoid of fat globules, and these milk neutrophils resemble their blood counterpart (Jain, 1976; Jain and Lasmanis, 1978). The findings reveal the potential of reducing bacterial mastitis by improving the phagocytic activity of milk neutrophils. This may be achieved by enhancing the opsonin concentration in milk perhaps by vaccination.

The phagocytic activity of neutrophils was not different when incubated with either pure or mixed bacterial cultures. However, when exposed to mixed bacterial cultures the neutrophils did not always phagocytize both species of bacteria. A poor preference was shown for *E. coli*, and this affinity for *E. coli* was much less in the presence of *Staph. aureus* than *Strep. agalactiae*. Previous studies on cattle neutrophils also demonstrated a low preference for phagocytizing *E. coli* in the presence of *Strep. agalactiae*, but not *Staph. aureus* (Silva, *et al.*, 1988). Microorganisms or their metabolic products can inhibit the phagocytic activity (Nonoyama, *et al.*, 1979; Gemmell, *et al.*, 1982; Ryu, *et al.*, 1984). The age of the neutrophils may also play a role in the efficiency of phagocytosis. The number of bacteria available for phagocytosis was not a factor because the optimum ratio of neutrophil:bacteria ratio used in this study.

PPBA was not seen in all phagocytically active neutrophils. Most neutrophils killed all the ingested bacteria whereas a few killed only some of the ingested bacteria. A relatively higher PPBA was observed for *E. coli* at 60 minutes. The PPBA elevated with prolonged incubation periods, which was also described for cattle cells (Mackie, *et al.*, 1982a). This activity was comparable between 30 and 60 minutes. In contrast, the speed of PPBA was relatively slower for *Staph. aureus*, which was seen by the elevation of PPBA when the incubation was prolonged from 45 to 60 minutes (36.5% and 39.2% respectively).

The method described herein, uses eosin to visualize the killed intracellular bacteria, which is simple and requires either an ordinary or a phase contrast microscope for observation. During the process of phagocytosis the medium bathing the bacterium is passively taken in and the bacteria will be killed by the intracellular microbicidal products (Jain, 1976). The present findings revealed that the bacteria which survived were able to exclude eosin, which was taken into the neutrophil along with the bacteria, and remained colourless even after fixing and staining with 2% MG. Previous studies on cattle neutrophils using radioassay technique and electronmicroscopy showed that an accurate estimation of the killing efficiency could be obtained only by direct observation of neutrophils (Mackie, *et al.*, 1982a; Mackie *et al.*, 1982b).

## Acknowledgements

This study was funded in part by the Swedish Agency for Research Cooperation with Developing Countries (SAREC) through NARESA and the University of Peradeniya. The author thanks K.A.M.J. Silva, K.R.J.K. Amararatne and Drs. R.O. Thattil and S. Kariyawasam for their assistance.

## References

Chamberlain, A. (1993) *Milk Production in the Tropics.* Longman Scientific and Technical, UK.

Chander, S. and Baxi, K.K. (1975) A note on diagnosis and treatment of subclinical mastitis in buffaloes. *Indian vet. J.* **52,** 847-849.

Dulin, A.M., Paape, M.J. and Nickerson, S.C. (1988) Comparison of phagocytosis and chemiluminescence by blood and mammary gland neutrophils from multiparous and nulliparous cows. *Am. J. vet. Res.* **49,** 172-177.

Fenizia, D., Guarino, A. and Izzi, R. (1988) Buffalo mastitis on rational and traditional farming. *Proc. Second World Buffalo Congress* **IV,** 52-56.

Gemmell, C.G., Peterson, P.K., Schmeling, D.J. and Quie, P.G. (1982) Effect of staphylococcal alpha toxin on phagocytosis of staphylococci by human polymorphonuclear leukocytes. *Infect. Immun.* **38,** 975-980.

Henricks, P.A.J., Verhoef, J. and Nijkamp, F.P. (1986) Modulation of phagocytic cell function. *Vet. Res. Comm.* **10,** 165-188.

Jain, N.C. (1976) Neutrophil leukocytes and inflammation of the bovine mammary gland. *Theriogenology* **6,** 153-173.

Jain, N.C. and Lasmanis, J. (1978) Phagocytosis of serum-resistant and serum-sensitive coliform bacteria (Klebsiella) by bovine neutrophils from blood and mastitic milk. *Am. J. vet. Res.* **39,** 425-427.

Jain, N.C., Jasper, D.E. and Carroll, E.J. (1967) Bactericidal activity for *Aerobacter aerogenes* of bovine serum and cell-free normal and mastitic milks. *Am. J. vet. Res.* **28,** 397-404.

Jasper, D.E., Jain, N.C. and Brazil, L.H. (1966) Clinical and laboratory observations on bovine mastitis due to mycoplasma. *J. Am. Vet. Med. Assoc.* **148,** 1017-1019.

Kapur, M.P., Sharma, A. and Bhardwaj, R.M. (1988) Bacteriology of clinical mastitis in buffaloes. *Proc. Second World Buffalo Congress* **IV,** 44-47.

Kerhli, M.E. Jr., Nonecke, B.J. and Roth, J.A. (1989) Alterations in bovine peripheral blood neutrophil function during the periparturient period. *Am. J. vet. Res.* **50,** 207-210.

Kerhli, M.E. Jr. and Schuster, D.E. (1994) Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *J. Dairy Sci.* **77,** 619-627.

Mackie, D.P., Pollock, D.A. and Logan, E.F. (1982a) In vitro bactericidal assay of bovine polymorphonuclear leukocytes gainst a group B streptococcus. *Res. Vet. Sci.* **33,** 240-242.

Mackie, D.P., Pearson, G.R., Curran, W.L., Pollock, D.A. and Lodan, E.F. (1982b) Electron microscopic visualization of the *in vitro* phagocytosis of group B streptococci by bovine polymorphonuclear leukocytes. *Res. Vet. Sci.* **33,** 333-337.

Nagahata, H., Makino, S., Takeda, S., Takahashi, H. and Noda, H. (1988) Assessment of neutrophil function in the dairy cow during the perinatal period. *J. Vet. Med.* Series B **35, 747-750.**

Niemialtowski, M., Nonnecke B.J. and Targowski, S.P.. (1988) Phagocytic activity of milk leukocytes during chronic staphylococcal mastitis. *J. Dairy Sci.* **71,** 780-787.

Nonoyama, S., Kojo, S., Mine, Y., Nishida, M., Goto, S. and Kuwahara, S. (1979) Inhibitory effect of *Pseudomonas aeruginosa* on the phagocytic and killing activity of rabbit polymorphonuclear leukocytes: mechanisms of action of a polymorphonuclear leukocyte inhibitor. *Infect. Immun.* **24,** 399-403.

Paape, M.J., Pearson, R.E., Wergin, W.P. and Guidry, A.J. (1976) Enhancement of chemotactic response of polymorphonuclear leukocytes into the mammary gland and isolation from milk. *J. Dairy Sci.* **60,** 53-62.

Paape, M.J., Wergin, W.P., Guidry, A.J. and Pearson, R.E. (1979) Leukocytes - Second line of defense against invading mastitis pathogens. *J. Dairy Sci.* **62,** 135-153.

Russell, M.W., Brooker, B.E. and Reiter, B. (1977) Electron microscopic observations of the interaction of casein micelles and milk fat globuleswith bovine polymorphonuclear leukocytes during the phagocytosis of staphylococci in milk. *J. Comp. Path.* **87,** 43-46.

Ryu, H., Kaeberle, M.L., Roth, J.A. and Griffith R.W. (1984) Effect of Type A *Pasteurella multocida* fractions on bovine polymorphonuclear leukocyte functions. *Infect. Immun.* **43,** 66-71.

Sears, M. (1984). Immunization and Immunity. In: Symposium on Bovine Mastitis. *Veterinary Clinics of North America: Large Animal Practice* **6,** 391-398.

Schalm, O.W., Carroll, E.J. and Jain, N.C. (1971) *Bovine Mastitis.* Lea and Febiger, Philadelphia, U.S.A

Shukla, P.C., Pande, M.B., Desai, H.B. and Desai, M.C. (1981) Haematological and chemical status of blood of local cattle and buffaloes of Panchmahals district of Gujrat State. *Indian J. Anim. Res.* **15,** 103-106.

Silva, I.D., Silva, K.F.S.T., Ambagala, A.P.N. and Cooray, R. (1995) Markers of inflammation in buffalo milk. *Proceedings of the Symposium on the role of buffalo in rural development in Asia.* This Proceedings.

Silva, I.D. and Silva, K.F.S.T. (1994) Total and differential cell counts in buffalo *(Bubalus bubalis)* milk. *Buffalo J.* **2,** 133-137.

Silva, I.D. and Jain, N.C. (1988) Phagocytic and nitroblue tetrazolium reductive properties of bovine neutrophils for mammary pathogens. *J. Dairy Sci.* **71,** 1625-1631.

Silva, I.D., Jain, N.C., Farver, T.B. and Zinkl, J.G. (1988) Phagocytic and postphagocytic activities of bovine neutrophils for pure and mixed bacterial cultures. *J. Dairy Sci.* **71,** 2513-2519.

Uppal, S.K., Singh, K.B., Roy, K.S., Nauriyal, D.C. and Bansal, B.K. (1994) Natural defence mechanisms against mastitis: a comparative histomorphology of buffalo and cow teat canal. *Buffalo J.* **2,** 125-131.

Wanasinghe, D.D. (1985) Mastitis among buffaloes in Sri Lanka. *Proc. of the First World Buffalo Congress* (Abstract) **IV,** 1331-1333.

Watson, D.L. (1976) The effect of cytophilic $IgG_2$ on phagocytosis by ovine polymorphonuclear leukocytes. *Immunology* **31,** 159-161.

