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SAREC/NARESA Regional Symposium on the Role of the Buffalo in Rural Development in Asia

11-15 December 1995

Plant Genetic Resources Center
Peradeniya, Sri Lanka



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Programme and Abstracts of papers

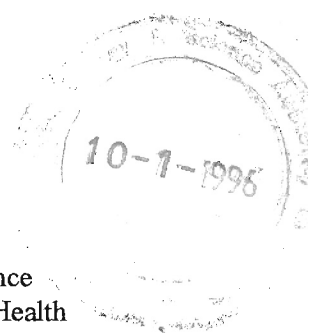


SAREC/NARESA Buffalo Research and Development Programme
Peradeniya, Sri Lanka

Publication No. 2

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Symposium Programme

11 - 15 December 1995
Plant Genetic Resources Centre
Peradeniya, Sri Lanka

11 December 1995 (Monday)

- 08.00 - 09.00** - **Registration**
- 09.00 - 10.05** - **Opening Ceremony**
- 09.00 - 09.10 - Welcome & Traditional lighting of oil lamp
09.10 - 09.20 - Address by Project Representative
09.20 - 09.30 - Address by Director General, NARESA
09.30 - 09.40 - Address by SAREC Representative
09.40 - 10.00 - Chief Guest's Address
10.00 - 10.05 - Vote of Thanks
- 10.05 - 10.30** - **Tea**
- 10.30 - 11.35** - **General Session** *Rapporteur: M.N.M. Ibrahim*
- Chairperson:** Priyani E. Soysa
- 10.30 - 11.15 - **Theme Paper:** A.W. Qureshi (FAO, Rome)
New approaches to research and development for sustainable agriculture and rural development.
- 11.15 - 11.35 - Activities of SAREC/NARESA Project - Phase III: *Dissemination of information on improved buffalo production systems to small-holder farmers.*
- 11.35 - 13.00** - **Session I - Production Systems and Uses** *Rapporteurs: M.N.M. Ibrahim
N.U. Horadagoda*
- Chairperson:** C. Devendra
- 11.35 - 12.20 - **Keynote Lecture 1:** Vicente Momongan (Philippines)
Buffalo production systems in SE Asia and possibilities for transfer of appropriate technologies to improve productivity - an overview.
- 12.20 - 12.40 - **Paper 1:** H. Abeygunawardena, *D.H.A. Subasinghe, S.S.E. Ranawana, M.W.A.P. Jayatilake, A.N.F. Perera, B.M.A.O. Perera
Transfer of technology in small-holder intensive buffalo farming: Results from a pilot project in Mahaweli System "H".

- 12.40 - 13.00 - **Paper 2:** C. Bogahawatta
Supply response of milk and management efficiency of cattle and buffalo production in Sri Lanka: A cross section study.
- 13.00 - 14.30 - **Lunch**
- 14.30 - 15.30 - **Session I - (Contd)**
Chairperson: Nguyen Van Thu
- 14.30 - 14.50 - **Paper 3:** *K.K. Pathirana, C.P. Kodikara, D.K.M.P. Dassanayake, S. Widanapathirana
A field survey and microbiological studies on Ruhunu curd.
- 14.50 - 15.10 - **Paper 4:** I.D. Silva, *A. Dangolla, K.F.S.T. Silva
Preliminary analytical observations on persistence of milk yield in buffalo in Sri Lanka.
- 15.10 - 15.30 - **Paper 5:** H.W. Cyril
Factors affecting carcass and meat quality of indigenous buffalo in Sri Lanka.
- 15.30 - 15.45 - **Tea**
- 15.45 - 17.00 - **Session II - Genotypes & Environment** *Rapporteurs: M.G. Jeyaruban
H. Peiris*
Chairperson: A.W. Qureshi
- 15.45 - 16.30 - **Keynote Lecture 2:** Charan Chantalakhana (Thailand)
Breeding strategies for optimum utilization of available resources in rural buffalo production systems.
- 16.30 - 17.00 - Address by I. Settergren (Sweden)
- 17.00 - 18.00 - **Get-together & Tea**

12 December 1995 (Tuesday)

- 09.00 - 10.00 - **Session II (Contd)**
Chairperson: V. Momongan
- 09.00 - 09.20 - **Paper 6:** *A.A.J. Rajaratne, S.S.E. Ranawana
Physiological responses of Lankan buffaloes to stress at work.
- 09.20 - 09.40 - **Paper 7:** *E.R.K. Perera, A.N.F. Perera
*Effectiveness of different cooling treatments in heat stress alleviation of water buffalo (*Bubalus bubalis*).*

- 09.40 - 10.00 - **Paper 8:** *A.A.J. Rajaratne, S.S.E. Ranawana
Physiological responses of Lankan buffaloes to dehydration.
- 10.00 - 10.20 - Tea**
- 10.20 - 12.25 - Session III- Nutrition & Feeding** *Rapporteurs: K.K. Pathirana
A.A.J. Rajaratne*
Chairperson: S.K. Ranjhan
- 10.20 - 11.05 - **Keynote Lecture 3:** Nguyen Van Thu (Vietnam)
New concepts and strategies in the utilization of agro-industrial by products in buffalo feeding.
- 11.05 - 11.25 - **Paper 9:** *S. Premaratne, P. Sivaram
Development of systems of supplementary feeding for buffaloes in Sri Lanka.
- 11.25 - 11.45 - **Paper 10:** *A.N.F. Perera, E.R.K. Perera
Natural and alternate feed sources for buffalo production in Sri Lanka.
- 11.45 - 12.05 - **Paper 11:** *J.G.S. Ranasinghe, R. Sivakanesan, H. Abeygunawardena, P. Mariathasan
Serum concentrations of progesterone and its precursor cholesterol in buffaloes.
- 12.05 - 12.25 - **Paper 12:** *E.R.K. Perera, A.N.F. Perera
Changes in growth, rumen characteristics and blood metabolites of indigenous buffalo heifers in response to supplementary feeding of urea treated straw.
- 12.25 - 13.00 - Poster Session**
- 13.00 - 14.00 - Lunch**
- 14.00 - 15.25 - Session III (Contd)**
Chairperson: C. Chantalakhana
- 14.00 - 14.45 - **Keynote Lecture 4:** S.K. Ranjhan (India)
Appropriate nutritional packages relevant to small-holder buffalo production systems in Asia.
- 14.45 - 15.05 - **Paper 13:** *H. Peiris, A. Perera
Study of the grazing behaviour and forage utilization of free range buffalo herds.
- 15.05 - 15.25 - **Paper 14:** *S.S.E. Ranawana, J. Dharmawardana, A.W.A.S. Abeysekara, A.A.J. Rajaratne, G.D.J.K. Gunaratne, E.M.C. Ekanayake
The nutritional status of indigenous buffaloes with respect to selected micronutrients.

