STUDIES ON THE EFFECT OF PROBIOTIC SUPPLEMENTATION TO COMPLETE RATIONS ON THE GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND RUMEN ENVIRONMENT IN NELLORE BROWN SHEEP

By

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This is to certify that the thesis entitled **"STUDIES ON THE EFFECT OF PROBIOTIC SUPPLEMENTATION TO COMPLETE RATIONS ON THE GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND RUMEN ENVIRONMENT IN NELLORE BROWN SHEEP"** submitted in partial fulfillment of the requirements for the degree of **"MASTER OF VETERINARY SCIENCE"** of the Achrya N.G.Ranga Agricultural University, Hyderbad, is a record of the bonafide research work carried out by **Mr.T.NAGAMALLESWARA RAO** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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ABSTRACT

Probiotics improve weight gain by improving nutrient availability. An attempt was made in the present ivestigation to study the effect of supplementing *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* (yea-sacc[1026]) individually and as combination of both to complete rations for sheep on voluntary feed intake, growth rate, feed efficiency, nutrient digestibility, N balance and rumen environment.

Three experiments in completely randomized design were conducted to evaluate the complete rations without or with the supplementation of the probiotics. A complete ration with 60:40 roughage to concentrate ratio was formulated with groundnut haulms (60), maize grain (20), groundnut cake (13.5), deoiled rice bran (3.5), mineral mixture (2) and salt (1.0%) and treated as control (CR-I). *Lactobacillus acidophilus* 1g (CR-2), yea-sacc[1026] 10g (CR-3) or *L. acidophilus* 0.5 g + yea-sacc[1026] 5g (CR-4) was supplemented to the control ration and treated as

experimental rations. The chemical composition of complete ration was DM, 91.3; OM, 79.4; CP, 13.9; CF, 21.9; EE, 2.5; TA, 11.9; NFE, 49.9; AIA, 7.9; Ca, 1.9; P,0.86 percent . The cell wall constituents were NDF, 43.3; ADF, 31.9; ADL, 9.8; Hemicellulose 11.3 and cellulose, 22.1%.

The results of the growth experiment with Nellore brown lambs (14.11 ± 0.53kg) fed for 70 days revealed that the DMI (g), ADG(g) and EFU for the lambs fed complete rations 1 to 4 were 1021. 101, 11.3; 996.0, 119, 9.5; 1183. 157, 8.3 and 998, 135, 8.1, respectively. Highest ($P < 0.05$) ADG was observed in CR-3 followed by CR-4, CR-2 and CR-1. The difference between CR-1 and CR-2 was significant ($P < 0.05$). Higher DMI might be the reason for higher growth rate in CR-3. Highest feed efficiency ($P < 0.05$) was observed in CR-4. However, the difference between CR-2, CR-3 and CR-4 was non significant. The feed cost per kg live weight gain were Rs.35.65, 30.87, 30.25 and 28.1, respectively, for CR-1 to CR-4.

The result of the second experiment with 16 Nellore brown rams (25.6kg ± 0.57) revealed that the digestibilities (%) of nutrients for complete rations 1 to 4 were DM, 66.9, 60.1, 70.7, 69.9; CP,60.7, 72.9, 78.6, 73.5; CF, 44.7, 50.8, 59.8,54.3; EE, 77.3, 80.1, 86.1, 82.7 and NFE, 70.3, 73.4, 80.4, 74. The digestibility (%) of cell wall fractions were 46.3, 52.3, 60.0, 54.3, for NDF, 44.9, 52.7, 60.2, 50.0, for ADF, 52.2, 57.4, 62.4, 60.1 for Hemicellulose and 49.4, 55.9, 59.5,56.4 for cellulose respectively for complete rations 1 to 4.

The digestibility (%) of CP in CR-1 was significantly lower ($P < 0.01$) than in CR-2, CR-3 or CR-4. Higher number of proteolytic bacteria might be the reason for higher protein digestibility in probiotic supplemented ration. The CF digestibility in CR-3 was higher ($P < 0.01$) than CR-1 which might be due to higher rate of fibre digestion and improved fermentation efficiency in yea-sacc supplemented rations. The increase in the digestibility of cell wall fractions in CR-3 ($P < 0.01$) than CR-1 might be due to increase in the number of cellulolytic bacteria.

The N retention (g/d, g/w $_{kg}^{0.75}$/ d or percent of intake) was significantly lower (P<0.05) in CR-1 (9.9,0.89,42.9) compared to CR-2 (15.5, 1.4, 54.8), CR-3 (16.4, 1.5, 63.1) or CR-4 (15.3,1.4,56.8). This might be due to stimulated proteolytic bacterial count in probiotic supplemented rations. Calcium intake (g/d) and retention (g/d) were 19.2, 2.74; 23.5, 3.1; 23.4, 3.5 and 22.6, 2.9. While P intake (g/d) and retention (g/d) were 8.9, 2.97; 10.9, 3.3; 10.8,4.1 and 10.5, 3.6, respectively for CR-1 to CR-4. The nutritive value in terms of DCP(%), TDN(%), DE and ME (Mcal/Kg) were 9.3, 58.4, 2.6, 2.1; 10.2, 62.4, 2.8, 2.3; 10.9, 69.7, 3.1, 2.5 and 10.2, 64.0, 2.8, 2.3, respectively, for CR-1 to CR-4.

A third experiment using 16 fistulated rams (23.9 ± 0.29 kg) was conducted to study the rumen metabolic profiles. The average value of p^H for 0,2,4,6,8 and 12 h post feeding intervals were 6.01 ± 0.10, 6.02 ±0.08, 6.19 ± 0.12 and 6.18 ± 0.13 for complete rations 1 to 4, respectively. The differences between the treatments were non-significant. The rumen NH_3 - N at different post feeding intervals among the complete rations was non-significant. Significantly higher (P<0.01) TVFA production (mm/l) was observed in CR-3 at 2(190.6), 4(157.9) and 6h(135.9) compared to CR-1 (143.0, 129.5, 100), CR-2 (153.6, 129.3, 112.4) or CR-4 (144.4, 130.8, 120.4) .Yea-sacc supplementation might have increased the values in CR-3 or 4. The total bacterial count in CR-4 (64.5 x 10^9 was significantly higher (P<0.01) than CR1 (36.0 x 10^9). The bacterial count of CR-2 (54.6 x 10^9) and CR-3 (61.9 x 10^9) were not different from CR-4. Supplementation of yea-sacc or yea-sacc + *L. acidophilus* have significantly increased the bacterial numbers.

It may be concluded that yea-sacc^{1026} or yea-sacc^{1026} + *L.acidophilus* supplementation to complete rations are beneficial as they have improved dry matter intake, nutrient utilization and growth rate leading to reduced cost per Kg live weight gain in Nellore brown sheep.