# STUDIES ON *Explanatum (Gigantocotyle) explanatum* INFECTION: PREVALENCE IN CATTLE AND BUFFALOES IN SRI LANKA AND PATHOLOGY IN NATURAL INFECTION

I.S. Abeygunawardena[1], D.J. Weilgama[2], N.U. Horadagoda[3] and H.M.H.L. Jayapadma[1]

[1]*Department of Veterinary Preclinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
[2]*Department of Parasitology, Faculty of Medicine,*
[3] *Department of Veterinary Paraclinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya,*
*SRI LANKA*

**Abstract:** *Explanatum (Gigantocotyle) explanatum* is one of the common trematode parasites in the liver of cattle and buffaloes and reported cases show a chronic debility and unthriftiness. Examinations were conducted to determine the prevalence and pathophysiology of the trematode infection. A survey of slaughter house material in Colombo and Kandy was carried out over a one year period commencing in April 1993. Post mortem material from several government farms and other field stations were also collected. Seventy percent of the carcasses examined were of cattle and the rest were of buffaloes. Livers and bile ducts of the cattle and buffaloes were examined for the presence of parasites. Out of 2297 carcasses of buffaloes examined, 784 (34.2%) were affected by the parasite, compared to 13 of 6097 carcasses of cattle (0.21%). The analysis of origin examined of animals or the carcasses revealed that the infection was found in all the agro-climatic zones. However, the prevalence rate was slightly higher (26.9%) in the dry zone compared to the wet zone (19.2%) and intermediate zone (23.2%). Animals over two years of age had a high rate of prevalence (85.7%) whilst only a few animals in the younger group were affected. Affected livers were dissected and parasites were collected for further laboratory studies. Only one species of parasite, *Explanatum explanatum* was identified. The affected livers were firm in consistency. The capsule was thick and opaque compared to that of unaffected livers. Further, some polyp-like protuberances on the epithelial surface of bile ducts were observed. The adult parasites were attached to these protuberances. The microscopy of hematoxylin and eosin stained sections revealed a mononuclear cell infiltration around portal triad with increased amounts of glandular tissue in the bile ducts. The preliminary results of this study suggest that *Explanatum explanatum* is a widespread liver parasite, primarily affecting buffaloes in Sri Lanka. Further studies should be done to determine the clinical aspect of the disease, to develop a serodiagnostic test and to assess economic losses due to the infection.

**Keywords:** Explanatum explanatum, buffalo, liver, prevalence

## INTRODUCTION

Trematode parasites of domestic ruminants have a world-wide distribution and in some countries are considered as a major constraint to ruminant productivity (Varma, 1957, Dargie, 1984). Parasites of Paramphistomatidae family have been found in rumen, reticulum, liver and bile ducts of many ruminant species and *E. gigantocotyle* is one of the common paramphistome parasites found in the liver and bile duct of ruminants, mainly cattle and buffaloes (Nasmark, 1937). This parasite has a world-wide distribution and has been recorded in the liver of buffaloes and cattle in Sri Lanka, India, Pakistan, Burma, Indo-China, Malaysia and the Philippines (Crusz, 1952; Kulasiri and Seneviratne, 1956, Zamri-saad *et al.,* 1988 and Khan and Anjum, 1994).

*Explanatum* (*Gigantocotyle) explanatum* requires the snails as its intermediate host, spending the major part of the life cycle in it, from which mature cercariae are discharged. They encyst on herbage and susceptible animal acquire the infection by grazing on them. Within the host animal, metacercariae are released and migrate into the bile duct where they develop into adults. Mature parasites lay eggs which pass through faeces into the external environment (Kunwar, 1958). The parasite does not appear to cause an acute infection but causes general debility which would affect productive and reproductive functions of the host animal causing substantial economic losses (Stoimeneu *et al.*, 1976). The infection is usually detected at routine postmortem examinations and at abattoirs where the infected livers are condemned, resulting in a direct loss to the meat industry.

The parasite was first reported in Sri Lanka by Crusz (1952) during an extensive survey. Subsequently, Kulasiri and Seneviratne (1956) conducted an abattoir study to examine effects of parasitism in buffaloes. Apart from these early reports, there has been no attempts made to determine the prevalence and pathophysiology of the infection.

This study was therefore, initiated to elucidate the pathophysiology of the disease with the ultimate goal of developing a diagnostic test to detect the infection in live animals. A further objective of the present study was to determine the current rate of infection in cattle and buffaloes in Sri Lanka.

## MATERIALS AND METHODS

### Survey

A survey of cattle and buffalo carcasses was conducted in government farms, field station and local farms. Carcasses presented for meat inspection at the Colombo and Kandy abattoirs were examined at weekly intervals over a one year period commencing in April 1993. Both cattle and buffalo carcasses were examined for the evidence of liver and bile duct parasites. Parasites from infected carcasses were randomly collected into bottles containing well water for laboratory identification. They were examined under a dissecting microscope for species identification as described by Kulasiri and Seneviratne (1956) and Eduardo (1985). Further, the origin of the infected animals, the macroscopic changes of affected livers and bile ducts were recorded and the approximate ages of the affected animals were estimated by using dentition formula. Tissue samples from the affected organs were collected into 10% formol-saline for histological examination.

## Laboratory Studies

*Preparation of tissue samples for microscopic examination*: Tissues fixed in 10% formol-saline were prepared for histological sectioning. The standard laboratory procedures were used and the tissue sections were stained with hematoxylin and eosin. The stained sections were examined under a light microscope to determine the tissue reactions to the parasite.

# RESULTS

The parasites were identified as *E. (gigantocotyle) explanatum* according to their morphological characteristics and the location in the liver of the host as described by Kulasiri and Seneviratne (1957).

*Incidence rate :* During the 12 month study period (April 1993 to April 1994) a total of 2297 buffalo carcasses and 6097 of cattle carcasses were examined. Of the 784 carcasses (34.2%) of buffaloes and 13 carcasses (0.21%) of cattle were found to be affected by the parasite (Table 1).

**Table 1.** Incidence of the infestation based on the abattoir survey.

| Species | No. livers examined | No. livers affected | % infected |
|---|---|---|---|
| Cattle | 6097 | 13 | 0.21 |
| Buffaloes | 2297 | 784 | 34.2 |

*Distribution of infected carcasses :* The rates of incidence in three major agroecological zones are given in Table 2. The highest incidence rate (26.9%) was recorded from the carcasses of animals brought from the dry zone, followed by the intermediate zone (23.2%) and wet zone (19.2%). The incidence rate was greater in animals above 2 years age than in the younger animals.

**Table 2.** Incidence of the infestations in different agro-climatic zones based on the abattoir survey.

| Agro-climatic Zones | % infected |
|---|---|
| Dry zone | 26.9 |
| Wet zone | 19.2 |
| Intermediate zone | 23.2 |

*Macroscopic appearance:* The gross appearance of the infected livers were unremarkable except for slight firmness in the consistency. On sectioning, the large bile ducts in all lobes were dilated, the walls were thickened and the lumen contained numerous adult parasites. The cystic duct was firm, tortuous and markedly dilated (Figure 1). On incision of the bile duct, the wall was found to be several times thicker than the unaffected bile duct and the lumen was packed with adult parasites that were closely attached to the mucosal surface by the posterior sucker (Figure 2). Dislodging the parasites, exposed the underlying mucosa that was studded by numerous protuberances which had a haemorrhagic surface. The height of these protuberances varied from 3 to 5 mm and represented the bile duct mucosa that is taken into the posterior sucker of adult parasites while being attached to the wall. The contents of the bile ducts were mucoid and bile stained while the gall bladder appeared unremarkable and was devoid of adult parasites.

*Histopathology:* In the infected livers the most conspicuous histological changes were confined to the bile ducts. Microscopic examination revealed that the thickening of the bile duct was due to the combination of several factors which included a proliferation of the connective tissue in the lamina propria, a marked hyperplasia of the epithelium and a diffuse infiltration of mononuclear cells.