- 15.25 - 15.45 - Tea
- 15.45 - 16.50 - **Session IV - Reproduction** *Rapporteurs: E.R.K. Perera
T.N. Jayatilaka*
- Chairperson: I. Settergren
- 15.45 - 16.30 - **Keynote Lecture 5:** Nasir Hussain Shah (Pakistan)
Experiences in improving reproduction in river type buffaloes.
- 16.30 - 16.50 - **Paper 15:** *H. Abeygunawardena, W.D. Abayawansa, B.M.A.O. Perera
Comparative study of reproduction and productive characteristics of indigenous swamp and exotic river buffaloes in Sri Lanka.

13 December 1995 (Wednesday)

Field Trip to Thambuttegama - Mahaweli System H

Coordinator: A.N.F. Perera

- 06.00 - Departure from ICEAH, Peradeniya.
- 09.30 - Arrival at Niraviya Farm
- 09.30 - 10.00** - **Tea**
- 10.00 - 12.30 - Visit to Intensive Buffalo Farm Modules at Thambuttegama
- 12.30 - 13.30** - **Lunch**
- 13.30 - Leave for Peradeniya (Via Dambulla Rock Temple)
- Approx. 18.30 - Arrival at ICE, Peradeniya

14 December 1995 (Thursday)

09.00 - 10.25 - **Session IV (Contd)**

Chairperson: D.K. Singh

- 09.00 - 09.20 - **Paper 16:** *D. Gunarajasingham, H. Abeygunawardena, V.Y. Kuruwita, B.M.A.O. Perera
Seasonal variations in seminal and testicular characteristics and plasma testosterone concentrations in buffalo bulls.
- 09.20 - 09.40 - **Paper 17:** *H. Abeygunawardena, V.Y. Kuruwita, B.M.A.O. Perera
Effects of different suckling regimes on postpartum fertility of buffalo cows and growth rates and mortality of calves.
- 09.40 - 10.00 - **Paper 18:** *H. Abeygunawardena, V.Y. Kuruwita, B.M.A.O. Perera
Effects of exogenous hormones on fertility of postpartum anoestrous buffaloes.

- 10.00 - 10.30** - **Tea**
- 10.30 - 12.25** - **Session V - Health & Diseases** *Rapporteurs: B.D.R. Wijewardana
A. Dangolla*
Chairperson: A. Rahaman
- 10.30 - 11.15 - **Keynote Lecture 6:** D.K. Singh (India)
Disease prevention strategies and recent innovations in the control of bacterial and viral diseases of buffaloes.
- 11.15 - 11.35 - **Paper 19:** *M.C.L. de Alwis, T.G. Wijewardana, N.U. Horadagoda, P. Abeynaïke, A.A. Vipulasiri, S.A. Thalagoda
Further studies on the epidemiology and immunology of Haemorrhagic Septicaemia in buffaloes.
- 11.35 - 11.55 - **Paper 20:** U. Ariyaratne, *S. Mahalingam
Characterization of strains of buffalo calf rota virus by polyacrylamide gel electrophoresis.
- 11.55 - 12.15 - **Paper 21:** *I.D. Silva, K.F.S.T. Silva, A.P.N. Ambagala and R. Cooray
Markers of inflammation in buffalo milk.
- 12.15 - 12.35 - **Paper 22:** *T.G. Wijewardana, B.D.R. Wijewardana, W.N.D.G.S. Appuhamy, K.R.V.P.M. Premaratne
Prevalence of leptospiral antibodies in buffaloes in Sri Lanka.
- 12.35 - 13.00 - Briefing on Group Discussions.
- 13.00 - 14.00** - **Lunch**
- 14.00 - 15.45** - **Session V (Contd)** *Rapporteurs: R. Sivakanesan
A.D.N. Chandrasiri*
Chairperson: N.H. Shah
- 14.00 - 14.45 - **Keynote Lecture 7:** Abdul Rahaman (India)
Implications of parasitic diseases to buffalo production and strategies for field application and control methods.
- 14.45 - 15.05 - **Paper 23:** *I.S. Abeygunawardena, D.J. Weilgama, H.M.H.L. Jayapadma, S.M. Seneviratne
Studies of Explanatum (Gigantocotyle) Explanatum infection: prevalence in cattle and buffaloes in Sri Lanka and pathology in natural infection.
- 15.05 - 15.25 - **Paper 24:** D.J. Weilgama
Observations on Schistosoma nasale infections in the vertebrate and snail hosts.
- 15.25 - 15.45** - **Tea**

15.45 - 16.45 - **Session V (Contd)**

Chairperson: M.C.L. de Alwis

15.45 - 16.05 - **Paper 25:** *N.U. Horadagoda, I.S. Gunawardena, A.P.N. Ambagala and D.M.S. Munasinghe
Haematological and biochemical profiles of adult female Lanka Buffaloes (Bubalus bubalis).

16.05 - 16.25 - **Paper 26:** D.D.N. de Silva
"Domosedan" as a sedative analgesic in indigenous buffalo.

16.25 - 16.45 - **Paper 27:** I.D. Silva
Functional efficiency of buffalo neutrophils.

15 December 1995 (Friday)

09.00 - 10.30 - Group Discussions on Conclusions and Recommendations

10.30 - 11.00 - Tea

11.00 - 13.00 - **Conclusions and Recommendations** *Rapporteurs: I.D. Silva
T.G. Wijewardana*

Chairperson: C. Devendra

11.00 - 12.00 - Presentations by Group Leaders

12.00 - 13.00 - General Discussion

13.00 - 13.15 - **Closing of Symposium**

13.15 - 14.15 - Lunch

**TRANSFER OF TECHNOLOGY IN SMALL-HOLDER INTENSIVE BUFFALO FARMING:
RESULTS FROM A PILOT PROJECT IN MAHAWELI SYSTEM "H"**

H. Abeygunawardena¹, *D.H.A. Subasinghe², S.S.E. Ranawana³, M.W.A.P. Jayatilake⁴,
A.N.F. Perera⁵ and B.M.A.O. Perera¹

¹*Department of Veterinary Clinical Studies, ⁴Department of Agricultural Economics and Extension,*