ABBREVIATIONS USED

ADF	..	Acid detergent fibre
ADG	..	Average daily gain
ADL	..	Acid detergent lignin
AIA	..	Acid insoluble ash
CA	..	Calcium
CF	..	Crude fibre
CM	..	Centimeter
CP	..	Crude protein
CR	..	Complete ration
CWC	..	Cell-Wall constituents
DCP	..	Digestible crude protein
DE	..	Digestible energy
DM	..	Dry matter
DMB	..	Dry matter basis
DMC	..	Direct microscopic count
DMI	..	Dry matter intake
DORB	..	Deoiled rice bran
EE	..	Ether extract
EFU	..	Efficiency of feed utilization
g	..	gram
g/d	..	grams/day
GNC	..	Groundnut cake
h	..	Hour
HC	..	Hemicellulose
kg	..	Kilogram

LSD	..	Latin square design
M	..	Million
Mcal	..	Mega calories
ML	..	Milli litre
MF	..	Microscopic facor
ME	..	Metabolizable energy
N	..	Nitrogen
NH_3-N	..	Ammonia Nitrogen
NDF	..	Neutral detergent fifbre
NFE	..	Nitrogen free extract
SRL	..	Strained Rumen Liquor
TA	..	Total ash
TCA	..	Trichloro acetic acid
OM	..	Organic matter
P	..	Phosphorus
TDN	..	Total digestible nutrients
W/W	..	Weight by weight
$W_{Kg}^{0.75}$	..	Metabolic body weight
YC	..	Yeast culture
µ m	..	Micro meters

CHAPTER I

1 INTRODUCTION

Sheep and goats are the most important ruminant species of economic value to the small and marginal farmers and landless labourers in India. The FAO estimates of 1994 indicate that there are 44.8 million sheep in India constituting 4% of the total sheep population of the world. By 2000 A.D. the projected sheep population is expected to be 51 million. Among the non-genetic factors affecting productivity in small ruminants, feeding and nutrition is the principal determinant of performance (Devendra, 1990).

In India sheep are managed in a traditional method of production system mainly based on grazing. There is need to popularise intensive system of management for sheep due to continuous depletion of grazing land. It is also necessary to economise the feeding under intensive system of management utilizing various. types of agro-industrial by product feeds and crop residues (Pradhan, 1997).

Complete feeds have particular application in situation where crop residues, agro-industrial by-products and non-conventional feeds are abundant. Their development ensures that a suitable feed ingredient can be blended to enable a balanced supply of nutrients to animals in a manner that those can be used efficiently.

A great deal of research has dealt in recent years with manipulation of microbial ecosystem of the rumen to improve production efficiency by ruminants.

Probiotics are among feed additives that offer potential as modifiers of ruminal fermentation. A probiotic is a live microbial feed supplement that improves the intestinal microbial balance of the host animal (Fuller, 1989). Naturally occurring live microorganisms include bacteria, fungi and yeast (Chiquette, 1995).

Lactic acid bacteria from part of the natural microbial population of the digestive tract of animals and are regarded as probiotics. They create an environment to enhance digestion and to stimulate immunity. Addition of viable yeast culture to diets has been shown to stabilize and stimulate rumen digestion specially fermentation of fibre. A combination of viable yeast culture and lactic acid producing bacteria has been shown effective in influencing gut microbial populations to help competetively exclude coliforms. Hence, an attempt was made with the following objectives.

To study the effect of supplementing *Lactobacillus acidophilus/ Saccharomyces cerevisiae* (Yea-sacc), a combination of Yea-Sacc plus *L.acidophilus* to complete rations for sheep on

1. Voluntary feed intake, growth rate and feed efficiency.
2. Nutrient digestibility and nitrogen balance and
3. Rumen environment (such as pH, VFA, NH_3-N, bacterial count) in Nellore brown sheep.

CHAPTER II

2 REVIEW OF LITERATURE

Feed additives have been used successfully to manipulate rumen microbial activity. Recently dietary supplementation of *probiotics* like *Yea-sacc* and *Lactobacillus acidophilus* were used in livestock feeding and obtained varied results.

Effect on growth

Mudgal *et al.* (1995) in their study on kids observed that crude protein and crude fiber were digested at higher rate on supplementation of *S.cerevisiae* compared to *L.acidophilus* but the combination of these two in equal proportions excelled overall individual addition. The addition of individual organisms increased growth rate and feed conversion efficiency over the control, but combination of both exhibited better results.

Chesson and Wallace (1996) reported that supplementing ruminant feeds with microorganisms can assist in establishing a healthy rumen microbial flora. In young animals, Lactic acid producing bacteria can inhibit colonization by pathogenic microorganisms by reducing pH, producing secondary metabolites harmful to pathogens and possibly by competing for nutrients and colonization sites. In older cattle augmenting feeds with microbes can increase growth rate.

Birch *et al.* (1994) studied the growth and carcass characteristics of newly received feeder lambs fed with *probiotics* and Vit E. In this study feeder lambs given *probiotics* were more feed efficient than control.

Podoshibyakin *et al.* (1991) observed that feeding preparation of propionibacterium and *Lactobacillus acidophilus* containing vitamin of group B, and Cu, Zn, Mn, Fe, Co, Ca, P and Carotenoids fed to ewes during winter at the rate of 0.45 g/kg body weight daily for 21 days before lambing and 20 days after wards has reduced the content of ketone bodies in blood and urine and the pH of rumen fluid. Further it improved the weight gain of the lambs.

Higgin Botham and Bath (1993) studied the effect of Direct - fed microbial feed additives, *Lactobacillus acidophilus* for calves and observed that calves given viable and non-viable microbial product tend to have higher gains in early stage of growth.

Abe *et al.* (1995) found that oral administration of calves with *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus* improved body weight gain and feed conversion efficiency as compared with untreated controls. The frequency of the occurrence of diarrhoea was decreased in the groups fed *probiotics*, however, there were no differences between calves fed *B.pseudolongum* and those fed *L.acidophilus*.

Grudkov *et al.* (1985) reported that when whey enriched by fermentation with *Lactobacillus acidophilus* was used in feed preparation for calves and lambs it helped to control gastro intestinal disorders and increased live weight gain.

Shrivanova and Machanova (1990) studied the lactic acid fermentation bacterium, *Lactobacillus acidophilus* of rumen origin in the cause of calf rearing. The trial was performed with 2 groups of six calves each in suckling period (10-60 days of age) and weaning (61-90 days of age). The calves of test group were given ploquindox (60 ppm) until the age of 60 days and they were given 3 applications of culture of *Lactobacillus acidophilus* (7/B strain), 30 ml after receiving first colostrum and 50 ml on the 3rd day and 10th day of age. The average daily gain (ADG) in the period of suckling were 0.600 ± 0.040 kg in the control group and 0.628 ± 0.044 kg in the test group which did not differ significantly.

Hussain (1991) reported that inoculation of *L.acidophilus* had accelerating effect on time of onset of puberty in male buffalo calves and observed that the inoculated animals reached puberty 2-3 months earlier than control.

Cruwagen *et al.* (1995) conducted an experiment to evaluate the effect of dietary supplementation with *Lactobacillus acidophilus* on young calves and reported that the daily gain during second week was affected.

Hamza *et al.* (1996) studied evaluation of diet containing *Lactobacillus acidophilus* on performance of young calves. Performance of calves was not significantly affected by treatment, although body weight (BW) increased mostly during weeks 7-9 for calves fed the mixed Lactobacilli and in weeks 10-12 for calves fed *L.acidophilus* 27 SC.

Adams *et al.* (1981) reported influence of viable Yeast cultures, sodiumbicarbonate and monensin on liquid dilution rate, rumen fermentation and feed lot performance of growing steers and digestibility in lambs. Average daily gain (ADG) and feed conversion ratios were higher in Yeast fed steers.

Rouzbehan *et al.* (1994) studied the effect of dietary inclusion of Yeast culture on growth and rumen metabolism of lambs given diets containing unground, pelleted, molasses, dried sugar beet pulp, and barley in various proportions. In this study Yea-sacc supplemented with barley fed lambs showed higher growth, DM intake, feed conversion and rate of digestion and superior feed conversion.

