

FEEDING TRIALS ON RABBITS USING A *SACCHAROMYCES CEREVISIAE* STRAIN

S. SEEVARATNAM, C. T. RAVIRAJAN AND K. BALASUBRAMANIAM
Department of Biochemistry, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka.

V. S. VARMAN
Department of Pathology, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka.

M. JAYARATNAM
Department of Chemistry, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka.

AND

K. JAYASEELAN
Department of Biochemistry, Faculty of Medicine, National University of Singapore.

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Abstract : A fast growing (growth rate $k = 0.63h^{-1}$), *Saccharomyces cerevisiae* strain Y₁₈ from grapes containing 52% protein was used in the feeding trials on rabbits. The total serum protein and albumin levels were the same and were not significantly different in all the four groups. The body weight measurements indicated that the mean effects due to control, yeast and casein diets levels are the same. But the mean effect due to low protein diet is lower than the control, yeast and casein diets levels. Histopathological studies of liver and kidney revealed no abnormal findings. It appears that this yeast protein has no deleterious effects.

Introduction

Protein malnutrition among children is the most common public health problem in developing countries. This is mainly due to the non-availability of good quality protein foods. This prompted us to look for cheap high quality microbial protein sources. Yeast was selected as the microorganism for this study. Yeasts contain lower level of nucleic acids and are rich in vitamins and minerals.³ The cultivation of microorganisms depends on the economic feasibility of producing a protein product which can compete with the conventional preparations of foods and feeds in the market. Baker's yeast (*Saccharomyces cerevisiae*) was grown in an aerated molasses ammonium salt medium for feed use in Germany during World War I (1914-1918). Aerobic yeasts, particularly *Candida utilis* (Torula yeast), were produced in Germany for food and feed during World War II (1939-1945).⁶

Numerous reviews and symposia describe the development of processes for utilizing raw materials, including simple sugars, starches, cellulose, agricultural and food processing wastes as carbon sources by yeasts and other microorganisms.¹

2. Experimental

2.1 Preparation of Yeast Powder

The *Saccharomyces cerevisiae* strain was grown in 10 l of the minimal medium⁴ containing 50g l^{-1} sucrose as the carbon source. The containers were aerated using a suction pump. The cells were harvested in the late exponential phase, usually after 18h, by centrifuging at 2460g for 15 min in a MSE refrigerated centrifuge at 4°C. The packed yeast cells were washed with normal saline and centrifuged again under same conditions. The cells were autoclaved at 103k Pa for 15 min and oven dried at 80°C for 16h. This was powdered using a micro mill.

2.2 Feeding of Rabbits

Young white 3 months old New Zealand rabbits were fed with a low protein diet containing 50% rice bran and 50% rice flour (7% protein) and adequate amounts of vitamins and minerals (Table 1). The vitamins and minerals were incorporated into the diet by mixing 10g of "Premix" which is commercially manufactured and sold as animal food. The rabbits were fed at the rate of 80g/diet/animal/day and water *ad libitum* for 4 weeks. They were then divided into four groups and each group had 4 rabbits. Low protein group animals were continued on low protein (7%) diet while the control group received a mixed high protein diet (16% protein).

Table 1. Composition of the diets fed to rabbits.

Group	Ingredients in diet	Diet (w/w) (%)	Protein (%)	Total Protein (%)
Low Protein	Rice flour	50	3.5	7
	Rice bran	50	3.5	
Control	Sorghum	30	2.7	16
	Rice bran	13.3	0.9	
	Rice flour	12	0.8	
	Soyabean meal	9	3.4	
	Fishmeal	7	2.6	
	Gingely meal	9	2.5	
	Milk powder	2	0.6	
	Coconut milk	11	2.2	
Yeast	Rice flour	40	2.5	16
	Rice bran	40	2.5	
	Yeast powder	20	11	
Casein	Rice flour	45	3	16
	Rice bran	45	3	
	Casein	10	10	

Note : 1) 80g diet was given/animal/day with 100g of cabbage.

2) The animals were supplied with adequate vitamins and minerals and water *ad libitum*. The minerals & vitamins were incorporated by mixing 10g of "Premix" which is commercially manufactured and sold as animal food.

Yeast group was fed on a rice diet (5% protein) supplemented with 11% yeast protein, while casein group received rice diet (6% protein) supplemented with casein (10%). Except the low protein group that received a 7% protein diet the rest of the groups had 16% protein in their diets (Table 1). All rabbits were supplied with adequate amounts of vitamins, minerals and water *ad libitum*. All rabbits in the different groups were maintained in their respective diets for a period of 8 weeks.

2.3 Body Weight and Serum Protein Levels

Body weights of these rabbits were recorded at ten day intervals. Blood samples from an ear vein were taken at 10 day intervals and analysed for

total protein by Lowry's method⁵ and albumin by bromocresol green (BCG) dye binding method.² Globulin levels were determined by the difference between the total protein and albumin.