⁵*Department of Animal Science, University of Peradeniya;*

²*Department of Animal Production and Health, Getambe;*

³*Veterinary Research Institute, Gannoruwa; Peradeniya*

The objective of this study was to develop a small holder intensive dairy buffalo management system in areas where grazing land availability is limited. As nutrition is the major constraint affecting the efficiency of production parameters, the system is based on the use of a cheaper alternative to traditional concentrate feeds. Nine small holder farmers in one settlement unit of Mahaweli system "H" were selected on the basis of a rapid rural appraisal and were each provided with three dairy buffaloes from state farms, assistance to construct a cattle shed and training on improved husbandry practices. The animals received a basal diet of green fodder and rice straw *ad libitum*; the relative proportions of these varied according to seasonal availability. In addition, a series of concentrate supplements were fed in sequence as follows: Diet 1 - concentrate feed given on the state farms (3 kg coconut poonac + 3 kg rice bran/cow/day) for one month; diet 2 - 1.2 kg/day of a paste containing 6% urea, 25% molasses, rice bran and minerals for four months; Diet 3 - same as diet 2 but with the addition of 50 g fish meal/day for 6 months; and Diet 4 - a lick-block containing 10% urea, 40% molasses, 3% fish meal, rice bran and minerals for 6 months.

The mean milk yield of the buffalo cows while on state farms (pre-project) was 3.9 ± 1.2 l/d (n=22, range 887 - 1417 l/lactation). The milk yields of cows after introduction to the nine small holder units showed wide variations, with mean yields for individual units on diets 2, 3 and 4 ranging from 2.6 - 5.9, 1.6 - 4.51 and 2.4 - 7.2 l/day respectively. When diets 2 and 3 were fed the daily average intake of roughages was 40 ± 14 and 41 ± 12 kg, while dry matter intakes were 9.9 ± 3 and 10.3 ± 3 kg respectively. Dry matter intake per day on the basis of kg body weight (BW), kg metabolic BW and kg/100 kg BW were 0.02 ± 0.01 and 0.03 ± 0.02 ; 0.15 ± 0.01 and 0.11 ± 0.04 ; and 2.4 ± 0.9 and 2.9 ± 1.0 , respectively. Overall, the animals were able to maintain the pre-project milk yields with diets 3 and 4, and there were no major changes in BW attributable to the different diets. The mean birth weight of calves was 23.7 kg (n=30) on state farms and 30.4 kg (n=19) on small-holdings, and their weight gains during different periods from birth to six months ranged from 183 - 510 g/d. Out of 16 animals which calved on the small holdings, the interval from calving to first detected oestrus ranged from 21 to 440 days. The interval was less than 60 days in 5 (31.3%) and more than 200 days in 7 (43.8%) animals. In the five animals which have been confirmed pregnant so far, the calving to conception interval ranged from 104 - 315 days.

The cost of concentrate feeding was Rs. 20.00 per day per cow, while that of diet 3 (1 kg paste with fish meal) was Rs. 4.50 per day. The daily intake of the lick-block (Diet 4) was around 700 g/day, resulting in a feeding cost of Rs. 5.00 per day. This study has shown that dairy buffaloes yielding 3-5 liters of milk per day can be maintained on a diet of green fodder, straw and a supplement containing urea, molasses, fish meal, rice bran and minerals, either in the form of a paste or lick-block, at approximately 25% of the cost of traditional concentrate feeding.

SUPPLY RESPONSE OF MILK AND MANAGEMENT EFFICIENCY OF CATTLE AND BUFFALO PRODUCTION IN SRI LANKA: A CROSS SECTION STUDY

C. Bogahawatte

*Department of Agriculture Economics and Extension,
Faculty of Agriculture, University of Peradeniya*

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. Hence the country is heavily dependent on imports of milk and milk products. In order to increase domestic milk production in cattle and buffalo farms and thereby reduce the dependence on milk imports, the government has introduced several measures. These include the payment of a minimum producer price for fresh milk, an expanded artificial insemination service, cattle and buffalo insurance scheme, an organised milk collection network and veterinary research and extension services.

The main objective of this study was to determine the supply response of milk and management efficiency of the 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 - March 1992. The supply response model consist of milk productivity and herd size equations estimated by Ordinary Least Squares. The management efficiency of buffalo and cattle production was estimated by using Timers method (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.

A FIELD SURVEY AND MICROBIOLOGICAL STUDIES ON RUHUNU CURD

*K.K. Pathirana¹, C.P. Kodikara², D.K.M.P Dassanayake¹ and S.Widanapathirana¹

¹*Department of Animal Science, Faculty of Agriculture,
University of Ruhuna, Kamburupitiya;*

²*Department of Microbiology, Faculty of Science, University of Kelaniya, Kelaniya*

Ruhunu curd although acclaimed as a quality product from ancient times, has not been subject to authentic study. Out 609 Grama Niladari (GN) divisions in the entire Hambantota district and Assistant Government Agent (AGA) division of Tanamalwila in Moneragala district, 511 GN divisions (84%) were covered in the study. All curd producers were identified by GN division, for basic information and a house to house survey was done on a 5% sample for details on curd production. Sixty samples of curd were subjected to microbiological studies. A total of 1,968 producers manufactured nearly 200,000 pots of curd per day. The highest number of producers and the number of pots were from Ambalantota while the lowest in both were from Okewela AGA division. Buffalo milk and the warm dry conditions prevalent in the area, largely contributed to the production of good 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 ($10-13 \times 10^{10}/g$) indicating the need to improve the hygienic conditions. Yeasts and moulds were present in all samples with very high counts ($1-5 \times 10^5/g$) 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 34×10^6 colony forming units. Indications are that with the introduction of low cost, simple scientific procedures, the quality of the curd could be improved.

PRELIMINARY ANALYTICAL OBSERVATIONS ON PERSISTENCE OF MILK YIELD IN BUFFALO IN SRI LANKA

I.D. Silva¹, *A. Dangolla¹ and K.F.S.T. Silva²

¹*Department of Veterinary Clinical Studies,
Faculty of Veterinary Medicine and Animal Science;*
²*Department of Animal Science, Faculty of Agriculture;
University of Peradeniya, Peradeniya*

The daily milk yield of 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. This communication examines the persistency of milk yield in 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 during the period between day 75 up to at least day 178 of the lactation period. The longest lactation period observed 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 liters 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 litres in 10 cows. However, in 9 cows the WMP increased at least twice to over 1.5 litres 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 $\geq 10\%$ 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 extraneous factors leading to such variations in daily milk yield.