Giger - Reverdin *et al.* (1996) studied the effect of *probiotic* Yeast in lactating ruminants and observed interaction with dietary nitrogen levels in 28 dairy goats and increased production by *probiotic* Yeast.

Kmet *et al.* (1993) reported that fungal *probiotics* based on *Saccharomyces cerevisiae* and *Aspergillus oryzae* are rapidly gaining acceptance as a means of improving productivity in adult ruminants, and dietary Yeast improved the performance of calves and lambs in terms of feed intake and live weight gain.

Gunther (1990) studied Yea-sacc ^{1026} success under German dairy conditions and reported that body weight changes and feed intakes between the control and Yea-sacc^{1026} fed groups were not significantly different. However, the average feed intake in Yea-sace fed animals was slightly higher over the control group of animals.

Sengupta *et al.* (1994) studied the effect of nutri-sacc (Yea-sacc[1026] + protected protein) on performance of lactating buffaloes and observed that the supplementation of Yea-sacc increased the milk yield but feed cost per kg fat-corrected milk yield were higher with the unsupplemented diet.

Mir and Mir (1994) reported effect of live Yeast culture and lasolacid supplementation on silage, corn silage and high - grain diets sequentially. Trials were conducted to determine the effect of supplementing feed lot diets with live-yeast culture (YC) Lasolacid (LAS) or YC+LAS on intake, growth and carcass characteristics on stress. Dietary additives in 96% corn silage or 75% dry-rolled barley-based diets increased ($P<0.05$) final weights and carcass weights of steers.

Mir and Mir (1994) studied the effect of addition of live Yeast *(Saccharomyces cerevisiae)* on growth and carcass quality of steers fed high-forage or high-grain diets and on feed digestibility and *in situ* degradability. In this study Yea-sacc (*Saccharomyces cervisiae)* supplementation increased digestibility of high dry-rolled barley grain for DM (65.4 vs 54.9), CP (60.0 vs 44.9) NDF (374, vs 22.1), ADF (40.3 vs 35.7) and daily intake g/kg (115.5 vs 106.8) than those fed high-forage diets.

Effect on Nutrient digestibility

Cole *et al.* (1992) observed influence of Yeast culture on feeder calves and lambs. Calves fed yeast culture tended to maintain heavier weight and higher DMI. Lambs fed yeast culture had greater DMI. Lambs fed yeast culture had greater ($P < 0.08$) N balance and tended to have greater Zn and Fe balance than control lambs.

Fisher *et al.* (1984) observed that addition of dried culture of *Lactobacillus acidophilus* as a silage additive to silage of cocksfoot grass/white clover resulted in higher digestibility of organic matter, acid detergent fibre and nitrogen in sheep.

Arambel (1988) reported effectiveness of yeast *(Saccharomyces Cerevisiae)* and fungal cultures *(Aspergillus oryzae)* in ruminant rations to improve the rumen fibre digestion, and concluded that such cultures could influence rumen fomentation, but the mode of action was not clearly understood.

Chademana and Offer (1990) studied the effect of dietary inclusion of yeast culture on digestion in sheep. Six mature sheep each fitted with a rumen cannula were assigned to six diets in a 6x6 latin - square design experiment to examine the effects of a yeast culture on ruminal metabolism, rumen liquid out flow rate, fiber digestion in the rumen and overall nutrient digestibility. The yeast culture (YC) was a commercial product composed of a yeast (*Saccharomyces cerevisiae*). Rumen pH, rumen liquid outflow rate, rumen ammonia concentration, total volatile fatty acids concentration and molar proportions of acetate, propionate and butyrate were not significantly affected by the inclusion of YC ($P < 0.05$) supplement.

Michael watkins (1993) reported yeast culture as a unique feed ingredient that improves the feeding value of feeds. He reported it as a very palatable feed ingredient and can increase rumen bacterial concentrations, results in greater feed utilization and improve animal production. His research indicated that dairy goat performance could be enhanced by feeding yeast culture.

Roa *et al.* (1997) studied the effect of fibre source and yeast culture on digestion and environment in the rumen of cattle fed alfalfa hay, coffee hull, or corn stalk with or without yeast culture *(Saccharomyces cerevisiae[1026])*. Addition of YS to alfalfa hay increased ($P < 0.05$) potentially digestive NDF (70.2 vs 56.1%) and potentially digestible CP (79.5 vs 71.4 %). For sorghum grain the YS increased ($P < 0.05$) potentially digested DM (94.7 vs 90.7%) potentially digested NDF (66.4 vs 50.7%) and potentially digested CP (85.7 vs 81.2%). Addition of Yea -Sacc[1026] increased ($P < 0.05$) NH_3-N ruminal concentration (Mg per 100 ml) for alfalfa hay (36.3 vs 27.7%) for coffee hulls (36.4 vs 26.9) and corn stalk (36.5 vs 28.2%) YS increased ($P < 0.05$) VFA concentration for alfalfa hay (88.2 vs 74.3 mm) and coffee hulls (69.6 vs 51.7 mm).

Arambel and Kent (1990) reported effect of yeast culture on nutrient digestibility and milk yield response in early to mid lactation dairy cows. In this study 20 Holstein dairy cows in early lactation were allocated equally to 1 - 2 treatments on the basis of age. All animals were given a total mixed ration, the ration for the treatment groups was top dressed with 90 g of yeast culture *(Saccharomyces cerevisiae)* per day. Treatment period was 10 wk. Mean daily DM intake, and body weight were not significantly affected by the treatment. No significant differences in digestibility were observed between treatment groups for CP, ADF and NDF.

Kamalamma *et al.* (1996) studied effect of feeding Yeast culture (yea - sacc[1026]) on rumen fermentation *in vitro* and production performance in crossbred dairy cows. In this study rumen fluid samples taken from cows fed on control diets (yeast unadapted, YU) or diets supplemented with yea-sacc[1026] (yeast adapted YA) were incubated *in vitro* with finger millet (*Eleusine coracana*) straw (FMS) or a commercial cattle feed (CCF). The 24 h cumulative gas production and digestibility of NDF and ADF were not different with YU and YA rumen inoculum for FMS and CCF. There were no differences in DM intake and body weight gain.

Kang *et al.* (1992) observed that Holstein dairy bulls about 120 kg when fed on concentrate diets without or with 0.25% live yeast culture *(Saccharomyces cerivisiae)* with 0.1% *probiotic A (Clostridium butyricum)* or *0.15% probiotic B (Lactobacillus acidophilus*) complex (*Probiotics + enzymes* + Yeast culture) during growing and finishing period to final weight of about 548 kg. Dry matter intake per kg live weight gain were highest ($P<0.05$) with live yeast diet. Daily gain, and nutrient dry matter (DM) crude protein (CP) total digestible nutrients (TDN) intake per kg live weight, were higher ($P < 0.05$) than control. Live yeast culture reduced time to market weight compared with other additives. Digestibility of DM, CP and CF was increased by additives.

Olson *et al.* (1994) in their study used twelve beef steers (368 ± 25.3 kg) and four beef heifers (559 ± 79.5 kg) fitted with ruminal cannulae to evaluate effect of yeast culture (YC) and advancing season on dietary chemical composition, intake and *insitu* CP and NDF degradation. Treatments were control and YC supplementation (28.4 g $steers^{-1}$ d^{-1} dosed ruminally). Steers grazed from late June to early November 1991 on mixed grass prairie. Experimental periods consisted of 10 d adaptation and 13 d collection. Forage samples collected from Yea-Sacc supplemented steers had greater ($P < 0.10$) soluble N and *in vitro* OM disaepperence than forage from controls thorugh out the grazing season.