2.4 Postmortem and Histopathological Studies

Rabbits from all four groups were anaesthetized, the body cavity opened and the internal organs examined. The liver and kidney were excised and prepared for histological examinations. Specimens from the liver and kidney were fixed in 10% formal saline for embedding in paraffin. Sections (4 μ m in thickness) were cut using a microtome (Shanton) and stained with haematoxylin and eosin (H & E) stain.

3. Results and Discussion

3.1 Weight Gain

Table 2 shows the weight gain of the rabbits. The results were statistically analysed by Analysis of Variance (Table 3). The observed F (F_{fratic}) value was greater than the table (F) value. Hence the H₀ null hypothesis was rejected and H₁ hypothesis (mean effects due to all the diets levels are not the same) was accepted. To find out the main effects of the diet further statistical analysis (Student 't' distribution) was done. From this analysis (Table 3) it was concluded that the mean effect due to low protein diet was lower than the mean effects due to control (μ_2), yeast (μ_3) and casein (μ_4) diets. But the mean effects due to control (μ_2), yeast (μ_3) and casein (μ_4) were the same. Similar results were obtained by the feeding studies of *Candida lipolytica* and *Candida tropicalis* grown on hydrocarbon with broiler chicks.⁷

Table 2. Weight gain of the rabbits.

Type of diets	Weight gain (g) / week		
	1st	2nd	3rd
Low	50	100	125
	35	265	35
	100	50	5
	50	150	5
Control	275	200	225
	350	225	0
	290	155	35
	300	150	230
Yeast	275	215	60
	175	225	75
	200	380	40
	300	180	100
Casein	200	250	50
	175	175	100
	220	230	155
	200	200	150

Table 3. The weight gain of the rabbits was analysed by analysis of variance and student 't' distribution.

The hypothesis

H(0): The mean effects due to all the diets levels are the same.

H(1): The mean effects due to all the diets levels are not the same.

ANOVA TABLE

Source	DF	SS	MS	F
DIETS	3	1.0785ES	35951	4.8
Error	44	3.3296ES	7567.3	
Total	47	44.08012		

F Test (Comparison of all the population means).

Observed value for H(0) 4.8

Theoretical value F (3,44) at 5% significant level 2.84

Hence H (0) hypothesis was rejected and H (1) accepted.

MAIN EFFECTS

Diet level	Mean	Count	S.E
1	80.833	12	25.1
2	202.917	12	25.1
3	185.417	12	25.1
4	175.417	12	25.1

Note : Diets level 1, Low protein : 2, Control : 3, Yeast : and 4, Casein.

Hypothesis (H ₀)	Hypothesis (H ₁)	Calculated t value $\frac{ \mu_1 - \mu_2 }{SE}$	Table Value (t ₄₄ , 0.05)	Conclusion
$\mu_1 = \mu_2$	$\mu_2 > \mu_1$	4.863	1.684	H ₁ accepted
$\mu_2 = \mu_3$	$\mu_2 > \mu_3$	0.697	1.684	H ₀ accepted
$\mu_3 = \mu_4$	$\mu_3 > \mu_4$	0.398	1.684	H ₀ accepted

Final Conclusion - $\mu_1 < \mu_2 = \mu_3 = \mu_4$

- (i) The mean effect due to low protein (μ_1) is lower than the mean effects due to Control (μ_2), Yeast (μ_3) and Casein (μ_4).
- (ii) The mean effects due to Control (μ_2) Yeast (μ_3) and Casein (μ_4) are the same.

3.2 Serum Protein Levels

Table 4 gives the blood serum protein levels. Total serum protein and albumin levels in all the four groups were similar and there were no significant differences between the groups. This could be due to all the groups getting more than the minimum amount of protein per day in their diets.

Table 4. Serum Protein Levels in Rabbits.

Group	Total Protein (g/dl) \pm S. D	Albumin (g/dl) \pm S. D	Globulin (g/dl) \pm S.D
Low Protein	7.00 \pm 0.05	4.40 \pm 0.14	2.60 \pm 0.04
Control	7.05 \pm 0.04	4.40 \pm 0.12	2.70 \pm 0.05
Yeast	7.10 \pm 0.05	4.40 \pm 0.11	2.70 \pm 0.03
Casein	6.95 \pm 0.10	4.05 \pm 0.04	2.80 \pm 0.03

Note : No significant changes in the serum protein levels ; serum protein levels were analysed at 10 days interval.

S.D - Standard deviation.

3.3 Postmortem and Histopathological Studies

All the animals appeared healthy and there were no observable changes in the internal organs. Sections from the liver of the rabbits fed with yeast proteins (Figure 1) showed normal architecture. There were no evidence for parenchymal cell necrosis.