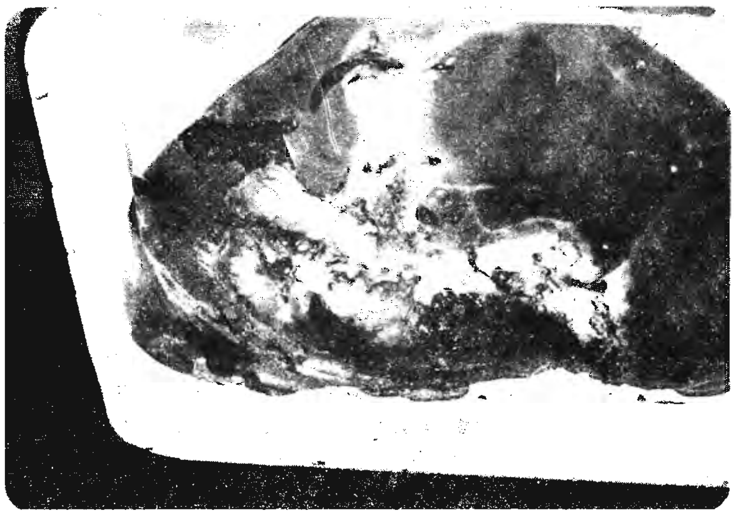


**Figure 1.** The tortuous and dilated cystic duct of the affected liver.

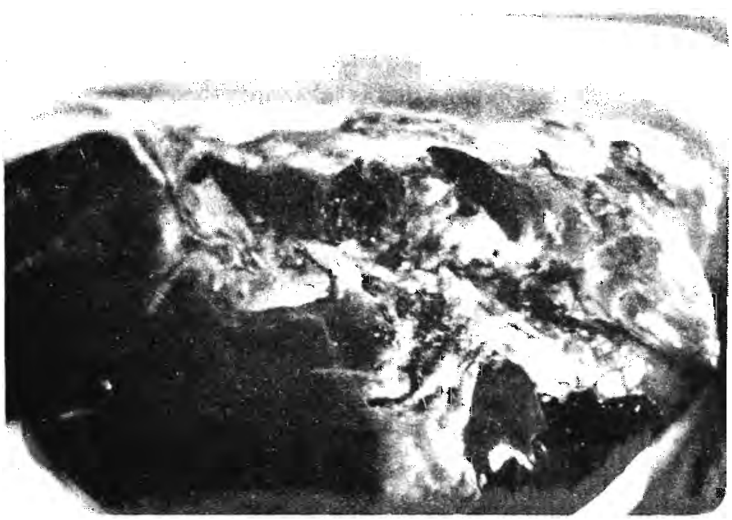


**Figure 2.** The bile duct of the affected liver containing numerous adult parasites.

The presence of polyp-like structures in the bile duct epithelium is another characteristic feature which represented the epithelium that was sucked into the concavity of the posterior sucker of the parasite (Figure 3). The surface of these protuberances like the rest of the bile duct epithelium was necrotic and covered with mucus while the underlying tissue showed a proliferation of connective tissue and a mononuclear cell infiltration with occasional polymorphonuclear leucocytes and eosinophils together with patchy haemorrhages (Figure 4 and 5). There was a marked hyperplasia of the epithelium and the cells were arranged into chords on the surface while in the more deeper layers it formed into glandular structures (Figure 6). The epithelium was dominated by goblet cells. The tissue immediately below the surface epithelium, particularly in the protuberances revealed the presence of a fibroblast proliferation together with new capillary formation, indicative of a repair process (Figure 7). In the lamina propria the most striking change was the marked proliferation of the connective tissue and the presence of hypertrophied medial arteries. Further there were numerous mononuclear cells which were arranged in layers parallel to the epithelial surface and present immediately below the epithelium and adjacent to the liver parenchyma. Occasionally, they were found in clumps or layers between the parallel layers of connective tissue. Despite the considerable pathological changes in the bile duct due to *E. explanatum* infection, the liver parenchymal tissue was relatively unaffected except for a mild to moderate mononuclear cell infiltration of the portal area (Figure 8). Further in a few liver sections, the thickening of the bile duct due to proliferation of connective tissue was also evident in the portal triad. Conversely, the hypertrophy of the arteries observed in the lamina propria was not present in the arteries of the portal triad. There was no microscopic evidence of the presence of parasites or its residual structures in the liver nor was there evidence for bile duct hyperplasia or hepatocyte regeneration which are characteristic features of the liver to injury (Kelly, 1993).

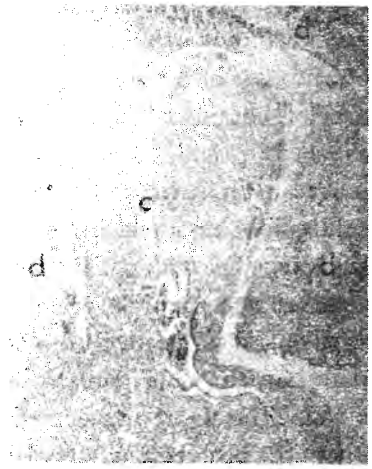



**Figure 3.** A transverse section of the bile duct showing a polyp-shaped projection of the mucosa (c) being drawn into and filling the concavity in the posterior sucker of the parasite (d). H and E x40.

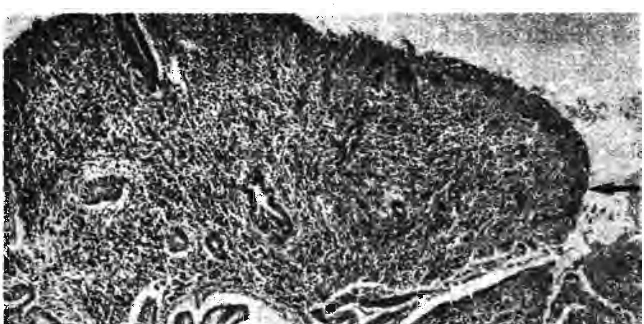


**Figure 4.** The polyp-shaped protuberance (arrow) on the mucosa of the bile duct in which the surface is necrotic and covered with mucus while the underlying tissue consists of hyperplastic mucus secreting glandular structures, mononuclear cell infiltration and a proliferation of connective tissue elements. H and E x40.

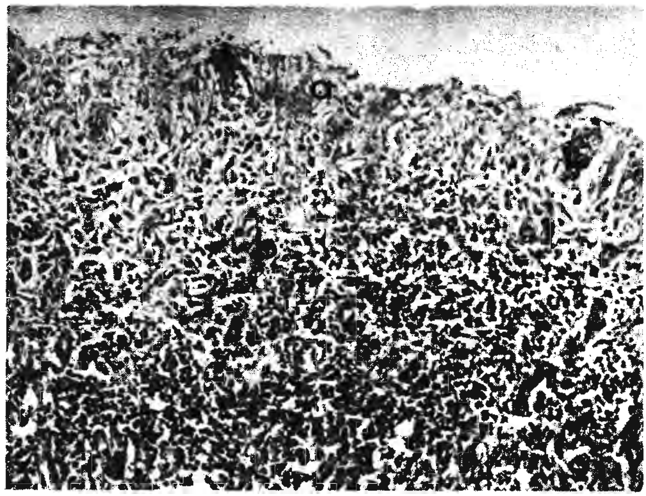


**Figure 5.** The surface of the bile duct epithelium showing necrosis (a) while in the underlying tissue there is a marked mononuclear cell infiltration. H and E x100.

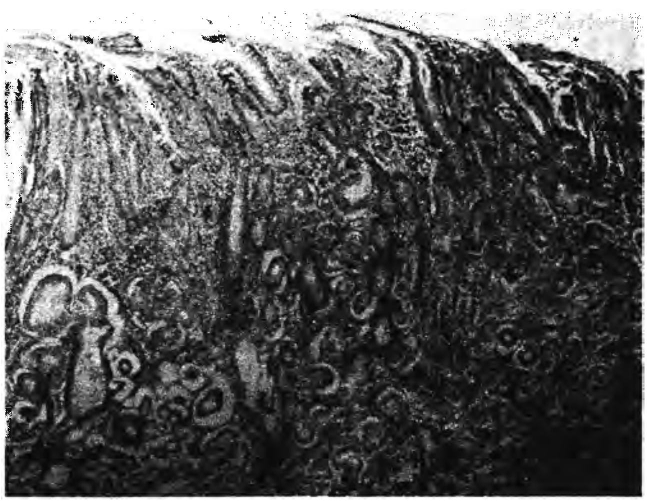


**Figure 6.** Section of the bile duct mucosa depicting a marked hyperplasia of the epithelium which have formed into projection that extend to the lumen and glandular structures in the deeper layers. There is medial hypertrophy of arteries (arrow head) of the lamina propria. H and E x40.

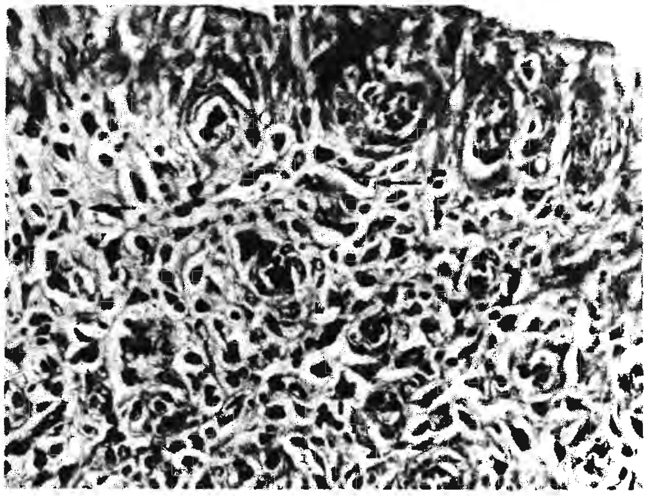


**Figure 7.** The fibroblast proliferation (arrow) and new capillary formation (arrow head) in the tissue underlying the necrotic surface of the bile duct mucosa. H and E x200.

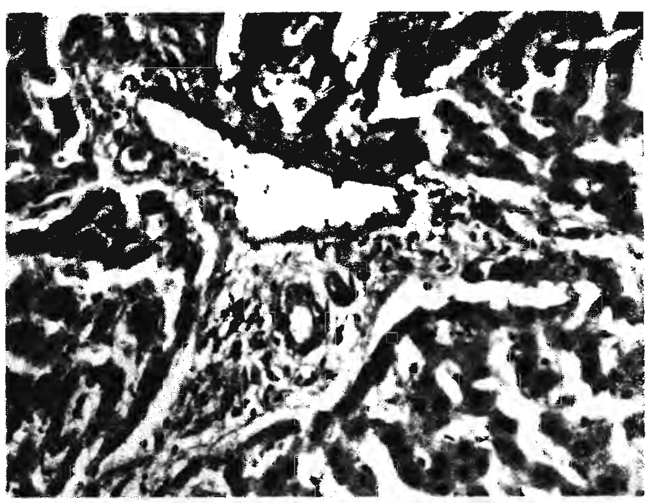


**Figure 8.** The portal tract of an infected liver showing a mild mononuclear cell infiltration. H and E x200.

## DISCUSSION

Presence of *E. (gigantocotyle ) explanatum* in buffaloes and cattle in Sri Lanka confirms the report of Cruz (1952) and Kulasiri and Seneviratne (1956). The estimate of the prevalence of the infection derived from the study indicated that buffaloes are affected more than cattle. Although, a direct comparison cannot be made on the prevalence rate, it is significantly higher in the dry zone. This may presumably be due to the higher concentration of intermediate host, snail in the dry zone compared to the other two zones. Further, the animals in the dry zone are managed extensively in communal grazing areas such as scrub land around village reservoirs during dry season, grasslands around stagnant water pools, paddy fields after the harvest and also in fallow paddy lands. These grazing areas provide ideal environmental conditions for propagation of the parasite and snails. In intermediate and wet zones cattle and buffaloes are managed more intensively and the chances of grazing on snail and miracidia infested pasture is less than that in the dry zone.