Plate *et al.* (1994) reported that addition of yeast culture with *saccharomyces cerevisiae* improved NDF fed oat straw based diet. It also increased ruminal protozoal population.

Moloney and Drenan (1994) studied the influence of basal diet on the effects of yeast culture on ruminal fermentation and digestibility in steers. Inclusion of yeast culture in diet containing high fibre/low protein (81.4 vs 79.6 mm/l) Increased VFA concentration. Digestibility of dry matter, organic metter and crude protein (CP) were higher (P<0.01) and acid detergent fibre was lower (P < 0.05) on diet containing low fibre and high protein (107.3 vs 110.3 mm) with yea-Sacc supplementing. It was concluded that dietary inclusion of yeast culture had a small influence on rumen fermentation parameters and *in vivo* digestibility but that its effect on nitrogen metabolism appeared to be dependent on nitrogen content of the basal diet.

Chiquette (1995) studied the effect of microbial supplements on ruminal and total tract digestibility, rumen fermentation, bacterial counts and bacterial colonization of fibrous feed in eight ruminally cannulated steers. The addition of *Aspergillus oryzae* in combination with *Saccharomyces cerevisiae* stimulated (P < 0.01) ruminal fermentation with high concentration of acetate (P < 0.01), propionate (P < 0.07) and total VFA (P < 0.01) when sampled prior to feeding. Ruminal pH was lower (P < 0.01) in animals receiving *Saccharomyces cerevisiae* combined with *Aspergillus oryzae*. The addition of direct fed microbials did not affect bacterial counts or bacterial colonization of roughage in the rumen.

Wohlt *et al.* (1991) reported yeast culture to improves the intakes and nutrient digestibility and performance by dairy cattle during early lactation. Digestibilities of protein and cellulose were improved in cows fed supplemental yeast, 6 Wk of lactation and higher average milk yield through 18 wk of lactation compared with control cows.

Effect on Nitrogen Retention

Jassim-Ram *et al.* (1985) in an experiment on 42 merino weathers weighing 35 kg given 920 g DM from roughage daily with out or with 24, 48, 72 g yeast *Saccharomyces cerevisiae* and 90 g crude protein or casein supplement reported that giving yeast at 24 g or 48 g did not significantly affect body weight or growth rate. However, they observed more N retention than the other groups.

Jassim - Ram *et al.* (1986) reported increased nitrogen retention by supplementing yeast (*Saccharomyces cerevisiae*) in merino sheep rations.

Flint and Wallace (1991) studied manipulating rumen micro organisms and rumen fermentation. It is now feasible to introduce new genetic information into certain species of rumen bacteria. Alterations in rumen fermentation, whether by genetic manipulation or other means may be of considerable environmental importance. Decreased methane production and increased nitrogen retention by ruminants would benefit the nutrition by the animal by retaining more energy and N in the body, and would reduce the contribution of methane to global warming and of nitrogenous excreta to ground water pollution.

Effect on Rumen Fermentation

Gray (1989) reported that the effects of yea-Sacc in the sheep rumen is to keep the acidity near to neutral, which is the optimum for microbial fermentation of fibrous type of foods. A more important effect was the rate of VFA production.

Gray and Ryan (1989) reported elevation of ruminal fluid pH in sheep fed hay or silage added with yea-Sacc culture at the rate of 2.5 g/head/day for at least 7 days. When yea-Sacc was administered at 5g/L level. Further they observed marked increase in volatile fatty acid production during yea-Sacc administration.

Newbold *et al.* (1995) reported that different strains of *Saccharomyces carevisiae* differ in their effect on ruminal bacterial number *in vitro,* and in sheep. A ruminal simulation device (Rusitec) was used to compare the effects of *Saccharomyces cerevisiae* strain NCYC 240, NCYC 694, NCYC 1026, NCYC 1088, and yea-Sacc (a commercial product containing *S.Cerevisiae*) on ruminal fermentation. All treatments simulated total and cellulolytic bacterial numbers. However, the simulation was only statistically significant for *S.cerevisiae NCYC 1026* with total bacterial numbers and *S.cerevisiae* NCYC 240 with cellulolytic bacteria ($P < 0.05$). Increased bacterial numbers were associated with an increase in the rate of straw degradation.

Harrison *et al.* (1988) conducted experiment with fistulated Holstein cows in a randomized block design to examine the effect of yeast culture supplement on ruminal metabolism and digestibility. Cows were fed on a diet of 40% corn silage and 60% concentrate (DM basis). Treatments were control (Supplement without yeast cells) and yeast culture supplement. Treatment periods were 6 WK. Ruminal pH, amonia, molar proportion of acetate and isovalerate and acetate : propionate ratio were lower.

Carro *et al.* (1992b) reported that in a cross over design 4 Friesian cows (640 kg) fitted with rumen and T-shaped duodenal canulae, were fed on grass silage, concentrate 50:50 on DM basis to study the effect of yeast culture (YC) from *Saccharomyces cerevisiae* (10 g/day) on rumen fermentation, digestibility and duodenal flow of nutrients. Rumen pH, ammonia and volatile fatty acids concentrations and molar proportions of acetate, propionate, butyrate and valerate were not effected by YC, but proportions of associates decreased ($P < 0.01$).

Nisbet *et al.* (1990) reported that yea-sacc[1026] filtrate increased production of acetate, propionate, total volatile fatty acids and growth yield of lactate grown cells.

Gombos *et al.* (1995) reported that yea-sacc[1026] (a live yeast culture probiotic) added to the diet of rumen cannulated cows at 10 g/head daily increased pH and decreased concentration of Lactic acid and Ammonia in rumen contents. But the level of response depend on the type of diet and on the roughage : concentrate ratio.

Girard (1996) observed that stimulation of rumen bacteria by specific yeast cultures (yea-sacc[1026]) is important in processes which lead to improved digestive functions. This stimulation is said to be mediated by live yeast cells or heat-labile component found in yeast culture preparations.

Besong *et al.* (1996) studied the effect of yeast product (about 11% DM) produced by a product of riboflavin synthesis incorporated at three concentrations. In a 3 x 3 L.S. design to determine the maximal inclusion rate and aceptability as a feed supplement in diets of lactating cows. Treatments containing (as fed basis) (1) no yeast product (control), (2) 20% dietary yeast product and (3) 40% dietary yeast product. The supplemented dietary yeast product had no effect on ruminal pH. However, the ratio of acetate to propionate decreased and propionate percentage tended to increase as supplementation of the yeast product increased.

Yoon and stern (1996) in an experiment with four lactating Holstein cows fitted with ruminal and duodenal cannulas in a 4 x 4 latin square design to examine the effect of supplemental yeast (*Saccharomyces cerevisiae*) and fungal *(Aspergillus oryzae)* culture on ruminal fermentation, microbial population and nutrient supply to the small intestine. Treatments were arranged in a 2 x 2 factorial as follows: (1) basal diet, (2) basal diet plus 57 g/d of yeast culture, (3) basal diet plus 3 g/d of fungal culture and (4) basal diet plus 57 g/d of yeast culture and 3g/d of fungal culture. yeast culture increased ruminal OM and CP digestion. Fiber digestion in the rumen was similar among treatments. Yeast culture stimulated proteolytic bacterial count. Results from this experiment demonstrated that yeast and fungal cultures could influence ruminal fermentation and microbial population.