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

H.W. Cyril

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

Two separate studies were conducted to study the factors affecting carcass quality and meat quality characters of indigenous buffaloes. For the first study, 60 randomly selected buffaloes (live weights ranging from 100 kg to 450 kg) were used and for the second study 90 randomly selected buffaloes were used. Various factors (eg. dressing percentage, meat to bone ratio, and organ percentage) affecting carcass quality 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 from 47.99 ± 0.14 to 54.35 ± 0.00 and meat to bone ratio increased from 2.39 ± 0.45 to 4.74 ± 0.00 . 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 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-quarter increased from 51.74 ± 3.13 to 57.94 ± 0.0 , while the percentage of hind-quarter 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 adults. Respective mean percentage 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 ± 1.69 kg), was found to be significantly lower than that of adult animals (8.79 ± 2.37 kg). 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 water and fat contents of meat. For male animals mean percentage values for water and fat content 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.

PHYSIOLOGICAL RESPONSES OF LANKAN BUFFALOES TO STRESS AT WORK

*A.A.J. Rajaratne¹ and S.S.E. Ranawana²

¹*Department of Physiology, Faculty of Medicine, University of Peradeniya,*

²*Veterinary Research Institute, Gannoruwa, Peradeniya*

A series of experiments were 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 buffaloes before and at 30 minute intervals of working. These measurements were made while buffaloes were engaged in different types of work namely threshing paddy, ploughing muddy fields and ploughing dry land.

Rectal temperature increased from 38.1°C to 39.2°C, respiration rate from 21 to 41 per minute and pulse rate from 63.3 to 81.8 per minute during one-hour period of ploughing mud fields. There was a significant reduction in respiration rate and pulse rate when the animals rested for a period of 30 minutes in mud fields.

Rectal temperature increased from 37.4° C to 39.7° C, respiration rate from 20.3 to 75.5 per minute and pulse rate from 66.5 to 82 per minute when animals ploughed dry land for a period of 1.5 hr. In this experiment too resting brought about a mark reduction in all the parameters measured.

When the animals were used for threshing paddy for a period of 2 hrs, the rectal temperature increased from 38.5° C to 40.5°C, respiration rate from 34.5 to 112 per minute and pulse rate from 68.9 to 107.3 per minute.

It is clear from these results that threshing paddy although not a very strenuous exercise, caused significantly higher stress in buffaloes compared to ploughing muddy fields. It is important to alleviate such stress in order to obtain maximal efficiency in buffaloes at work. When ploughing, water was used as method of alleviating stress in working buffaloes. There was a significant reduction in the pulse rate and respiration rate but the effect on rectal temperature was not significant.

The above results point to heat stress as the main stress factor in working buffaloes. 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.

EFFECTIVENESS OF DIFFERENT COOLING TREATMENTS IN HEAT STRESS ALLEVIATION OF WATER BUFFALO (*BUBALUS BUBALIS*)

*E.R.K. Perera and A.N.F. Perera

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An experiment was conducted to evaluate the physiological responses of water buffalo to application of different cooling treatments in order to find out an effective method of heat stress alleviation for stall-fed buffaloes. The following cooling treatments were applied and compared with no cooling (control) using a minimum of three heifers per treatment: covering with wet gunnies (T1), six different sprinkler applications (T2-T6) using a combination of 30, 60 and 90 sec sprinkling durations and 3 or 6 repeated sprinkling cycles, and 540 sec continuous sprinkling (T7). Among treatment groups, the heifers were balanced by age (2.0 ± 0.6 yr) and body weight (155 ± 10.0 kg). Data on skin temperature (ST), respiration rate (RR), heart rate (HR) and rectal temperature (RT) were obtained for all animals at -10, 0, +15, +30, +45 and +60 min following application of each treatment. Data on ambient temperature was recorded at the same time intervals. Each treatment was repeated on five different days.

Skin temperature reduced ($p < .01$) in response to all sprinkling treatments. Three repeated cycles of 30 or 60 sec sprinkling was not effective in reducing HR, RR or RT significantly. However, 6 repeated sprinkling cycles of 30 and 60 sec duration and both 3 and 6 repeated sprinkling cycles of 90 sec duration as well as 540 sec continuous sprinkling were effective in reducing RR, HR and RT significantly ($p < .05$). Following the withdrawal of the cooling treatments, ST, RR, HR and RT increased with time to reach the precooling values at different rates. The results suggest that, out of the tested cooling methods, 3 or 6 repeated sprinkling cycles of 90 sec duration and 540 sec continuous sprinkling were more effective in heat stress alleviation of stall-fed water buffaloes.

PHYSIOLOGICAL RESPONSES OF LANKAN BUFFALOES TO DEHYDRATION

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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 output. Total mean water intake, output and balance in the normal hydration was 21.8 l/day, 19.46 l/day and 2.32 l/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.

**DEVELOPMENT OF SYSTEMS OF SUPPLEMENTARY FEEDING
FOR BUFFALOES IN SRI LANKA**

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Swamp buffalo being an important source of draft power and, to a limited extent a source of milk, improving the productivity of these animals 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 objectives of this study were to develop systems of supplementary feeding for buffaloes in Sri Lanka.

Four rumen fistulated male buffaloes were used to study the intake, digestibility and growth response of buffalo fed rice straw, supplemented with non-protein nitrogen and energy (Latin Square Design). Treatments consisted of 2% urea supplemented straw or plain straw with or without Jack seeds. Feed offered, refused and feces excreted were measured and the dry matter intake and digestibility were calculated. Urine was collected to measure the N balance of 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.

In another experiment with milking buffaloes the effect of different fodders on the dry matter intake of straw was studied.

Dry matter intake of urea supplemented straw with Jack seeds was higher compared to plain straw (102 vs 87 g/kg W^{0.75}) whereas, dry matter digestibility of plain straw with Jack seeds was higher compared to plain straw (55% vs 46%). Inclusion of Jack 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 gliricidia.

In conclusion, it can be said that supplementation of straw based diets with urea and gliricidia improved the intake, digestibility and growth of buffaloes.

**NATURAL AND ALTERNATE FEED SOURCES FOR
BUFFALO PRODUCTION IN SRI LANKA**

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The total buffalo population in Sri Lanka is 0.85 million, and more than 75% are managed under free grazing in natural grazing lands on natural pastures or on other natural herbs. An experiment was conducted to evaluate the production and seasonal variation in dry matter yield (DMY) and feeding value of pastures and Forbes in different natural grazing lands. A series of experiments were also conducted to evaluate the methods available to upgrade the feeding value of crop residues to feed buffaloes as a supplementary feed.