The pathological effects of *G. explanatum* in particular, in buffaloes has not been fully reported by previous investigators. The characteristic macroscopic lesions were the dilated bile ducts which were full of mature parasites and haemorrhagic protuberances to which the adult parasites were anchored. Microscopic examination of affected tissues showed marked hyperplasia of the epithelium with focal areas of mucoid metaplasia. This appears to be due to the tissue response to minimise the effect of irritation caused by parasites adhere to the mucosal surface. Despite the presence of a large number of parasites in the bile duct there appeared to be no obstruction to the flow of bile, presumably because of the dilatation of the bile duct. In contrast to fascioliasis, there appears to be very little damage to the hepatic parenchyma as revealed by both the macroscopic and microscopic appearance in the tissues although the parasite enters the liver through the peritoneum.

In the present study, parasites were located only in the bile ducts while Kulasiri and Seneviratne (1956) reported the presence of parasites in the liver substance and around veins in a few instances, in addition to that in bile ducts. These authors correctly pointed out that the presence of the parasite within the liver parenchyma reflected an incidental finding as evident by the lack of a tissue response and attributed it to a post mortem migration. Conversely, the presence of dead parasite amidst a round cell infiltration may not necessarily represent *G. explantum* as similar lesions have been encountered in abattoir studies of cattle liver disease (Wettimuny and Athureliya, 1976) and may very well represent aberrant migration of parasites such as oesophagostomum species that have been arrested within the liver parenchyma.

The macroscopic and microscopic findings indicate that the most remarkable lesions were apparent obstruction of bile ducts by the mature parasites, dilatation of the ducts and necrosis of the bile duct epithelium, thickening of the epithelium of the bile duct because of connective tissue proliferation and inflammatory cell infiltration. Liver parenchyma appears to be unaffected. The obstruction to bile flow may be compensated by compensatory dilatation of the duct system. The pathological changes make the affected liver unsuitable for consumption which cause a direct economic loss to the meat industry. The effect of these lesions on the physiological functions such as digestion and hepatic functions need to be investigated. For this type of a study, a test for detection of animals with the parasite is of vital importance.

The results of this study indicate that the prevalence rate is higher in the buffalo population and small percentage of cattle are affected by this parasite. The infestation with the parasite results in the condemnation of livers of buffaloes and cattle at routine meat inspection, which result in a direct loss to meat industry. However, the macroscopic and microscopic findings do not lend much support to answer the question of whether the infestation causes any derailment of digestive and hepatic function. Nevertheless, this question needs to be addressed in future studies and the development of a diagnostic test to detect infestation in live animals is a prerequisite for such studies.

## Acknowledgments

The authors thank Dr. I.V.P. Dharmawardena, Colombo abattoir for cooperation in this study, Mr. M.A. Navaratna, Department of Veterinary Preclinical Studies for his excellent technical assistance in histological sectioning and Dr. H. Abeygunawardena for guidance and critical evaluation of the script.

The study was supported by SAREC/NARESA research grant - BF/80 and partly by the Peradeniya university research grant RG/7/91/Vt.

## References

Creplin, F.C.H. (1847) Besohreibung zweier neuen Amphistomen-Arten aus dem Zebu-Ochsen. Arch. Naturgesch, *Beulin J.* **13,** (Vol.1), 30-35 (W.L. 1782)

Crusz, H. (1952) The Nature, incidence and geographical distribution of Amphistomic intestations in neat cattle, buffaloes and goats in Ceylon. *Ceylon J. Sci.* (B) **25,** 59-73.

Dargie, J.E. (1986). The impact on production and mechanisms of pathogenesis of trematode infections in cattle and sheep: In: Parasitology - quo vadit? *Proceedings of the Sixth International Congress of Parasitology.* M.J. Howell (Editor), Australian Academy of Science, Canberra.

Eduardo, S. L. (1985), The taxonomy of the family paramphistomatidae Fischoeder, 1901, with special reference to the morphology of species occuring in ruminants. IV Revision of the genus *Gigantocotyle* Nasmark, 1937 and elevation of the subgenus *Explanatum* Fukui 1929 to full generic status. *Syst. Parasite,* **6,** 3-26.

Kelly, W.R. (1993) The liver and biliary system. In: *Pathology of Domestic Animals.* 4th Edition. (Eds) Jubb, K.V.F., Kennedy, P.C. and Palmer, N. Academic Press, London. pp 319-409.

Khan, A. and Anjum, A.D. (1994) Liver paramphistomiasis in a buffalo. *Buffalo J.* **2,** 185-188.

Kulasiri, C. and Seneviratne, R.D. (1956) *Gigantocotyle explanatum Creplin* (Trematoda: paramphistomidae) infection of the liver of the buffalo in Ceylon. *J. comp. Path.* **66,** 83-87.

Kunwar, S.S. (1958). A Redescription and life-history of *Gigantocotyle explanatum* (Creplin,1847) Nasmark, 1937 (Trematoda: Paramphistomidae). *Indian J. Parasitol.* **44,** 210-224.

Nasmark, K.E. (1937) "A revision of the trematode family Paramphistomidae", *Zool. Bidrag.* Uppsala, **16,** 301-566. (W.L. 23836).

Soulsby, E.J.L. (1962). *Helminths, Arthropods and Protozoa of Domestic Animals.* 9 Ed.

Stoimenov, K. Graev, A. and Simov, A. (1976) Anthelmintic treatment of Paramphispmatid infection. *Veterinarna Sbirka.* **74,** 41-43.

Varma, A.K. (1957) On a collection of Paramphistomes from domesticated animals in Bihar. *Indian J. vet. Sci.* **27,** part 1.

Wettimuny, S. G.de S. and Athureliya, D.S. (1975) An abattoir study of liver diseases of cattle. *Ceylon vet. J.* **23,** 53-56.

Zamri-saad, M., Saharee, A.A. and Abdullah, M.N. (1988) Disease conditions in farm and smallholder buffaloes of Serdang, Malaysia. *Pertanika* **11,** 51-55.

NA - 143 - 487

## SESSION V - Health and Diseases

### Panel Discussion

**Q:** Abdul Rahaman - What is the incidence of schistomiasis among buffaloes and cattle in Sri Lanka? The reason for asking this question is because the country is so green and lush. I am sure there is lot of water available for snails to thrive.

**A:** D.J. Weilgama - In Sri Lanka we do not see very many clinical cases except perhaps in the Kandy area where we have had some cases in cattle as well as in buffaloes. In the dry zone because of seasonality in the rainfall, there are certain periods when live snails are not seen at all. They are all dead. Then they come up with the rains. It may be that the infection picks up at that time but the important thing is that the infection stays on for years and years.

**Q:** D.J. Weilgama - How important is hydatidosis in man in India?

**A:** Abdul Rahaman - In a survey we conducted 10 years ago there was a 17% incidence of hydatidosis in patients admitted to a Bangalore sanatorium. We have a very high incidence of *Echinococcus granulosus* in dogs, compared to all other types of cestodes. Hydatidosis in cattle, buffaloes and sheep is also high. The combination of cysticercosis and hydatidosis are considered as very important zoonotic problems in India.

**Q:** I.D. Silva - What is the impact of Babesiosis on the dairy industry?

**A:** Abdul Rahaman - Babesiosis is not a major problem in India as compared to Theileriasis. At present bovine babesiosis is detected in time and appropriate treatment is instituted. Theileriasis is a major threat to our livestock and the control of the disease is primarily through the vaccination.

**Q:** N. Horadagoda - What is the incidence of *Dictyocaulus viviparous* infection in India?

**A:** Abdul Rahaman - Lung worm infection is confined to the cooler regions of India especially in the northern parts of the country. However, even in these areas it is considered to be less important than gastrointestinal parasitic infections in both cattle and buffaloes.

**Comment:** N.H. Shah - In general, buffaloes are as susceptible to parasitic infections as cattle but we have observed that buffaloes are more susceptible to liver fluke infection than cattle. On the other hand, buffaloes appear to be more resistant to some ectoparasitic infections such as warble fly infestation.

**Q:** N.H. Shah - Have you any experience on the effect of parasitism on reproduction in buffaloes?

**A:** Abdul Rahaman - In our studies, over 80 per cent of animals with parasitic infections have infertility problems. Therefore as a routine we administer anthelmintics together with the specific treatment for infertility. The response to this kind of treatment has been good. Therefore we believe there is a relationship beween infertility and parasitic infestation.

**Comment:** D.K. Singh - We conducted a nationwide survey on parasitism and concluded that the incidence of parasitic infections was directly related to rainfall. For example in Kerala, parasitism was highest a fortnight after the rains while in other parts of the country too similar patterns were observed. We also conducted a trial on a very large farm with 400 to 500 animals to study the effect of parasitism on reproduction. In this experiment one group of animals were wormed while the other remained as controls. When the lactation records of the two groups were compared we noted that in the group that received the worm treatment the milk yield had increased up to 800 ml per day and it continued to remain high over a period of 33 months.

**Comment:** D.K. Singh - Removal of parasitiasis (parasitic infection without overt clinical signs) has several advantages. Firstly, it increases the milk yield up to 800 ml per day. Secondly, there is also an increased weight gain in the animals; weight gain in calves can be as much as 50 g per day. Thirdly, there was a lenghtening of the lactation period by 10 days and further there was a 65 day reduction in the calving to conception period. The aforementioned information has been published in the Buffalo Journal.

**Q:** D.H.A. Subasinghe - What is the importance of coccidiosis in buffalo calves?

**A:** Abdul Rahaman - Coccidiosis as you are aware affects young animals and mortality is high in calves of buffaloes and cattle. Usually the significance of coccidiosis is not fully appreciated as compared to ascariasis, trichostrongylasis and moniasiasis but there are several reports from my country which emphasises the importance of coccidiosis as a serious clinical disease in calves.