Putnam *et al.* (1997) used eight early lactation primiparous Holstein cows fitted with ruminal and duodenal cannulae to study dry matter intake which tended to increase as amount of yeast culture increased. However, yeast culture had no effect on ruminal pH, concentration of NH_3 and volatile fatty acids in ruminal fluid or ruminal digestibility.

Callaway and Martin (1997) studied the effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. Yeast culture filtrate increased the concentration of acetate and total volatile fatty acids that were produced by *Selenomonas ruminantium* (HD_4) and increased the concentrations of propionate and total volatile fatty acids that were produced by S. ruminantium. Collectively these results suggest that yeast culture provides soluble growth factors (ie. organic acids, B.Vitamins and Amino acids) that stimulate growth of ruminal bacteria that utilize lactate and digest cellulose.

Dawson *et al.* (1990) reported effect of two microbial feed supplements containing yeast and *Lactobacilli* on roughage fed ruminal microbial activities. The pH tended to be greater ($P<0.13$) in continuous culture receiving yeast culture supplement than in cultures receiving the unsupplemented diet. Concentration of cellulolytic micro organisms in culture and the rumen of steers receiving supplements containing yeast were 5 to 40 times greater than those observed in cultures of steers receiving the unsupplemented diet. However, neither supplement consistently alter the relative concentration of volatile fatty acids or ammonia in continuous cultures and in the rumen of steers.

Carro *et al.* (1992a) studied influence of yeast culture on the in vitro fermentation of diets containing variable portions of concentrates. Using the rumen simulation technique diets containing a forage : concentrate ratio (DM basis) of 70 : 30 (Low-concentrate) 50 : 50 (medium-concentrate) and (30 : 70 high-concentrate) were incubated without or with yeast culture (YC), (*Saccharomyces cerevisiae* 15 mg/g D.M). Overall addition of YC had a small influence on fermentation of Low-concentrate diet, but molar proportion of volatile fatty acids

(VFA) were affected ($P < 0.05$). When medium concentrate diet was supplemented with YC, methane production ($P < 0.06$); Protozoal concentration ($P < 0.03$) and acetate concentration ($P < 0.01$) were reduced, while butyrate and velarate concentration were increased ($P < 0.01$). Adding YC to high concentrate diet increased DM and NDF degredabilities ($P < 0.01$), VFA production ($P < 0.05$), methane production ($P < 0.07$) and concentration of protozoa ($P < 0.02$) but output of ammonia reduced significantly ($P < 0.10$).

Effect on Total Bacterial Count

Williams and Lyons (1988) studied biochemical mode of action of yeast culture. In this review possible mechanism of action of yeast cultures, defined as the living yeast cell and the medium upon which it was grown in ruminant digestion were discussed. Facultative anaerobes, such as *Saccharomyces cerevisiae* had been found to grow in the rumen and to influence cellulolysis by increasing bacterial number. They observed that they might increase efficiency of rumen fermentation.

Williams (1989) conducted experiments to explain the mode of action of yeast by identifying properties of yeast cell biochemistry that may integrate with rumen metabolism and result in an increased feed intake, increased metabolizability of the diet and or increased protein supply to the duodenum interaction between pH and yeast growth, effects of yeast culture upon cellulolysis, fibre degradation and methane production in the rumen. It was suggested that addition of yea-sacc[1026] to the rumen increased the number of bacteria especially cellulolytic bacteria in the rumen possibly by assisting in H_2 transfer than by allowing increased cellulolysis and reducing losses of H_2 as methane.

Kumar *et al.* (1994) reported the effect of inclusion of live yeast culture (YC), (*Saccharomyces cerevisiae* plus growth medium) in a high concentrate diet given to buffalo (Bubalus bubalis) calves on the rumen microbial population and fermentation pattern and *in sacco* dry matter disappearance of dietary constituents. The number of total bacteria, viable, bacteria, cellulolytic bacteria, amylolytic bacteria and protozoa were increased proportionately. The concentration of total volatile fatty acids particularly at 4h post feeding ($P < 0.01$), acetate ($P < 0.01$) and propionate and the acetate to propionate ratio were higher in the YC group as compared with the control group.

CHAPTER III

3 MATERIALS AND METHODS

The research was designed to asses the effect of supplementing probiotics, *Lactobacillus acidophilus* and *Yea-sacc* [1026] to a complete ration for sheep, on growth performance, rumen environment and nutrient digestibility. A complete ration was formulated with 60:40 roughage to concentrate ratio.

Procurement of Ingredients

Groundnut haulms were procured from villages around Tirupati, Chittoor District. Maize, groundnut cake and deoiled rice bran were purchased from Regional Poultry, Demonstration and Research Farm Chittoor. *Lactobacillus acidophilus* obtained from National Collection of Dairy Cultures Dairy Microbiology Division. NDRI-KARNAL, in freeze dried form and propagated at Dairy Technology Laboratory, College of Veterinary Science, Tirupati. Yea-Sacc[1026] was supplied by vet-care, Bangalore.

Maintanance and Preservation of Bacterial Culture

Lactobacillus acidophilus strain NDRI-III was propagated by using skim milk with 11 percent solids (Yadav *et al.*, 1993). Eleven grams of fresh skim milk powder was dissolved in 100 ml of distilled water, dispersed in test tubes and autoclaved for 10 minutes at 10 PSI and -115°c on the first day. The tubes were left at room temperature. On the second day the skim milk tubes were examined for clots. The tubes without clots were steamed for 30 minutes to ensure the total distruction of bacteria present in skim milk.

The sterilized skim milk tubes were used for propagation of starter culture. The ampule containing the culture was broken and asceptically added to the sterilized skim milk tubes with a pasteur pipette, mixed well and incubated at 37°c for 24 h. The cloted skim milk was labelled as mother culture and from this culture subsequent propagations were made using sterile skimmilk.

The time of incubation was so adjusted to contain the number of viable cells in one gram of culture around $5x10^9$ cells. The bacterial counts were made by standard plate count method (spc). Fresh cultures were propagated with required number of bacteria one day prior to feeding the experimental sheep.

Preparation of Yeast Culture

Yea-Sacc[1026] was obtained from M/s Vet-care Bangalore in 10 gm bolus form. The bolus was powdered using pestle and mortor as per the required :ity. Each bolus contained $25x10^9$ live yeast-cells.

ration of Complete rations

Four complete rations were formulated keeping the roughage to concentrate ratio at 60:40. Groundnut haulms was the sole roughage source. The other feed ingredients in the complete ration were, maize (20%), groundnut cake (13.5%), deoiled rice bran (3.5%), mineral mixture (2%) and salt (1%). Rovimix was added to the rations at 25 g/q. This ration was used as the control ration (CR-I). Complete ration 2,3 and 4 were supplemented with *Lactobacillus acidophilus* one gram, yea-sacc[1026] ten grams, and *Lacicbacillus acidophilus* half a gram and yea-sacc[1026] 5 grams, respectively.