The annual DMY of natural grasses and Forbes ranged from 800 - 1800 kg/ha, with a crude protein (CP) content of 80 - 140 g/kg DM and dry matter digestibility (DMD) of 350 - 670 g/kg. Rice straw ensiled with Biogas Slurry Liquid up to 14 days improved CP from 44 to 64 g/kg and DMD from 295 to 421 g/kg. Treatment of Palm Press Fibre (PPF) with Sodium Hydroxide (0 - 60 g/kg) or Urea (0 - 80 g/kg) increased the DMD from 225 to 357 and 225 to 319 g/kg, respectively. Urea treated PPF had a higher CP content (55 vs 300 g/kg) due to urea nitrogen.

Sugar cane by products, less exploited but high potential feed resources, were also studied. The CP of green cane tops, bagasse, bagsilo and molasses were 51, 16, 14 and 24 g/kg, respectively. The effect of different levels of urea (0 - 60 g urea/kg) and ensiling duration (0 - 21 days) on bagasse was evaluated. The level of urea increased the DMD but the duration of ensiling had no effect.

These results suggest the possibility of using different treatments on available crop residues to improve their feeding value in order to use them as supplementary feeds in buffalo production systems.

SERUM CONCENTRATIONS OF PROGESTERONE AND ITS PRECURSOR CHOLESTEROL IN BUFFALOES

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The dependence of steroidogenesis in mammalian ovary of 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 the control group were cycling by 150 days whereas 5 out of 18 in the supplemented group were cycling by 133 days. The mean body weights of the supplemented group were slightly higher than the control group but the differences were not statistically significant.

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.

**CHANGES IN GROWTH, RUMEN CHARACTERISTICS AND METABOLITES OF
INDIGENOUS BUFFALO HEIFERS IN RESPONSE TO SUPPLEMENTARY FEEDING OF
UREA-TREATED STRAW**

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An experiment was conducted to investigate the response of feed intake, rumen parameters, blood metabolites and growth of indigenous buffalo heifers to supplementary feeding of urea-treated straw. Twelve indigenous buffalo heifers weighing 89.0 ± 8.9 kg and age 12.0 ± 0.5 months were allotted to two equal groups. All the animals were offered Guinea grass (*Panicum maximum ecotype A*) *ad libitum* during day time. At night, the treatment group was given 4% urea treated straw, while the control group was given untreated straw. Daily group feed intake of Guinea grass and straw was recorded. Representative samples of Guinea grass, straw and refusals were obtained periodically for proximate analysis. Individual body weights were recorded monthly. Rumen samples and blood samples were obtained every fourth week to determine rumen pH, rumen ammonia-N ($\text{NH}_3\text{-N}$), blood urea nitrogen (BUN) and beta-hydroxybutyrate (BHB).

Urea treated straw had higher ($P < 0.01$) crude protein content than untreated straw. The heifers in 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 those in the control group. Rumen ammonia content was higher ($P \leq 0.05$) in the treatment group. Rumen pH, circulating BHB, and BUN were not different between the groups, but trends in rumen pH and BUN in the two groups suggested better utilization of available dietary protein by the treatment group heifers. The latter had superior ($P \leq 0.05$) total body weight gain compared to control animals. 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.

**STUDY OF THE GRAZING BEHAVIOUR AND FORAGE UTILIZATION
OF FREE RANGE BUFFALO HERDS**

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Buffalo farming under the free range system is one of the common management practices in Sri Lanka due to limited land availability for pasture/fodder cultivation. As a result the free range animals mainly depend on the natural herbage. Since supplementary feeding is not a common practice under small holder systems, a study was conducted to investigate the grazing behaviour of free range buffalo herds in relation to the forage quality.

Eight experiments were conducted using 24 buffaloes under field conditions covering both dry and wet seasons of the year. Their behaviour was monitored in relation to the herbage quality over 24 hour periods in each study.

Results revealed that the free range buffaloes on natural forages under coconut palms spent more time ($P < 0.01$) on grazing, selecting feed and ruminating when compared to those on improved forages (*B. milliformis*) under similar conditions. Regression analysis indicated that there was a high correlation between the grazing time and the nitrogen content of forages ($r = 0.96$), and also with the resting time of free range animals and the lignin content of the forages ($r = 0.92$).

Results indicate that the close relationship between the quality of herbage and the grazing behaviour of free range buffalo could be used as a measurement to assess the forage quality under field conditions. Secondly, the quality of natural herbage was very poor and therefore, introducing suitable supplementary feed for free range animals would be an advantage.

**THE NUTRITIONAL STATUS OF INDIGENOUS BUFFALOES
WITH RESPECT TO SELECTED MICRONUTRIENTS**

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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, Kuliyaipitiya/Pannala, Mihintale, Tissamaharama & 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 was collected.

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 was 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.

Measurements of chest girth showed that in the dry zone locations of Mihintale and Tissa/Thanamalwila, the animals lost weight in August/September. Hematocrit values, an index of general body condition, were also low in the Tissamaharama cows during this time. Values for PCV were below normal values in many of the Mihintale calves. 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.

COMPARATIVE STUDY OF REPRODUCTION AND PRODUCTIVE CHARACTERISTICS OF INDIGENOUS SWAMP AND EXOTIC RIVER BUFFALOES IN SRI LANKA.

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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 progenies, primarily males, were 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 results are summarized in the table.

	Surti	Murrah	Nili Ravi	Indigenous
Birth weight (kg)	27.1 ± 4.0	27.6 ± 2.7	30.1 ± 5.1	17.9 ± 2.8
Prepubertal growth rate (kg/d)	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)	149.3 ± 79.4	177.4 ± 83.2	136.9 ± 90.7	--
Calving interval (days)	481.8 ± 157.7	509.8 ± 183.6	490.5 ± 126.0	506.8 ± 157.9
Lactation yield (liters)	1003.0 ± 391.0	1052.1 ± 496.4	1760.9 ± 581.4	--
Lactation length (days)	267.3 ± 72.0	286.6 ± 92.0	309.0 ± 97.3	--

Birth weight and prepubertal growth rate were highest in N and lowest in L. Average age at first calving was lower in L than in all river types. Average calving intervals of all 4 were above 16 months. 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.

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.

SEASONAL VARIATIONS IN SEMINAL AND TESTICULAR CHARACTERISTICS AND PLASMA TESTOSTERONE CONCENTRATIONS IN BUFFALO BULLS.