**Comment:** D.K. Singh - It would be interesting to know how anaemia proceeds following infection with blood protozoa in cattle and buffaloes. It will have bearing on future studies. That should be the next step that you should follow.

**Q:** P. Amerasinghe - Did you see any parasites in the faeces of these animals?

**A:** N.U. Horadagoda - Yes, some of these animals did have parasitic eggs but they were within limits of what one would find in apparently normal animals.

**Q:** N.U. Horadagoda - Did you have any fatalities when trying to develop a dosage for buffaloes? Secondly, are there any contraindications for the usage of the drug in practice.

**A:** D.D.N. De Silva - Fortunately we did not have to any fatalities as we worked well within the safe range of the drug as described by the manufacturers. However, with the high dosage one animal started regurgitating and there was a possibility for the aspiration of ruminal contents. We managed to avoid this by elevating the head with bails of straw.

The answer to the second question is that the drug induces myometrial contractions in pregnancy hence it is contraindicated in pregnant animals. However, we used this drug on early pregnant animals but did not have any problem, as they went through the complete pregnancy and calved down normally. In this respect the drug behaves similar to Xylazine which can cause abortion.

**Q:** A. Horadagoda - What is the cost of the drug?

**A:** D.D.N. De Silva - The price is comparable to Xylazine. The price of a 20ml vial is around Rs. 1000.00( ~20 USD).

**Comment:** T.G. Wijewardana - I used the dosage recommended by you to collect tonsillar washings but the animals continued to struggle.

**A:** D.D.N. De Silva - Was the animal excited before the injection was given? If that was the case then the drug will not work because either the animal was already in an excited state or the validity period of the drug may have expired.

**A:** T.G. Wijewardana - Not to my knowledge.

**Q:** M.C.L. De Alwis - Would you recommend a larger dosage?

**A:** D.D.N. De Silva - I would not recommend a dosage in excess of 0.4 to 0.6 ml/100kg.

**Q:** M.C.L. De Alwis - I presume this is the first time that this drug has been used on buffaloes. What were the other comparable drugs that were available up to this time for this kind of purpose?

**A:** D.D.N. De Silva - Xylazine is a useful alternative but it is not available in the market. Even if it is available with agents, they will only entertain large orders.

**Q:** M.C.L. De Alwis - Apart from the free availability are there any other advantages of this product over other comparable preparations?

**A:** D.D.N. De Silva - I am not able to answer that question because there have been unpredictable results with Xylazine as mentioned by Dr. T.G. Wijewardena.

**Q:** D.K. Singh - What was the unpredictable result with Xylazine?

**A:** D.D.N. De Silva - Xylazine does not appear to sedate aggressive buffaloes even after repeated administrations.

**Q:** D.K. Singh - What is the safety margin of Xylazine?

**A:** D.D.N. De Silva - For mild sedation we use 0.25ml/50kg, for moderate sedation 0.5ml/50kg, 1.0ml/50kg for deep sedation and 2.0ml/50kg to induce anaesthesia. It is dose dependent.

**Q:** D.K. Singh - So you feel Xylazine is not safe.

**A:** D.D.N.De Silva - I did not say that. You have to be cautious. You have to have resuscitative measures at hand.

**Comment:** D.K. Singh - As regards to your problem of non-availability of Xylazine I can help because Xylazine is one of our products and I can send you a few free samples.

**A:** D.D.N. de Silva - Thank you.

**Q:** D.K. Singh - Dr. I.D. Silva, what is the post phagocytic time that you are talking of?

**A:** I.D. Silva - We checked it for 15, 30 and 45 minutes. It was the same although the percentage increased, the trend was the same.

**Q:** D.K. Singh - Not mere killing but internalization you said there was a difference in the three types. *Staphylococcus aureus* is internalized but not killed as fast as *E. coli* . Is that right?

**A:** I.D. Silva - Yes.

**Comment:** D.K. Singh - I think you are unfair by yourself when you say the work was academic. Your work is exciting and very nicely presented.

**A:** I.D. Silva - Thank you.

**Q:** D.K. Singh - I would like to know firstly, whether you could increase the rate of phagocytosis if you add opsonin and secondly, if you immunize an animal intramuscularly by the agents that are involved in mastitis, most common ones, whether you will increase the rate of phagocytosis in mastitis and enhance early recovery.

**A:** I.D. Silva - Yes, because I opsonised the bacteria using the serum of the same animal, while of course it was a clinically healthy and apparently normal animal. So if you did use serum from an immunised animal it would have more opsonin.

**Q:** D.K. Singh - What I am trying to find is whether there is a potential of immunizing if not fully at least partially against the most common organisms causing mastitis?

**A:** I.D. Silva - Yes there is.

**Comment:** M.C.L. De Alwis - Yes, that is a very interesting observation of Dr. Singh. A practical approach to the problem.

NA - 143 - 493

# SESSION VI - CONCLUSIONS AND RECOMMENDATIONS

The final (fifth) day of the Symposium was devoted to a series of objective-oriented large-group and small-group discussions. Initially, the discussions were directed, based on the knowledge gained so far under the SAREC/NARESA Buffalo Research Programme in Sri Lanka and experiences in other Asian countries, towards determining:

(a) which technologies can be applied at the small-farm level,
(b) how these can be built into practical packages, and
(c) the mechanisms for their transfer to farmers.

Subsequently, the participants assembled in four separate discussion groups: production systems; nutrition and feeding; breeding and reproduction; and health and diseases. Each group was requested to identify further constraints to improving specific small-farm systems, and to recommend areas requiring further research leading to technologies which can be transferred to farmers.

Finally, the conclusions and recommendations from each group were presented to all participants, discussed and modified as required to obtain consensus. These are presented below.

## 1. Production Systems and Uses

### *1.1. Conclusions*

1.1.1 Heat and humidity, and to a lesser extent work load, were identified as the main stress factors in working buffaloes. The temperature/humidity index was an important criterion in assessing climatic stress. The latter can be ameliorated by intermittent sprinkling of water.

1.1.2. Warm and dry conditions were favourable for the production of Ruhunu curd. However, there is a need to improve the hygienic conditions during processing.

1.1.3. The success of on-farm trials, where farmer participation was obtained for adaptive trials and generation of appropriate technology packages, have been demonstrated. The farming systems approach to the transfer of such technologies proved superior to the traditional approach.

### 1.2. *Recommendations*

1.2.1. There is a need to focus on the constraints and limitations in introducing technologies at the small-farm level, to identify appropriate methods and to understand the reasons why farmers hesitate to adopt certain technologies. Therefore research approaches should be re-oriented so as to bring about active farmer participation in development of appropriate technologies.

1.2.2. Socio-economic evaluation of production systems should be undertaken, including consumer preference, markets and alternative production systems.

1.2.3. The possibilities for integration of livestock and forestry should receive attention.

1.2.4. In the light of structural changes and adjustments occurring in the socio-economic milieu, there is a need to look at the service delivery systems in the livestock sub-sector.

1.2.5. The impact of macro issues like world trends, pricing structure and policies at international and national level, taxation and duty levies on the livestock industry need to be emphasized.

## 2. Nutrition and Feeding

### 2.1. *Conclusions*

2.1.1. The availability of a large volume of biomass in the ecosystem in Sri Lanka and the possibilities of utilizing this biomass in ruminant production systems was recognized. However, adequate information on the feed resource bases in the different farming systems in Sri Lanka is a prerequisite for increasing the utilization of the available biomass.

2.1.2. A considerable amount of research information is now available on procedures to utilize cereal straw as a ruminant feed for productive purposes. However, much of this is based on-station studies. The failure by scientists to develop appropriate technologies through conduct of on-farm adaptive research with active farmer participation was identified as the reason for apathy of farmers in adopting recommended procedures.

2.1.3. The possibilities that exist to increase the dry matter intake, digestibility, nutrient utilization and performance of animals fed rice straw and poor quality roughage supplemented with urea, leguminous fodder and non-conventional energy sources has been clearly demonstrated.

2.1.4. There is inadequate information on the existing feeding practices for year-round feeding in the different farming systems, and on the ability of these feeding systems to meet the nutrient requirements to sustain as well as increase production.

2.1.5. Information has been generated on procedures for utilization of locally available non-conventional feed resources.

### *2.2. Recommendations*

2.2.1. Methodologies need to be developed to assess the feed resources bases in the different farming systems in Sri Lanka.

2.2.2. Research on optimum utilization of potential feed resources should be continued, taking into consideration the limitations of utilization.

2.2.3. Studies on the existing feeding practices in the different farming systems and their adequacy to meet the nutrient requirements for production should be initiated.

2.2.4. Appropriate nutritional packages should be developed for different production objectives (milk, meat, draught) and physiological states (growing, pregnant and lactating animals) under intensive and semi-intensive systems for year-round feeding.

2.2.5. There is a need to focus attention on the development of food and feeding systems and on the economics of feeding systems.

## 3. Breeding and Reproduction

### *3.1. Conclusions*

3.1.1. The data presented provided several approaches that can be applied in herd health and fertility improvement programmes to maintain reproductive performance and to implement preventive measures.

3.1.2. Indigenous buffaloes of Sri Lanka, when compared with exotic breeds, have lower birth weights and growth rates, but have their first calf at an earlier age. Among the three exotic breeds studied (Surti, Murrah and Nili-Ravi), the Nili Ravi had the highest growth rate, shortest calving to first service interval and highest milk yield. There were no significant differences between the other two breeds.

3.1.3. Different suckling and weaning regimes had marked influences on the fertility of indigenous buffaloes and survival of their calves. Weaning at day 45 improved fertility significantly but also resulted in high calf mortality. Weaning at 60 or 90-120 days did not improve fertility. Restricted suckling once or twice a day improved fertility significantly and did not increase calf mortality, although initial growth rate of calves was lower with once a day suckling. Restricted suckling is being practiced by farmers in certain regions of Sri Lanka and can be easily incorporated into existing farming systems in other areas.