EXPERIMENT - I

Growth Study

To study the effect of probiotics on growth performance a completely randomized experiment was conducted for 70 days using twenty Nellore brown lambs (14.11 ± 0.53 kg). The lambs were divided into four groups of five animals each. They were allotted to one of the experimental rations at random. The lambs were housed in separate pens (2Mx1M) each provided with a waterer and a feeder. The complete rations were offered *adlibitum* twice daily at 8.30 am and 2.30 pm. *Lactobacillus acidophilus* (1g) was added to 100g Complete ration II (CR-2) and mixed thoroughly and fed to the sheep. After consumption of this remaining ration was fed *adlibitum*. In complete ration III (CR-3) yea-sacc[1026] (10g) was added to 100 gm of the feed, mixed thoughly and fed to the sheep. Once this 1s consumed the remaining ration was fed *adlibitum*. For complete ration-IV (CR-4) half a gram of *Lactobacillus acidophilus* and 5g of yea-sacc[1026] were added to 100g of the feed and fed after mixing thoroughly. After consumption of this the remaining portion of CR-4 was fed *adlibitum*. The left overs were weighed on the next morning to calculate the actual consumption of ration by the lambs. The lambs were weighed at weekly intervals before feed was offered in the morning to record the weight gain.

EXPERIMENT - II

Nutrient Utilization and nitrogen balance of rams fed complete rations containing the probiotics.

Metabolism Trail

In a completely randomized design experiment 16 Nellore brown rams (25.06 ± 0.57 kg) were used to evaluate the complete rations for their nutrient digestibility and nitrogen balance. The complete rations 1-4 were the same as those used in Experiment-I.

The rams were confined to individual pens of 2Mx1M with facility for feeding and watering. A 15-day preliminary period and a 7-day collection period were observed. The rams were alloted to one of the complete rations at random and fed *adlibitum* twice daily at 8.30 am and 2.30 pm. The left overs were weighed on the next day morning to evaluate the exact quantity of respective complete rations consumed. The rams were shifted to metabolism cages one day prior to and during the collection period.

The live weight of rams were recorded before the start and at the end of each period prior to offering feed and water. Fresh drinking water was made available at all the times. During each period of metabolism trial faeces voided in 24 h was collected with the help of faecal collection bags, harnessed to the rams. The daily urine output of each animal was measured by collecting in the glass bottles kept at bottom of each metabolism cage. Few drops of concentrate hydrochloric acid were added to each urine collection bottle daily as a preservative. Samples of feed, faeces and urine were preserved for further analysis.

EXPERIMENT - III

Rumen Metabolic Profile Studies

In a third experiment the effect of supplementing probiotics to the complete rations at different levels on the rumen metabolic profiles was studied using four adult (23.95 ± 0.29 kg) permanently fistulated rams. The study was conducted in four trials. An adoptation period of 15 days preceeded to collection period was followed in each period and collection was carried out on 2 consecutive days.

Rumen liquors was collected from fistulated rams at 0 (before feeding) 2,4,6,8 and 12 h post feeding. The collected rumen liquor was filtered through four layered muslin cloth and resultant liquid was designated as strained rumen liquor (SRL). The SRL was drawn into a sterile polythene bottle at each collection. The pH and NH_3-N of SRL was determined immediately. Total bacterial count was done 4h immediately after collection. For this bacterial count the SRL was drawn into sterile screwcap pad test tubes.

One millilitre of saturated mercuric chloride solution was added to each tube to check the microbial activity. The SRL was stored in polythene bottles at sub-zero temperature for analysing the Total Volatile Fatty Acid (TVFA) Concentration.

ANALYTICAL PROCEDURE

Total Bacterial Count

The total number of bacteria present in the SRL collected 4h immediately was arrived by Direct microscopic count (DMC) using modified method of BIS [ISI : 1479 (PART I) - 1960].

The sample of rumen liquor was serially diluted using normal saline dilution blanks to the appropriate dilution so as to ensure the total number of organisms in the microscopic field do not exceed 30 organisms. Using sterile pipette 0.01 ml sample was taken from the appropriate dilution and was mixed with small quantity of Nigrosine strain (Moir 1951) and spread uniformly over the entire 1 square centimeter area from the slide and dried.

The dried film was examined under oil immersion objective by placing one drop of immersion oil on the film. Single organisms were counted on the microscopic field at random from all parts of the film. The number of fields counted was based on the number of bacterial cells in each field. The average number of cells per field was modified by the microscopic factor to give the Direct microscopic count per milliliter.

The microscopic factor (M.F) of the microscope was estimated using stage micro meter with 16 mm objective. The microscopic factor was calculated by using the formula.

Calculation

$$MF = \frac{\text{Area of Smear (mm)}}{\text{Area of Microscopic field } (\pi r^2)} \times \frac{v}{\text{Vol. of sample}}$$

$$= \frac{100 \text{ s.q. mmx7}}{22 \times 0.065 \times 0.065} \times \frac{1}{0.00001\text{m}}$$

$$= \frac{700}{22 \times 0.065 \times 0.065 \times 0.0001}$$

$$= \frac{7,00,00,000}{0.09295} = 7.53093060 \times 10^8 = 7.531 \times 10^8$$

$$= X \times 7.531 \times 10^8$$

X = Average number of Cells for field

pH of the Rumen fluid

pH of the SRL was measured immediately after collection. The SRL was taken in a 25 ml beaker and the pH was recorded with digital pH meter. (Model-DJ-707. DIGISUN Electronics, Hyderabad which was standardized to pH7.0.)

Ammonia nitrogen

Ammonia nitrogen from the SRL was estimated by micro diffusion method of Conway (1957) using mixed indicator (Livingston *et al.*, 1964).

Total volatile fatty acids (TVFA) in rumen fluid

The VFA of rumen liquor were determined with the help of Merkhams distillation apparatus following the method of Barnett and Reid (1956). Two ml of supernatant of centrifuged (3000 rpm for 10 min) SRL was distilled in Merkhams distillation apparatus in the presence of 2ml scarisbrick buffer and 1 ml of 2% caprilic alcohol. Distillate (100 ml) ice crystals and titrate against N/50 NaOH using phenolpthalin as indicator. A blank was run using distilled water in place of SRL.

Calculations : TVFA/ml SRL = A x 0.02/2 meq

A = ml N/50 NaOH Corrected for blank value

Collection of Samples

Feed

Samples of experimental rations fed were collected daily and composited for chemical analysis. The left over feed during each day of the collection period was weighed.

Faeces

Faeces voided by each ram during 24 h was weighed at 8.30 AM and a 5% aliquot was composited in polythene bags and frozen in a deep freez. At the end of each collection period, respective faecal samples were oven dried, ground through medium mesh screen of a Willey mill and placed in airtight-polythene bottles until analysed.

Urine

Urine voided by individual rams during 24 h was measured at 8.30 AM and a 2% aliquot of urine was composited in glass bottles and kept in refrigerator till analysed.

Chemical Analysis

Samples of complete rations, groundnut haulms, maize, groundnut cake and deoiled rice bran were analysed for proximate principles (AOAC 1980) samples of fresh faeces and aliquots of urine were used to determine the nitrogen content. The cell-wall constituents of groundnut haulms, maize, groundnut cake and deoiled rice bran and faeces were determined as per the procedure of Goering and Van Soest (1970). Calcium and phosphorus in feed samples and dung were determined by the methods suggested by Talapatra *et al.* (1940). Calcium and phosphorus in urine were estimated by using the method of Ferro and Ham (1957) and Fiske and Subbarow (1925), respectively.

Statistical Analysis

The data were subjected to analysis of variance (Snedecor and Cochran, 1968) and means were tested for significance by LSD.

CHAPTER - IV

4 RESULTS

4.1 CHEMICAL COMPOSITION OF FEED INGREDIENTS

The chemical composition of different feed ingredients used in the experiment is presented in Table 1.