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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 AI Centre at Kundasale were used. Semen samples (n=132) were collected using an artificial vagina at weekly intervals for twelve months, and were evaluated for volume, colour, density, mass activity, motility and concentration. Monthly collections (n=36) were used to study sperm morphology (unstained or stained with modified William's stain), cells other than sperms (Haematoxylin- Eosin) and live and dead status (Nigrosine-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 such variations were observed in the percentages of head, mid piece and tail abnormalities.

In study 2, three indigenous buffalo bulls aged 3 - 4 years (158 - 238 kg body weight) were used to study changes in plasma testosterone secretion. Serial blood samples were collected at 30 min intervals from 0800 to 1130 hrs once every month over twelve months. Testosterone was measured by Radioimmunoassay (Coat-A-Count, DPC, USA). Monthly mean plasma testosterone levels remained below the detection limit of the assay (0.04 ng/ml) and peak elevation of testosterone was seen in December (0.5 ± 0.4 ng/ml).

In study 3, seasonal changes in testicular morphology were examined during hot (August) and wet (December) seasons. Testes were removed from adult buffalo bulls at 3 - 4 year age (n = 3), tissue samples from three locations taken and preserved in Bouin's solution, processed and stained with H-E stain. The mean diameters of seminiferous tubules (n = 30 per testis) during the month of August and December respectively were 177.2 ± 15.2 and 193.7 ± 14.2 , and this difference was significant ($P < 0.01$). However, no differences due to season were detected in the specific stage viii of the seminiferous epithelial cycle.

EFFECTS OF DIFFERENT SUCKLING REGIMES ON POSTPARTUM FERTILITY OF BUFFALO COWS AND GROWTH RATES AND MORTALITY OF BUFFALO CALVES

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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 free suckling by calves. A 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 (FS45); FS and weaning by day 60 (FS60); FS and weaning by day 90-120 (FS90); 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 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. Pregnancy rates at 90, 120 and 150 days postpartum and calf mortality rates are presented in table 1.

Treatment	n	Pregnancy Rate (%)			Calf Mortality (%)
		90 days	120 days	150 days	
FS	74	19	20	23	5.4
FS45	11	54	73	73	55.0
FS60	16	6	31	37	31.0
FS90	18	5	17	27	33.0
OS	29	38	59	59	6.8
TS	22	31	45	59	4.5

FS resulted in the lowest pregnancy rates. FS60 and FS90 did not improve postpartum fertility. Weaning at all 3 ages resulted in substantial calf mortality which was highest with FS45. OS resulted in lower body weights as a result of slower growth rates during the first 3 months, but there was no difference from 4 months onwards. TS did not result in any significant reduction in growth rate compared with FS. In conclusion, the results of this study show that once a day as well as twice a day suckling could be adopted as suitable suckling regimes to improve postpartum fertility in indigenous buffaloes.

EFFECTS OF EXOGENOUS HORMONES ON FERTILITY OF POSTPARTUM ANOESTROUS BUFFALOES

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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 treatment 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; (d) progesterone releasing silastic ear implant (Synchromate B, Intervet) for 11 days and 500 IU of PMSG at the time of implant removal; and (e) untreated controls. After the treatments the animals were allowed to run with stud bulls in their natural habitat. Subsequent calvings were recorded and the number of pregnancies occurring in each group during the first 90 days after treatment were determined. The results are given in the table.

In a separate trial, 9 cows were given treatment (d), kept separate 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 very distinct signs of oestrus following implant removal and 8 (89%) had ovulations as indicated by plasma progesterone. However, the ovulations were spread over several days and two animals had abnormal luteal phases with both relapsing in to acyclicity.

Treatment	n	No. pregnant at 90 days	Pregnancy rate (%)
a. GnRH	10	0	0
b. GnRH + FSH	12	0	0
c. PRID + PMSG	23	19	83
d. Synchromate + PMSG	44	32	73
e. Control	45	15	33

The lack of response to GnRH treatment could be due to refractoriness of the ovary and pituitary, and the lack of tertiary follicles. However, 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.

These results indicate that short term progesterone treatment in conjunction with PMSG 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. In particular, the time sequence of events in relation to implant removal, LH surge, ovulation and oestrus need to be determined precisely in order to use this treatment more effectively with planned breeding.

**FURTHER STUDIES ON THE EPIDEMIOLOGY AND IMMUNOLOGY
OF HAEMORRHAGIC SEPTICAEMIA IN BUFFALOES**

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The existence of "Latent" and "Active" carriers in haemorrhagic septicaemia (HS), and the intermittent conversion of latent carriers into an active state was demonstrated in earlier studies. The factors which influenced such conversion were yet obscure. Further it was found that the carrier state could not be cleared by treatment using antibiotics to which the organism was sensitive *in vitro*.

Investigations were therefore carried out to determine the exact site of location of the Pasteurellae within the tonsil, and the factors which caused their appearance in the nasopharynx.

The HS Vaccine produced in Sri Lanka was last evaluated in the late 1970s. Since then several modifications have been made to the production procedures. It was therefore considered necessary to study the immune response of Buffaloes to the current vaccine.

The carrier state was established in 13 buffaloes by exposure to a marked strain of *P. 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. Thirty eight out of 47 antibody free calves, were vaccinated with HS oil adjuvant vaccine. The serological response of these and 9 unvaccinated controls was 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 using 10⁸ CFU by the subcutaneous route.

It was found that the pasteurellae were localised in the crypts of the tonsils. Treatment of latent carriers with steroids and Neostigmin failed to activate latent carriers.

Protection to challenge at 9 m and 12 m was 50% and 67% respectively. No correlation was observed between serological titres and response to challenge.

**CHARACTERIZATION OF STRAINS OF BUFFALO CALF ROTAVIRUS BY
POLYACRYLAMIDE GEL ELECTROPHORESIS**

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The objective of the present study was to detect non group A ('atypical') rotaviruses as well as 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. 139/175 (79.4%) of these calves were diarrhoeic at the time of sampling whilst the rest were non diarrhoeic, but in contact with them.

14/175 (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 (6) of these strains were different from each other. These strains were circulating in farms, 60-100 km 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 is hard to come by. However electropherotyping of strains of rotaviruses may not indicate variation in antigenicity of these strains.

MARKERS OF INFLAMMATION IN BUFFALO MILK

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Monitoring of somatic cell counts (SCC) and serum factors in milk is a standard practice used to detect mastitis in cattle, because immunity in milk against invading pathogens is asserted by the phagocytes and serum factors. This communication describes such markers in buffaloes which are useful in the early detection of subclinical mastitis.