3.1.4. Treatment of buffalo cows exhibiting prolonged post-partum anoestrus with Gn-RH gave poor results, while treatment with progesterone-releasing implants followed by PMSG gave satisfactory fertility; the latter can be cost-effective in dairy buffaloes. Animals to be used for such treatments must be chosen with care and, considering the spread of ovulation observed, natural mating is preferable to fixed-time artificial insemination.

3.1.5. In Pakistan, there is a demand for herd fertility improvement programmes from the larger commercial farmers. Low reproductive efficiency such as an increase in calving interval from 365 days to 635 days decreased the annual milk yield per animal by 2 kg per day.

### *3.2. Recommendations*

3.2.1. Since buffalo farmers will need to adopt more intensive management systems in the future, for which dual-purpose (milk and meat) animals will be required, a vigorous breed improvement programme should be undertaken in the villages.

3.2.2. There should be an active process of selection of bulls for breeding purposes. The two parameters which should be used in selection are milk production and reproductive performance. For the latter, the calving interval should receive priority, as this will be a determinant of lifetime productivity.

3.2.3. In the promotion of artificial insemination (AI), the initial focus should be with herds which are currently producing milk under intensive or semi-intensive conditions, such as in the peri-urban areas. In the extensively managed herds in rural areas, alternative methods of breed improvement based on natural service should initially be continued until management conditions become favourable for a transition to breeding by AI.

3.2.4. As the breeding programme progresses, studies should be undertaken on the comparative milk yield, draught performance and reproductive efficiency of female buffaloes of different genotypes, including pure breeds and crosses.

3.2.5. Based on the studies reported here, the technologies which can be used under village small-holder farming conditions in Sri Lanka to improve the reproductive performance of buffaloes include: (a) improved nutrition during the pre-pubertal period in heifers and the pre- and post-partum period in cows, (b) adoption of restricted suckling, (c) improvement of heat detection and timing of natural service or AI, and (d) the judicious use of hormones in selected cases.

## 4. Health and Diseases

### *4.1. Conclusions*

4.1.1. Most diseases in cattle also occur in buffaloes but there are differences in the severity and manifestation as illustrated by the data presented. Attempts have been made to explain these differences, to understand the disease process and to develop techniques for their early detection in buffaloes.

4.1.2. Detomidine HCl (Domosedan) was found to be an effective sedative/analgesic drug that can be used safely in buffaloes in preoperative medication, minor surgical procedures, transportation and in restraint of intractable animals for clinical examination.

4.1.3. Studies on the epidemiology of haemorrahagic septicaemia (HS), indicated that *Pasteurella multocida* in latent carriers were located exclusively in the tonsillar crypts and that steroid-induced immunosuppression was not successful in converting latent carriers to active carriers. Vaccination with oil adjuvant vaccine protected the animals against HS up to 12 months, but there was no correlation between the response to direct challenge and the antibody titres measured by the indirect haemagglutination test, the passive mouse protection test or the ELISA.

4.1.4. The haematological parameters in the Lanka Buffalo were comparable to that of river type buffaloes, and were characterized by a low icterus index, an increased erythrocyte sedimentation rate and a predominance of lymphocytes over neutrophils. The values of several serum biochemical constituents were similar to that of cattle, except for AST, ALP and ALT which were higher in the buffalo. In contrast to other domesticated ruminants, the buffalo has a high serum ALT concentration.

4.1.5. The neutrophils of the buffalo which appear in milk during the early stages of acute mastitis were as efficient as blood neutrophils in phagocytosing bacteria, with the highest percent phagocytosis for *Staphylococcus aureus* and similar percentages for *Streptococcus agalactiae* and *E. coli*. Experimentally induced acute, subclinical mastitis in the buffalo caused an elevation of the somatic cell count, electrical conductivity, chloride ion percentage and adenosine 5'-triphosphate of milk while there was no significant alteration in the acidity percentage and pH.

4.1.6. Rotavirus infection is recognized as an important cause of buffalo calf diarrhoea and studies reported herein indicated that polyacrylamide gel electrophoresis (PAGE) is a useful technique to distinguish isolates and outbreaks of the disease and that it is also an alternative technique to detect atypical rotaviruses.

4.1.7. An abattoir survey revealed that approximately one third of buffalo livers were infected with the trematode parasite, *Explanatum explanatum*. Animals from all agro-ecological zones were infected and the incidence was low in animals below two years. Another study which examined the incidence of *Schistosoma nasale* noted a higher infection rate in buffaloes when compared to cattle; a circumoval precipitin test (COPT) was developed to detect *S. nasale* infections in both cattle and buffaloes.

4.1.8. Studies on the seroprevalence of leptospirosis in 12 districts of Sri Lanka indicated an overall incidence of 42 %. The most common serovars of *Leptospira* encountered were *weerasingha*, *pomona* and *hardjo*.

### *4.2. Recommendations*

4.2.1. Current knowledge on disease prevention and control should be incorporated into cost-effective health care packages that are applicable to different age groups of animals (calves, heifers and adults).

4.2.2. The effect of gastrointestinal parasites (other than *T. vitulorum*) on the productivity of adult buffaloes should be determined and the impact of external parasites investigated.

4.2.3. A serum bank should be established with a view to sero-surveillance of important diseases caused by bacteria (Brucellosis, Leptospirosis) and viruses (Foot-and-Mouth Disease and Rinderpest).

4.2.4. Surveys should be conducted to determine the incidence of subclinical mastitis in buffaloes and to assess its impact on milk production. A cow-side test that could be used by farmers to detect subclinical mastitis needs to be developed.

4.2.5. A multidisciplinary approach should be adopted to investigate the causes of high mortality in buffalo calves.

## 5. General Recommendations

5.1. The active participation of private sector enterprises and organizations should be obtained in research and development activities relating to the buffalo.

5.2. Linkages between farmers, extension workers and researchers should be strengthened and a continuous dialogue between these groups should be promoted in the generation, testing and transfer of technologies.

5.3. Opportunities for exchange and dissemination of information should be promoted through linkages between institutions and libraries of the South and South East Asian countries.

NA - 143 - 501

# IMMUNOLOGICAL RESPONSE OF BUFFALO COWS TO *TOXOCARA VITULORUM* - ANTIGENIC ANALYSIS*

P.H. Amarasinghe

*Department of Zoology, Faculty of Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

Parasitological, haematological and immunological responses of pregnant and non-pregnant indigenous buffaloes (N = 12) to natural and experimental infections with *Toxocara vitulorum* were studied. Antigens responsible for eliciting an antibody response during natural infections were investigated. Immunogenicity of these antigens was studied in a mouse model.

A strong gel diffusion precipitin response (GPR) to infective egg extract was seen in the naturally infected buffalo cows. In these animals the GPR and Enzyme-linked Immunosorbent Assay titres rose prior to parturition and fell at the time of parturition. Also, the nature (intensity and number of bands) of the reactions in individual animals showed a direct relationship to the GPR titres. In contrast, the precipitin reactions of the Murrah buffaloes were faint while the neat cattle were negative. These anti-*T. vitulorum* antibodies were predominantly $IgG_1$ class of immunoglobulins.

A few eggs were seen in the faeces of three parturient animals. Haematological values showed a wide range among buffaloes, and there were no significant differences in the haematological values between the naturally and the experimentally infected buffaloes.

Antigens from excretory-secretory products of infective larvae, infective egg and adult worm extracts and perienteric fluid of adult *T. vitulorum* were analysed. Oral-secretory products were highly antigenic

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy

as seen in the circum-oral precipitin reactions. Of the extracts prepared, infective egg extracts gave the strongest precipitin reactions indicating a recurrent exposure to this antigen in nature. In comparison the reactions with adult antigen extracts were weak. Different antigen preparations shared antigenic components, but at least two stage specific components were present in the infective egg extracts. The antigenic sites in the adult parasite were located in the cuticle, sensory region of the muscle layer and ovary, as revealed by immunofluorescence studies.

Although individual levels of protection varied between antigens, parenteral immunization with adult antigens and oral immunization with infective eggs were able to confer a significant ($p = < 0{\cdot}001$) degree of immunity in the mouse model. Perienteric fluid conferred 100% protection to a challenge dose of infective larvae. The protective nature of the perienteric fluid of the adult parasite needs further investigation as it could prove to be an important antigen in the immunization of the natural host.

# PRODUCTION SYSTEMS AND REPRODUCTIVE PERFORMANCE OF INDIGENOUS BUFFALOES IN SRI LANKA*

L.N.A. de Silva

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

A field survey was conducted on the indigenous buffalo of Sri Lanka, to determine the systems of their management, patterns of utilisation and reproductive performance and the interrelationship among these factors. The sample comprised 11,863 buffaloes (little more than 1% of the national population) kept on 528 holdings distributed in 16 of the 24 districts of the country. Buffalo farmers were visited, a questionnaire administrated and stock numbers were physically verified. Rectal examination was done on 1,300 breedable females in the sample in order to determine their reproductive status.

Almost all of the buffalo farmers were primarily engaged in paddy cultivation in small holdings, and supporting an average of 7.5 people. Their resources in terms of land, capital and stock were limited. Buffaloes were managed extensively (free grazing only) or semi extensively (tethered with or without free grazing). The main source of feed consisted of low quality herbage obtained from grazing; no supplementary concentrate feed was provided. The overall average herd size was 22·5 (range 5·3 - 53·6). On average, a herd was composed of 17·1% of 0 - 1 year olds, 13·0% of 1 - 2 year old, 9·1% of adult heifers, 32·9% of cows, 14·9% of castrated males and 13·1% of entire males.