The Dry matter (DM), Crude Protein, (CP), Ether Extract (EE), Crude Fibre (CF), Total Ash (TA) and Acid Insoluble Ash (AIA) contents of groundnut haulms were 91.20, 9.26, 1.51, 31.59, 11.38 and 8.59 percent, respectively. The DM, CP, EE, CF, TA, AIA content of maize grain were 91.80, 10.29, 3.74, 2.66, 2.31 and 0.7 percent, respectively. The DM, CP, EE, CF, TA and AIA content of Groundnut cake were 91.78, 42.6, 5.68, 6.99, 7.6 and 3.16 percent, respectively. The DM, CP, EE, CF, TA and AIA contents of Deoiled Rice bran were 90.31, 15.62, 1.24, 16.44, 15.58 and 7.22 percent, respectively.

4.1.1 Calcium and Phosphorus Contents of Feed Ingredients

The calcium and phosphorus contents of feed ingredients are presented in Table 1. The calcium and phosphorus contents of groundnut haulms were 1.45 and 0.46% respectively. The calcium and phosphorus contents of maize grain were 0.59 0.34%. Calcium and phosphorus contents GN cake and deoiled rice bran were 0.32, 0.93 and 0.42, 1.55% respectively.

Table 1 Chemical Composition (%) of Feed Ingredients used in Complete Rations

Component	Groundnut haulms	Maize grain	Groundnut cake	Deoiled rice bran
OM	88.62	97.69	92.4	84.38
DM	91.20	91.80	91.78	90.31
CP	9.26	10.29	42.67	15.62
EE	1.51	3.74	5.68	1.24
CF	31.59	2.66	6.99	16.44
TOTAL ASH	11.38	2.31	7.6	15.62
NFE	46.26	81.00	37.06	51.08
AIA	8.59	0.7	3.16	7.22
Ca	1.45	0.59	0.32	0.42
P	0.46	0.34	0.93	1.55

On dry matter basis except for dry matter.

4.2 CELL WALL CONSTITUENTS OF COMPLETE RATIONS

The cell wall constituents of complete rations are presented in Table 2. The percent of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignine (ADL), Hemicellulose and cellulose content of ground nut haulms were 48.90, 40.08, 12.42, 8.82 and 27.66 percent, respectively. The NDF, ADF, ADL, Hemicellulose and cellulose contents of maize grain were 36.45, 12.76, 6.02, 23.69 and 6.74 percent, respectively. The NDF, ADF, ADL. Hemicellulose and cellulose content of groundnut cake were 43.33, 15.09, 6.96, 28.24 and 8.13 percent, respectively. The NDF, ADF, ADL, Hemicellulose and cellulose content of deoiled rice bran were 51.23, 30.60, 7.39, 20.63 and 23.21 percent, respectively.

4.3 INGREDIENT COMPOSITION OF COMPLETE RATIONS

Four complete rations (CR) were formulated (Table 3) supplementing varying levels of probiotics. Complete ration 1 (control) contained groundnut haulms, 60; Maize, 20; groundnut cake 13.5; deoiled rice bran, 3.5 and mineral mixture + salt 3%. The complete rations 2 to 4 were similar in their feed ingredient composition except that *Lacto bacillus acidophilus* was supplemented (1g) to CR-II, yea - sacc [1026](10g)to CR-III and 0.5g *Lacto bacillus acidophilus* +5g yea-sacc[1026] to CR-IV.

Table 2 Cell wall constituents (%) of Feed Ingredients used in the Complete Ration

Component	Groundnut haulms	Maize grain	Groundnut cake	Deoiled rice bran
NDF	48.90	36.45	43.33	51.23
ADF	40.08	12.76	15.09	30.60
ADL	12.42	6.02	6.96	7.39
Hemicellulose (NDF-ADF)	8.82	23.69	28.24	20.63
Cellulose (ADF-ADL)	27.66	6.74	8.13	23.21

Table 3 Ingredient composition (%) of complete rations containing varying levels of probiotics

Ingredient	CR_1 (Control)	CR_2 (Lactobacillus acidophilus 1g)	CR_3 (Yee-Sacc 1026 (10g)	CR_4 (L.acidophilus 0.5g + Yee-Sacc 1026 5g)
Groundnut haulms	60	60	60	60
Maize grain	20	20	20	20
Groundnut cake	13.5	13.5	13.5	13.5
Deoiled rice bran	3.5	3.5	3.5	3.5
Mineral mixture[a]	2.0	2.0	2.0	2.0
Salt[b]	1	1	1	1
Rovimix[c]		25g		
L. acidophilus(g)	-	1	-	0.5
Yee-Sacc[1026](g)	-	-	10.0	5.0

a Mineral mixture contained : Ca 22%, P 9%, Cobalt 0.01%, Copper 0.06%, Iron 0.4%, Iodine 0.2%, Zinc 0.15% and Mn 0.09%.

b Salt contains 22% Nacl

c Rovimix. AB_2 D_3 Vitamin feed supplement. Each gram contained vit A 40,000 I.U., Vit B_2 25 mg; Vit D_3 6000 I.U.

Chemical composition of complete ration (control) on DMB is presented in Table 4. The DM, OM, CP, CF, EE, TA, NFE and AIA contents were. 91.26, 79.39, 13.90, 21.86, 2.46, 11.87, 49.91 and 7.93 percent, respectively. The calcium and phosphorus contents of complete ration were 1.86 and 0.86 percent, respectively.

4.3.1 Cell-wall constituents of complete ration

The cell-wall constituents of complete ration are presented in Table 5. The percent NDF, ADF, ADL, Hemicellulose and cellulose contents were 43.26, 31.92, 9.82, 11.34 and 22.1, respectively.

Table 4 Chemical composition (% of DM) of the complete ration[a]

Component	Control
Dry matter	91.26
Organic Matter	79.39
Crude Protein	13.90
Crude Fibre	21.86
Ether Extract	2.46
Total Ash	11.87
Nitrogen free extract	49.91
Acid Insoluble Ash	7.93
Calcium	1.86
Phosphorus	0.86

a on dry matter basis except for dry matter.

Table 5 Cell-wall constituents of complete ration (% of DM)

Component	Control
NDF	43.26
ADF	31.92
ADL	9.82
Hemi cellulose (NDF-ADF)	11.34
Cellulose (ADF-ADL)	22.1

4.4 GROWTH EXPERIMENT

The effect of supplementing probiotics to complete ration on the feed intake, growth rate and feed efficiency in Nellore brown lambs for a period of 70 days is presented in Table 6.

The weight gains of lambs fed four experimental rations (CR-1 to CR-4) were 7.06 ± 0.83, 8.36 ± 0.93, 10.98 ± 0.31 and 9.44 ± 1.05 Kg, respectively. The difference between the weight gain of lambs fed CR-3 was significantly higher ($P < 0.05$) than CR-1, CR-2 or CR-4. However, the differences in weight gain between CR-1, CR-2 and CR-4 were non significant.

The average daily gain (ADG) was higher ($P < 0.05$) in CR_{-3} fed lambs, compared to the lambs fed CR-1, CR-2 or CR-4. However, the differences between CR-1 and CR-2, CR-1 and CR-4, CR-2 and CR-4 were also not significant.

Total feed consumed by the lambs fed complete rations 1-4 was 78.34 ± 7.75, 76.4 ± 6.69, 90.76 ± 3.39 and 76.54 ± 8.82 Kg, respectively. Although the lambs fed CR-3 consumed higher quantity of feed the difference between the feed consumption by the lambs fed different rations was non-significant.