The SCC in normal buffalo milk ranged from 50,000 to 375,000/ml, of which 56% (22 - 88) were neutrophils which are the most active phagocytes in the body. The lymphocytes constituted 28% (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 (6.1 - 7.0). The electrical conductivity (EC) and chloride ion percentage (CI%) were 3.86 milli Siemens/cm and 0.11%, respectively, in normal milk.

Acute inflammation permits leukocytes and serum factors in blood to enter the lacteal secretion. Experimentally induced acute, subclinical mastitis elevated the SCC from 50,000 to 75×10^6 /ml, and 90% of those were neutrophils. The adenosine 5'-triphosphate (ATP) values elevated with the increasing SCC. The entry of serum into milk preceded 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 raise in CI% to 0.14% were also observed before a detectable elevation of SCC. Significant alterations were not detected in acidity% and pH. Therefore SCC, EC, CI%, and ATP are makers of inflammation in buffalo milk.

PREVALENCE OF LEPTOSPIRAL ANTIBODIES IN BUFFALOES IN SRI LANKA

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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 determined by the data extracted from government hospitals, Anuradhapura, Badulla, Colombo, Galle, Gampaha, Kandy, Kegalle, Kalutara, Kurunegala, Matara, Polonnaruwa and Ratnapura districts were selected for the survey. From each selected Assistant Government Agent's division within a district, serum was collected from 0.4% of the buffalo population, giving a total of 1500. Each sample was examined by the microscopic agglutination test, against a panel of 10 serovars of leptospira that had been isolated from Sri Lanka, which were recommended and provided by the WHO/FAO Reference Centre for Leptospirosis in Australia. Those showing an agglutination at a dilution of 1 in 50 or above were considered positive.

The overall prevalence was 41.93%. The highest was in Badulla district (72.91%) while the lowest was in Kegalle district (11.84%). The most common serovar was *weerasinghe* (30.20%) followed by *pomona* (26.5%) and *hardjo* (24.16%). The least common serovar was *ceylonica*.

The serovars *pomona* and *hardjo* are confined to cattle and buffaloes. While *hardjo* has been incriminated with abortions and still births in cattle, *pomona* has been associated with the clinical disease. In the past, animals with a history of abortions or still births were examined only for Brucellosis and, as the results of the present study indicate, in instances where the animal was found to be negative for Brucellosis, the causative agent could have been leptospira.

**STUDIES ON *EXPLANATUM (GIGANTOCOTYLE) EXPLANATUM* INFECTION:
PREVALENCE IN CATTLE AND BUFFALOES IN SRI LANKA AND
PATHOLOGY IN NATURAL INFECTION**

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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 one year period commencing in April 1993. Postmortem material from several government farms were also collected. Seventy percent of the carcasses presented for meat inspection at the slaughter houses were of cattle and the rest were of buffaloes. Livers and bile ducts of the cattle and buffaloes were examined macroscopically for the presence of parasite. 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 sources of animals or the carcasses submitted to the slaughter houses revealed that the infection was found in all the agro-climatic zones. However, the incidence rate was slightly higher (26.9%) in dry zone compared to 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 few animals in younger group were affected. Affected livers were dissected and parasites were collected for further laboratory characterization. Only one type of parasite, *Explanatum explanatum* was identified so far. The affected livers were soft in consistency, the capsule was thicker and opaque compared to unaffected livers. Further, it was observed that some polyp-like protuberances on the epithelial surface of bile ducts. The adult parasites were attached to these protuberances. The microscopy of haematoxylin and eosin stained sections revealed lymphocyte and macrophage infiltration around portal triad with increased amount of glandular tissue in bile duct areas. The preliminary results of this study suggest that *Explanatum explanatum* is a widespread liver parasite, primarily affecting buffaloes in Sri Lanka. Further studies are in progress to determine the clinical aspects of the disease and to develop a serodiagnostic test and to assess economic losses due to the infection.

**OBSERVATIONS ON *SCHISTOSOMA NASALE* INFECTIONS IN
THE VERTEBRATE AND SNAIL HOSTS**

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Nasal schistosomiasis due to *Schistosoma nasale* has been observed in cattle and buffaloes in Sri Lanka. In cattle the disease is often severe and could even be fatal. Studies were made on the prevalence of *S. nasale* in cattle and buffaloes at the Colombo abattoir and the Buffalo Research Farm at Narangalla. At the former, from August to December, *S. nasale* was detected in 5.9% of cattle and in 23.9% of buffaloes. The prevalence rate, however, in buffaloes at the Research Farm was 91%. Monthly examination of nasal secretions of these infected buffaloes showed *S. nasale* eggs persisting up to eight months. The only clinical sign seen was a thick nasal discharge. "Snoring" was not present.

Snails belonging to six species were examined and in nature only *Indoplanorbis exustus* was infected. The infection rate, however, was low (0.55%). Laboratory reared snails (F₁ Gen.) of *I. exustus* from four different areas Narammala, Peradeniya, Nikaweratiya and Girandurukotte were tested and only snails from Peradeniya and Girandurukotte were susceptible. Infection rates of upto 50% were obtained. Attempts to infect snails of *Bithynia sp.* and *Bulimus sp.* were not successful.

Miracidial doses of 1, 4, 6 and 10 per snail produced infections in *I. exustus* but higher doses (20/snail) caused heavy snail mortality. The prepatent period varied from 30 to 59 days post exposure and cercarial emergence peaked in the morning hours (8.00 to 1.30 hr). The cercaria were observed to migrate through the 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) and Indirect Fluorescent Antibody Test (IFAT) were developed and both were found to be useful in detecting *S. nasale* infections in cattle and buffaloes. COPT produced clear precipitin reactions around schistosomulae and yielded better diagnostic results than the IFAT.

**HAEMATOLOGICAL AND BIOCHEMICAL PROFILES OF ADULT
FEMALE LANKA BUFFALOES (*BUBALUS BUBALIS*)**

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This study was undertaken 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 79 apparently healthy female buffaloes (2-7 years) at the Narangalla buffalo research farm were assayed for haematological parameters using standard methods. The serum biochemical constituents were analysed in 120 animals, both at Narangalla and the Mawela farm, Peradeniya. The serum analytes were assayed using biochemical kits from Randox Laboratories, UK.