The main use of buffaloes was in tillage. On average, 92% of the farmers used their animals in land preparation (ploughing/puddling/levelling) in paddy cultivation and 82% used them in threshing the harvest. The mean age at initial use was 3·1 years and 82·9% farmers used both males and females. Number of buffalo days

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Master of Philosophy.

required to plough one acre of land varied from 4·0 to 7·8 days. Puddling generally required twice as many buffalo days as ploughing. The mean duration of work was 52·3 days/year. Milking of buffaloes was restricted to some districts. The overall mean lactation yield was 1·5 litres/day and lactation length varied from 5 to 6 months.The overall means for reproductive performance were: age at first calving 45·7 months, annual calving rate 57·8% and calving interval 18·9 months. Fertility was found to be comparatively higher in groups of buffaloes subjected to milking and limited suckling (calf separated during part of the day), and in those not used for work.

Haemorrhagic septicaemia and gastro-intestinal parasitism were reported to be the major disease problems. Proportion of farmers using vaccination to control infectious disease was 64·9%. Annual mortality rates among age groups 0 - 1 year, 1 - 2 years and adults were 25·4, 21·5 and 8·0% respectively.

# STUDIES ON THE COMPOSITION OF INDIGENOUS BUFFALO MILK IN SRI LANKA*

A. Horadagoda

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

This study was undertaken to establish norms for chemical characteristics and physical properties of indigenous (Lanka) buffalo milk. A total of 566 samples of milk collected from buffaloes managed under both field conditions and at the university experimental farm were analysed using standard techniques in dairy chemistry.

The overall values (mean ± S.E.M., g/l) for major constituents of indigenous buffalo milk, irrespective of the stage of lactation, the lactation number and age were: fat 74·5 ± 0·62; total proteins 51·6 ± 0·15; casein 44·0 ± 0·19; lactose 43·0 ± 0·02; ash 7·2 ± 0·02; solids-non-fat (SNF) 96·2 ± 0·46 and total solids 174·0 ± 0·57. The average values (mean ± S.E.M., mg/l) of the minor constituents studied under the same conditions were: sodium 456 ± 99·6; potassium 1213 ± 356; total phosphorus 1500 ± 400; inorganic phosphate 1000 ± 200; calcium 1555 ± 350 and magnesium 325 ± 96. The mean vitamin A content was 1·20 ± 0·1 IU/ml. The results of the physical properties revealed that the specific gravity of indigenous buffalo milk was 1·033 ± 0·003 while the surface tension, electrical conductivity and viscosity were 46·52 ± 3·4 dynes/cm, 37·05 x $10^{-4}$ ± 5·00 mhos and 1·9633 ± 0·243 centipoises, respectively at 27°C. The average pH of milk at 27°C was 6·42 while the fat globular size varied between 5 and 10 μm at this temperature. The titrable acidity and ethanol stability were 0·202 and 50 per cent, respectively. All constituents of milk except for lactose and ash varied with the stage of lactation, lactation number and age. The average milk yield of indigenous buffalo cows was 3·58 litres per day. when estimated by measuring water turn-over in their calves.

---

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Master of Philosophy.

The composition of colostrum was markedly different from that of milk. The average values (g/l) for parameters examined were: fat 63; total proteins 150; casein 80; lactose 30; ash 11·0; SNF 200; total solids 260 and vitamin A 3·30 IU/ml. At 27°C, the viscosity and surface tension of colostrum was 4·067 ± 0·461 centipoises and 29·35 ± 1·92 dynes/cm, respectively.

The result revealed that the fat percentage of the Lanka buffalo milk was similar to that of the Murrah buffalo while the protein content was high, and comparable to swamp buffaloes of the Philippines and China. The elevated protein concentration in the Lanka buffalo indicate that the alcohol test is not a suitable platform test for assessing the quality of milk from this breed.

# IMMUNOPATHOLOGICAL STUDIES OF *TOXOCARA VITULORUM* IN BUFFALO CALVES AND RODENTS*

M.A. Masoodi

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

Studies on the migratory behaviour of larvae of *Toxocara vitulorum* in mice demonstrated extensive somatic migration into the liver, lungs, kidney, brain and other visceral organs despite restrictions of larval development at the second (infective) stage.

Immunization of mice with two small doses of *Toxocara vitulorum* or *Toxocara canis* induced a strong resistance to a challenge with a large infection of *Toxocara vitulorum*. The manifestation of immunity in mice differed with the type of immunization. Mice with homologous immunization were able to inhibit the establishment of the larvae in the liver while mice with the heterologous immunization did not have a significant affect on the establishment of the larvae in the liver.

Monitoring of *Toxocara vitulorum* eggs in the faeces of buffalo calves from birth revealed that eggs of the parasites appeared around 19 - 21 days and that it was preceded by eggs of Strongyloides species which appeared in the faeces between day 9 and 16. The patent period *of Toxocara vitulorum* infection lasted up to 2·5 - 3 months with marked variation in the peak egg count. Colostral antibodies passively transmitted to calves from the dam do not appear to have any effect in protecting infections acquired by calves either pre-, peri- or postnatally. Reinfection of buffalo calves with large doses of infective eggs of *Toxocara vitulorum* after they had cleansed themselves of a natural infection acquired from birth, did not result in a patent infection. Instead, it induced strong precipitin reaction in the calves.

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.

Buffalo calf serum collected at different stages of natural and experimental infections when fractionated by gel filtration yielded three main peaks. The first peak consisted largely of IgM and $\alpha 2$-macroglobulin while the second peak contained IgG's. The third peak consisted mostly of non-antibody proteins. Further fractionation of the second gel filtration peak (IgG's) by ion exchange chromatography produced two peaks representing the two subclasses, $IgG_1$ and $IgG_2$. Enzyme Linked Immunosorbent Assay ( ELISA) demonstrated antibodies both in the IgM and IgG fractions with a higher $IgG_1$ activity as opposed to $IgG_2$. Gel diffusion and *in vitro* larval preacceptance were also confined to the $IgG_1$ and not to the $IgG_2$.

# CLINICAL AND ENDOCRINOLOGICAL STUDIES ON POSTPARTUM OVARIAN ACTIVITY IN LANKA BUFFALOES (*BUBALUS BUBALIS*)

V. Mohan

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
SRI LANKA

Although Lanka buffaloes raised under village conditions in Sri Lanka generally have poor fertility, short calving intervals and high fertility have been reported in certain locations. Four experiments were conducted to determine reasons for the differences in fertility in different locations; endocrinological changes during the postpartum (PP) period; and effects of suckling on resumption of ovarian activity and other fertility indices.

Sixteen buffalo cows from a "low fertility" area and a "high fertility" area (n=8 each) were brought to the SAREC/NARESA farm and maintained over a period of 3 years under uniform management. Ovarian activity was monitored by oestrus observation, rectal palpation and measurement of plasma progesterone. Calvings were distributed throughout the year and there were no differences between the groups ($p>0{\cdot}05$) in calving interval, time taken for uterine involution, duration of postpartum anoestrus, or the number of services required for conception. In 14 buffalo cows sequential blood samples were collected on days 7, 14, 21 and 28 PP at 15 min intervals for 8 hours, two injections of 12·5 µg GnRH given i/v two hours apart, and sampling continued for a further 4 hours. Plasma LH was measured by a heterologous (bovine) RIA and assay validity was checked with a specific bioimmunoassay (*in vitro* secretion of testosterone by mouse Leydig cells). LH remained below the detection limit of the assay with no pulses up to day 28 PP, and no LH response was detectable following the low doses of GnRH.

---

*** This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.**

Eighteen pluriparous buffalo cows were allotted to three suckling regimes: AS - continuous (*ad libitum*); RS - restricted (two periods of 20 minutes per day); and ZS - zero ( calves weaned within 15 days). Blood was collected on days 30, 45 and 90 PP at 20 min intervals for 8 hours, followed by two doses of GnRH and sampling as previously. Rectal examination and measurement of plasma progesterone were done weekly. Mean LH before GnRH treatment was below the detection limit on days 15 and 30 PP, around 0·5 ng/ml on day 45 and around 0·6 ng/ml on day 60 PP. There was no difference ($p>0·05$) in the mean LH values between the groups, but the response to GnRH was lower ($p<0·05$) in AS compared with that of RS and ZS on days 45 and 60. A further 18 pluriparous buffaloes were assigned to one of three treatment groups: AS (*ad libitum*); RS (restricted); and AS/S (AS with supplementary feed). The pre GnRH mean LH increased in all three groups with the days PP. Mean LH for RS was higher ($p<0·05$) than that for other two groups. Resumption of pulsatile LH release was followed by ovarian activity. The pituitary response to exogenous GnRH was greater in RS.

These results confirm that long calving intervals in Lanka buffaloes are mainly due to long periods of postpartum anoestrus, which could be due to delayed reappearance of pulsatile LH secretion caused by managemental factors such as suckling. Restricted calf suckling could be a practical and effective method for improving the efficiency of reproduction in Lanka buffaloes.

# IMMUNOLOGICAL RESPONSE OF BUFFALO COWS AND CALVES TO *TOXOCARA VITULORUM**

R.P.V.J. Rajapakse

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
SRI LANKA

Maternal transfer of *Toxocara vitulorum* antibodies from naturally infected buffalo cows to their calves was studied in 12 buffalo cow-calf pairs. The antibody response to *T. vitulorum* was determined by means of the enzyme linked immunosorbent assay (ELISA) and gel precipitin test (GPT). Excretory and secretory antigen from infective larvae (TVL2ES) was used as antigens. Specificity of antigen was evaluated by lymphocyte transformation test as well as immunofluorescent test.

The effect of maternal antibodies on *T. vitulorum* infection in the calves was elucidated. Infection was monitored by McMaster technique. The calves which suckled colostrum had high ELISA titres and showed low eggs per gram (epg) in their faeces at and during the patency compared with calves which suckled low titred colostrum. Thus a highly significant inverse correlation between colostral and calf serum antibody titres and peak epg of the calves was evident ($p<0{\cdot}01$).

*In vitro* and *in vivo* action of colostrum immunoglobulins and their subclasses were determined in a mouse model. Whole colostrum antibody showed a highly protective activity against oral challenge infection. The $IgG_1$, $IgG_2$ and IgM types showed a significant protective activity against an oral challenge ($P<0{\cdot}01$, $<0{\cdot}01$, $<0{\cdot}001$) but $IgG_1$ was relatively more protective than IgM and $IgG_2$.