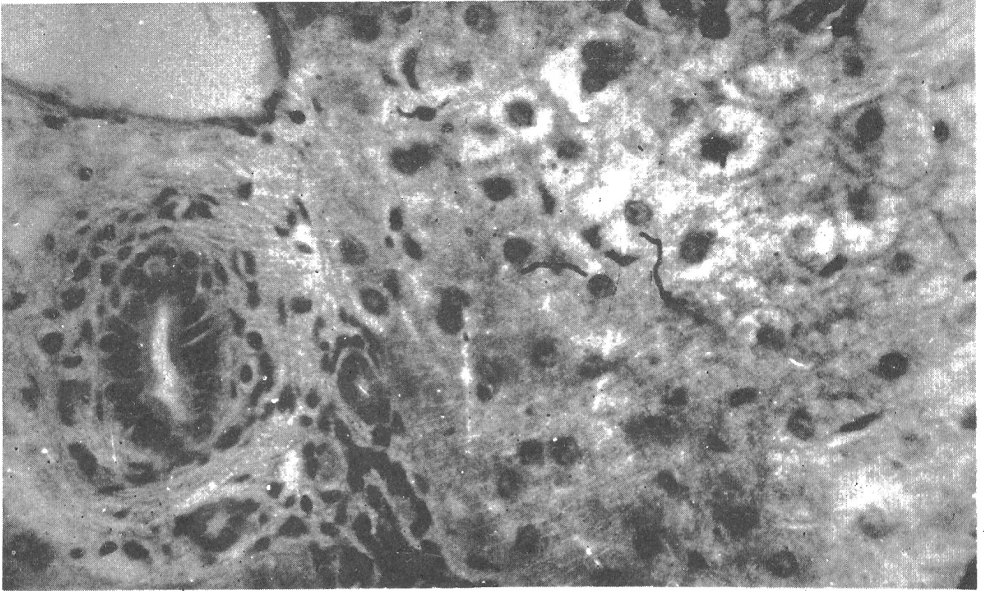


Figure 1. Section of the liver of the rabbit on a yeast protein diet (16%) stained with H & E.

Microscope enlargement x 100

on printing 36 : 24 mm enlarged to 127 : 82 mm.

However, the portal tracts contained a moderate to heavy infiltration of lymphocytes together with occasional plasma cells. The central veins and arteries did not show any abnormalities. The kidney section (Figure 2) showed no abnormality.

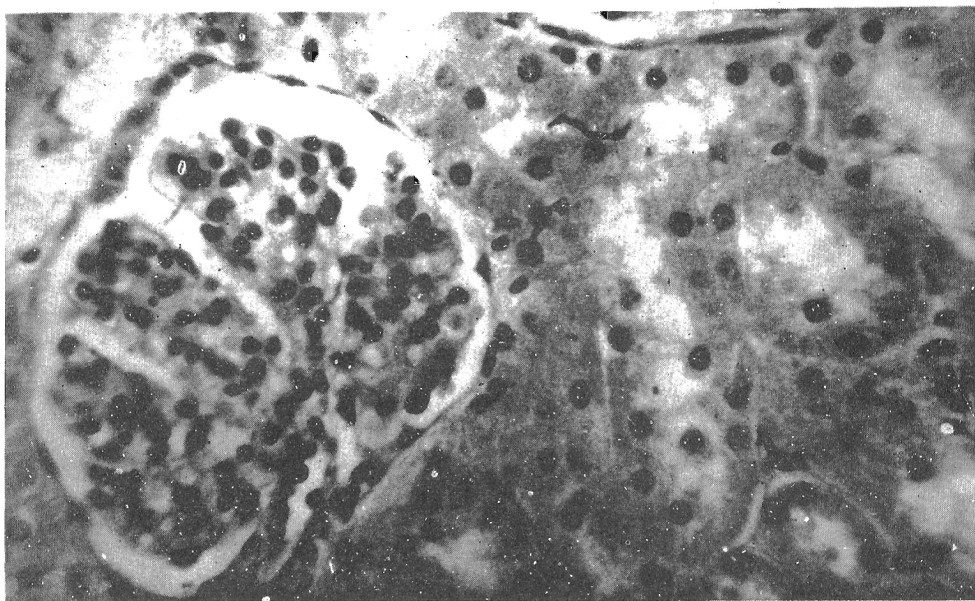


Figure 2. Section of the kidney (through glomerulus) of the rabbits on yeast protein diet (16%) stained with H & E.

Microscope enlargement x 100

on printing 36 : 24 enlarged to 127 : 82 mm.

This shows that there are no deleterious substances in the yeast protein produced by strain Y_{18} at 11% concentration. Further studies have to be carried out with increased supplementation of yeast protein to detect any toxic compounds that may be present in lower amounts.

Gastro-intestinal disturbances usually arise by the consumption of living microbial cells which can survive gastric acidity and which on entering the intestine can multiply and ferment carbohydrates liberating CO_2 and toxic amines. They may also utilize B group vitamins at the expense of the host. All these effects are prevented by autoclaving at $120^{\circ}C$ for 15 min before incorporating into feed.

4. Conclusion

Feeding trials on rabbits using *S. cerevisiae* strain Y₁₈ shows, that the strain has no deleterious substances and it appears that this yeast diet is comparable to the good quality casein.

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