Irrespective of age, the reference values (mean \pm standard deviation) for haematological parameters were: red blood cells ($10^{12}/l$) 5.34 ± 1.23 , haemoglobin (g/dl) 11.88 ± 1.69 , packed cell volume (l/l) 0.31 ± 0.05 , erythrocyte sedimentation rate (mm/hr) 31.17 ± 22.2 , plasma protein (g/l) 76 ± 11.5 , Fibrinogen (g/l) 6.8 ± 2.7 , icterus index 5.66 ± 2.23 , white blood cells ($10^9/l$) $11,642 \pm 3991$, lymphocytes (%) 54.05 ± 11.31 or ($10^9/l$) 6171 ± 3540 , neutrophils (%) 36.23 ± 11.04 or ($10^9/l$) 3797 ± 1928 , monocytes (%) 3.48 ± 2.26 or ($10^9/l$) 388 ± 357 , eosinophils (%) 6.79 ± 5.51 or ($10^9/l$) 682 ± 580 and basophils (%) 0.38 ± 0.3 or ($10^9/l$) 39 ± 24 .

The reference values (mean \pm standard deviation) for biochemical constituents were: albumin 31.64 ± 4.54 g/l, aspartate aminotransferase (AST) 105 ± 36.56 U/l, alanine aminotransferase (AST) 36.2 ± 11.87 U/l, creatine phosphokinase 66.75 ± 35.47 U/l, alkaline phosphate (ALP) 118.56 ± 64.29 U/l, gamma glutamyl transferase 18.77 ± 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 4.93 ± 4.4 (total), 2.05 ± 1.71 (direct) $\mu\text{mol/l}$.

The results of this study indicates that the haematological parameters in the Lanka Buffalo are similar to that reported for the swamp and river type buffaloes in Asia, characterised by a low icterus index, and increased ESR and a predominance of lymphocytes over neutrophils. The biochemical values closely correspond to values obtained for the swamp buffalo in Australasia. These are also comparable to values reported for 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.

"DOMOSEDAN" AS A SEDATIVE ANALGESIC IN INDIGENOUS BUFFALO

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Detomidine HCL (Domosedan, Farnos, Finland) is a Imidazole derivative which acts upon α -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 which are intractable, for various purposes such as restraint for easy handling, transportation, immobilization, preoperative medication and analgesia prompted the author to carry out the present study.

Four dosages namely, 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 randomly divided into 4 groups and whose ages ranged from 8 months to 8 years. Rectal temperature, pulse rate and respiratory rate were measured prior to and 01, 05, 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 above intervals after injection.

The results indicated that the dosage of 0.1 ml/100 kg caused only a mild sedation which was unlikely to be of any practical use, whereas dosages 0.2 and 0.4 ml/100 kg were useful to produce desirable sedative analgesic effects for clinical examination, preoperative medication, minor surgical procedures and transportation. The highest dosage of 0.8 ml/100 kg produced a very deep sedation and marked analgesia always associated with recumbency which was useful for complete immobilization of the animal. In general detomidine caused bradycardia, reduced pulse pressure, salivation and with higher dosages regurgitation. The degree of sedation and analgesia were dose dependent.

FUNCTIONAL EFFICIENCY OF BUFFALO NEUTROPHILS

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Neutrophils are the most active phagocytes in the body. The phagocytic and postphagocytic bactericidal activities of buffalo neutrophils were measured to study their functional efficiency. The following 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 *E. coli* in pure and mixed cultures.

The percent phagocytosis (PP) was optimum at 45 minutes and was highest for *Staph. aureus* (86%) followed by *Streptococcus agalactiae* (77%) and *Escherichia coli* (74%), in pure cultures. The phagocytic efficiency was similar in the presence of pure or mixed cultures. However, with mixed cultures a majority (40%) of neutrophils phagocytised both species and the rest showed preferential phagocytosis of one species. Neutrophils incubated with mixed cultures of *E. coli* and *Staph. aureus* showed a significant preference for *Staph. aureus* with a PP of approximately 40%. Similarly, when incubated with mixed cultures of *E. coli* and *Strep. agalactiae*, a significant preference was shown for *Strep. agalactiae* with a PP of approximately 32%. 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 were optimum at 60 minutes. The degree of resistance to PPBA was similar for all three bacteria, but was relatively lower for *E. coli*.

Neutrophils which appear in milk during the early stages of acute mastitis 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*.

IMMUNOLOGICAL RESPONSE OF BUFFALO COWS TO *TOXOCARA VITULORUM* - ANTIGENIC ANALYSIS*

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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 IgG₁ 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 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 ($p < 0.001$) 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.

*This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.

**PRODUCTION SYSTEMS AND REPRODUCTIVE PERFORMANCE OF INDIGENOUS
BUFFALOES IN SRI LANKA***

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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 administered 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 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.

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**STUDIES ON THE COMPOSITION OF INDIGENOUS BUFFALO MILK
IN SRI LANKA***

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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 \pm 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 \pm 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, $37.05 \times 10^{-4} \pm 5.00$ 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 μ m 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.

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.

*This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Master of Philosophy.

IMMUNOPATHOLOGICAL STUDIES OF *TOXOCARA VITULORUM* IN BUFFALO CALVES AND RODENTS*

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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.

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 α 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, IgG₁ and IgG₂. Enzyme Linked Immunosorbent Assay (ELISA) demonstrated antibodies both in the IgM and IgG fractions with a higher IgG₁ activity as opposed to IgG₂. Gel diffusion and *in vitro* larval preacceptance were also confined to the IgG₁ and not to the IgG₂.

*This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy

CLINICAL AND ENDOCRINOLOGICAL STUDIES ON POSTPARTUM OVARIAN ACTIVITY IN LANKA BUFFALOES (*BUBALUS BUBALIS*)

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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 ($p>0.05$) 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.

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 managerial factors such as suckling. Restricted calf suckling could be a practical and effective method for improving the efficiency of reproduction in Lanka buffaloes.

*This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy

**IMMUNOLOGICAL RESPONSE OF BUFFALO COWS AND CALVES
TO *TOXOCARA VITULORUM****

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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 colostrum and calf serum antibody titres and peak epg of the calves was evident ($p < 0.01$).

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 IgG₁, IgG₂ and IgM types showed a significant protective activity against an oral challenge ($P < 0.01$, < 0.01 , < 0.001) but IgG₁ was relatively more protective than IgM and IgG₂.

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 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*.

*This abstract is based on the thesis submitted to the University of Peradeniya for the degree of Doctor of Philosophy.

**IMMUNOLOGICAL RESPONSES OF MICE, RABBITS AND BUFFALO CALVES TO
TOXOCARA (NEOASCARIS) VITULORUM INFECTION***

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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 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.

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