Further, immunogenicity of somatic antigen preparations from infective larvae (TVL2) and TVL2ES were determined in the mouse model. Mice were immunised with the TVL2ES and TVL2 antigens

---

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.

using four weekly doses (0·36 mg/dose). Immunisation by the oral route with infective eggs using four doses of five hundred eggs administered weekly conferred a significant degree of immunity to an oral challenge with *T. vitulorum* infective eggs. However, the level of protection conferred by these antigens varied. In particular TVL2ES conferred almost 100% immunity against a challenge infection on day five.

Finally, using *in vitro* circum-larval precipitin tests it was shown that only infective larvae of *T. vitulorum* and *T. vitulorum* larvae isolated from milk showed oral and body precipitates. It was further confirmed by immunofluorescent staining technique that both infective larvae and milk larvae *of T. vitulorum* showed fluorescence in their orifices as well as cuticle but not *T. canis* or *Strongyloides* larvae. Therefore it is suggested that *in vitro* circum-larval precipitin test is a good technique for the diagnosis of *T. vitulorum*.

# IMMUNOLOGICAL RESPONSES OF MICE, RABBITS AND BUFFALO CALVES TO *TOXOCARA (NEOASCARIS) VITULORUM* INFECTION*

B.T. Samarasinghe

*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

Immunological response of mice, rabbits and buffalo calves to *Toxocara vitulorum* infection was studied by enzyme-linked immunosorbent assay (ELISA), gel-diffusion precipitins (GPT), immunoelectrophoresis and *in vitro* larval precipitins (IVP) tests. Several antigens of *T. vitulorum* including embryonated egg antigen, whole worm antigen, and perienteric fluid (PF) from the adult *T. vitulorum* were used. In a few instances, embryonated egg antigen of *T. canis* (TCE) was used to study any cross-reactivity. The immunoglobulin classes in the sera of buffalo calves at different stages of natural and experimental infections were also characterized by gel filtration and ion exchange chromatography followed by immunoelectrophoresis. In rabbits, attempts were made to study the serum immunoglobulin classes by ELISA using commercial horse-radish peroxidase (HRP) conjugated IgG F(ab') 2 (H and L) and IgM (chain specific).

The *in vitro* action of sera from rabbits and buffalo calves infected with *T. vitulorum* on the infective-stages of *T. vitulorum* larvae was examined to ascertain the specificity of the reaction. These studies were further supported by immunofluorescent tests. The trend in the antibody response of buffalo calves measured by ELISA was correlated with that of faecal *T. vitulorum* egg counts.

Serum antibodies to *T. vitulorum* were detected in buffalo calves by ELISA from about 24 hours after birth. The antibody titres increased with the rise in egg counts but continued to increase even after egg counts had fallen to zero levels. On reinfection, the ELISA response remained

* This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.

unchanged. Experimental infection of rabbits with *T. vitulorum* yielded an initial IgM type response which was short lived followed by a persistent IgG type response. The GPT and IVP reactions were used to ascertain the trend and nature of the antibody response in rabbits and buffalo calves. The antibodies to *T. vitulorum* infection in rabbits reacted with TVE and TCE in GPT test in the presence of 0.85 percent NaCl in the gel, whereas the buffalo antibodies to *T. vitulorum* precipitated well with the same antigens in the presence of 8 percent NaCl.

The *in vitro* precipitin test using sera from *T. vitulorum* infected rabbits and buffaloes revealed the presence of precipitates at the natural orifices and body of infective *T. vitulorum* larvae but not in *T. canis* larvae. This indicates the species specificity of the test. The indirect immunofluorescent studies on the larvae suggests that circumlarval precipitates are formed by a reaction of serum antibodies presumably with the excretions and secretions from the larvae. The fluorescent reaction was very marked around the oesophagus and the lips, but the cuticle showed comparatively less fluorescence.

Colostrum from buffalo cows reared under free grazing conditions throughout their gestation period revealed precipitins to TVE antigens, which was identical with that elicited in buffalo calves by an experimental infection. These buffalo calves which were naturally infected with *T. vitulorum* at birth had cleansed themselves of the patent infection at the time of experimental infection. *In vitro* precipitins however, were not observed in the sera of the calves before the experimental infection.

NA-143-515

# STUDIES ON ROTAVIRUS INFECTION OF BUFFALO CALVES IN SRI LANKA*

N.P. Sunil Chandra

*Department of Veterinary Paraclinical Studies,*
*Faculty of Veterinary Medicine and Animal Science,*
*University of Peradeniya, Peradeniya*
*SRI LANKA*

Faecal samples collected from 150 buffalo calves 1 to 150 days old were examined by the Enzyme Linked Immunosorbent Assay (ELISA) test for group A, rotavirus antigen. 27.3 per cent of these calves were having diarrhoea at the time of sampling. The rest were non- diarrhoeic, but incontact with them. Antigen was detected in 36.6 percent of diarrhoeic animals, and in 11.9 percent non-diarrhoeic animals. There was a significant association between the presence of antigen in faeces and diarrhoea in these animals ($P<0.001$). By the screening ELISA test using monoclonal antibodies the strongly positive specimens belonged to subgroup I rotavirus. The weakly positive specimens however, could not be subgrouped. Antigen was detected in diarrhoeic calves in the age group 1-60 days, but none in the 61-150 day group, indicating, the former group to be more susceptible to clinical disease. Apparently healthy calves, too, were infected with buffalo rotaviruses.

This is the first study establishing the presence of rotavirus in buffalo calves in Sri-Lanka, and its association with diarrhoeas in these animals. Three strains of rotavirus were isolated from faecal material collected from five diarrhoeic buffalo calves in MA104 cells. For virus isolation, pretreatment with trypsin, incorporation of trypsin in the maintenance medium, and rolling of the inoculated cell cultures at 37°C was necessary. These strains required adaptation before distinct cytopathic effects were produced. One of the isolates 11C selected for study was also known to be a group A rotavirus by ELISA, with subgroup I specificity.

---

*** This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Master of Philosophy.**

Using post infection sera obtained from mothers, cattle and buffalo, a close antigenic relationship was observed between this isolate and U.K. bovine rotavirus whereas the buffalo and human subgroup II rotavirus strains were distinct. The test used to distinguish these strains was the blocking ELISA test. Antirotaviral antibodies in the sera of buffalo calves rose to very high levels from negative levels on the 1st days of their life, subsequent to colostrum suckling as tested by the blocking ELISA test. These maternally derived antibodies declined to negativity by the 33rd to 56th day. Five buffalo calves which were diarrhoeic and in which antigen was detected, were excreting virus in spite of having circulating antibodies. Virus was not detectable for more than seven days, in a natural rotavirus infection studied. In 63.4 per cent of the diarrhoeic animals rotavirus was not detected, indicating that other enteropathogens too, other than rotavirus, were associated in these diarrhoeas.

## List of Participants

*University of Peradeniya, Sri Lanka*
Abeygunawardena, H.
Ambagala, A.P.N.
Amerasinghe, P.
Bogahawatte, C.
Dangolla, A.
de Silva, D.D.N.
de Silva, L.N.A.
Gunawardena, I.S.
Horadagoda, A.
Horadagoda, N.U.
Ibrahim, M.N.M.
Jayatilake, M.W.A.P.
Kuruwita, V.Y.
Mahalingam, S.
Munasinghe, D.M.S.
Perera, A.N.F.
Perera, B.M.A.O.
Perera, E.R.K.
Premaratne, S.
Rajaguru, A.S.B.
Rajapakse, R.P.V.J.
Rajaratne, A.A.J.
Ranasinghe, J.G.S.
Silva, I.D.
Silva, K.F.S.T.
Sivaram, P.
Sivakanesan, R.
Weilgama, D.J.

*Department of Animal Production and Health, Sri Lanka*
Abeysekara, A.W.A.S.
Bandara, D.H.M.U.S.
Chandrasiri, A.D.N.
de Alwis, M.C.L.
Dharmawardena, J.
Ekanayake, E.M.C.
Jayasinghe, S.R.
Jeyaruban, M.G.

Kendaragama, K.M.T.
Kodituwakku, A.O.
Liyanagamage, A.G.
Perera, A.
Ranawana, S.S.E.
Thalagoda, S.A.
Thilakaratne, G.M.N.
Vipulasiri, A.A.
Wijewantha, P.
Wijewardana, B.D.R.
Wijewardana, T.G.

*SAREC/NARESA Project*
Siriwardene, J.A. de S.
Subasinghe, D.H.A.

*NARESA*
Soysa, P.E.
Karunasinghe, A.W.J.
Premakumara, S.

*SAREC - Sweden*
Duell, R.
Settergren, I.
Sher, Afzal

*Mahaweli Authority of Sri Lanka*
Dayaratne, D.K.
Dhanapala, S.B.

*Agrarian Research and Training Institute, Sri Lanka*
Jayatilake, T.N.

*Coconut Triangle Milk Producers' Union*
Perera, W.

*University of Jayawardenapura, Sri Lanka*
Peiris, H.

*University of Kelaniya, Sri Lanka*
Kodikara, C.P.

*University of Ruhuna, Sri Lanka*
Pathirana, K.K.

*Foreign Participants*
Bunyavejchewin, P. - Thailand
Chantalakhana, C. - Thailand
Devendra, C. - Malaysia
Momongan, V. - The Philippines
Rahaman, Abdul - India
Ranjhan, S.K. - India
Singh, D.K. - India
Shah, Nasir Hussain - Pakistan
Thu, Nguyen Van - Vietnam

*Secretarial Staff*
Alexander, B.
Dayananda, W.K.R.
Herath, J.
Karunaratne, A.M.
Nizreen, R.
Ratnayake, G.
Ruwanpathirana, R.
Siriwardena, D.M.
Weeratunga, J.
Wijetunga, N.

## Authors Index