Dry matter intake expressed in kg/100 kg body weight or $g/w_{kg}^{0.75}$ of lambs fed complete rations 1-4 were 5.76 ± 0.12, 117.85 ± 4.65; 5.49 ± 0.14, 113.05 ± 3.78; 6.04 ± 0.30, 126.96 ± 4.72 and 5.36 ± 0.37, 111.32 ± 8.65, respectively.

The Dry matter intake of lambs expressed in g/d, kg/100 kg weight or $g/w_{kg}^{0.75}$ did not show any significant ($P > 0.05$) difference among different treatments.

The feed efficiency of lambs fed CR-1 to CR-4 was 11.32 ± 0.8, 9.47 ± 0.93, 8.27 ± 0.19 and 8.12 ± 0.29, respectively. The feed efficiency of CR-1 was lower than CR-2, CR-3 or CR-4. However, feed efficiencies of CR-2, CR-3 or CR-4 were significantly higher than that of lambs fed CR-1. The difference between the lambs fed CR-2, CR-3 or CR-4 were non significant in the feed efficiency.

The cost per kg feed (Rs/Kg) and cost per kg live weight gain (Rs/kg) for CR-1 to CR-4 were 3.15, 35.65 ± 2.56; 3.26, 30.87 ± 3.04; 3.66, 30.25 ± 0.68 and 3.46, 28.10 ± 1.00, respectively.

Table 6 Growth performance of Nellore brown lambs fed different probiotics *(Lactobacillus acidophilus* and Yea-sacc^{1026}) on nutrient digestibility of complete rations

	CR-1 (Control)	CR-2 (L.acidophilus 1g)	CR-3 (Yea-Sacc^{1026} 10g)	CR-4 (L.acidophilus 0.5g + Yea-Sacc^{1026} +5g)	SEM
Initial body weight (kg)	14.10 ± 1.06	13.96 ± 1.31	14.26 ± 0.98	13.72 ± 0.81	-
Final body weight (kg)	21.16 ± 1.68	22.32 ± 1.72	25.24 ± 1.22	23.16 ± 1.56	-
Weight gain * (kg)	7.06^{a} ± 0.83	8.36^{a,b} ± 0.93	10.98^{b} ± 0.31	9.44^{a,b} ± 1.05	0.83
Average daily gain * (g)	100.86^{a} ± 11.86	119.43^{a} ± 13.32	156.86^{b} ± 4.48	134.86^{a,b} ± 15.02	11.87
Total feed consumed (kg)	78.34 ± 7.75	76.4 ± 6.69	90.76 ± 3.39	76.54 ± 8.82	6.97
Average daily feed consumption (g/d)	1119.11 ± 110.76	1091.43 ± 95.54	1296.57 ± 48.39	1093.43 ± 126.05	99.53
Dry Matter intake (g/d)	1021.30 ± 101.08	996.04 ± 87.19	1183.25 ± 44.17	997.86 ± 115.03	90.83
Dry Matter intake per $W^{0.75}$ kg (g/d)	117.85 ± 4.65	113.05 ± 3.78	126.96 ± 4.72	111.32 ± 8.65	5.77
Dry Matter/100 kg body weight (kg)	5.76 ± 0.12	5.49 ± 0.14	6.04 ± 0.30	5.36 ± 0.37	0.25
Feed efficiency *	11.32^{a} ± 0.81	9.47^{b} ± 0.93	8.27^{b} ± 0.19	8.13^{b} ± 0.29	0.63
Cost of feed (Rs/kg)	3.15	3.26	3.66	3.46	-
Cost per kg live weight gain (Rs/kg)	35.65 ± 2.56	30.87 ± 3.04	30.25 ± 0.68	28.10 ± 1.00	2.52

a, b Values in the rows bearing different superscripts differ significantly * $P < 0.05$

4.5 Nutrient Digestibility

The date on nutrient digestibility of rams supplemented with different probiotics in complete rations is presented in Table 7. The average DM digestibility values were 66.86 ± 2.28, 60.07 ± 0.97, 70.66 ± 1.32 and 69.59 ± 1.56 for complete rations 1 to 4, respectively. The DM digestibility of CR-3 was higher than that of CR-1, CR-2 or CR-4. However, the difference were not significant (P > .05).

The apparent disability of CP for the complete rations 1 to 4 were 60.70 ± 1.73, 72.99 ± 0.94, 78.60 ± 2.52 and 73.48 ± 0.97%, respectively. The CP digestibility of CR-3, CR-4 or CR-2 was significantly higher (P < 0.01) than that of CR-1. The differences among the complete rations CR-2, CR-3 and CR-4 were not significant.

The digestibility value of CF for CR-1 to CR-4 were 44.66 ± 1.46, 50.79 ± 1.33, 59.78 ± 3.42 and 54.34 ± 3.26 percent, respectively. The CF digestibility of CR-3 was significantly higher (P < 0.01) than those of CR-1, CR-2 or CR-4. The difference among complete ration CR-4, CR-2 and CR-4 were not significant.

The average digestibility value of EE for CR-1 to CR-4 were 77.27 ± 1.46, 80.87 ± 1.46, 86.08 ± 1.52 and 82.69 ± 2.48 respectively. The EE digestibility of CR-3 was significantly higher (P < 0.01) than those of CR-1, CR-2 or CR-4. The differences in the digestibility of EE among the complete rations CR-1, CR-2 and CR-4 were not significant.

The average digestibility value of NFE for CR-1 to CR-4 were 70.30 ± 2.56, 73.39 ± 1.92, 80.44 ± 2.93 and 74.77 ± 4.5, respectively. Though the percent of NFE digestibility of CR-3 was higher the difference among the treatments was not-significant.

Table 7 Effect of supplementing different probiotics *(Lacto bacillus acidophilus,* Yea-sacc[1026]) on Nutrient digestibility of complete rations.

	CR-1 Control	CR-2 (L acidophilus 1g)	CR-3 (Yea-sacc[1026] 10g)	CR-4 (L. acidophilus + yea-sacc[1026] 0.5g+5)	SEM
Dry matter	66.86 ± 2.28	60.07 ± 0.97	70.66 ± 1.32	69.59 ± 1.56	1.60
Crude protein * *	60.70^{a} ± 1.73	72.99^{b} ± 0.94	78.60^{b} ± 2.52	73.48^{b} ± 0.97	1.67
Crude Fibre * *	44.66^{a} ± 1.46	50.79^{ab} ± 1.33	59.78^{b} ± 3.42	54.34^{ab} ± 3.26	2.56
Ether Extract * *	77.27^{a} ± 1.46	80.87^{a} ± 1.46	86.08^{b} ± 1.52	82.69^{ab} ± 2.48	1.78
Nitrogen Free Extract	70.30 ± 2.56	73.39 ± 1.92	80.44 ± 2.93	74.77 ± 4.5	3.13

a, b Values in the rows bearing different superscripts differ singnificantly * * (P < 0.01)

4.5.1 Digestibility of cell-wall constituents of complete rations

The digestibilities of cell-wall constituents for CR-1 to CR-4 were presented in Table 8.

The data on apparent digestibility of cell-wall constituents revealed that the digestibility of NDF for CR-1 to CR-4 were 46.32 ± 2.88, 52.31 ± 1.50, 59.96 ± 2.07 and 56.44 ± 2.43 percent, respectively. The NDF digestibility of CR-3 was significantly higher ($P < 0.01$) than that of CR-1. The difference between CR-1 and CR-2 was not significant. The difference between CR-1 and CR-3 or CR-4 was significant ($P < 0.01$). The differences among the complete rations CR-2, CR-3 and CR-4 were non significant.

The digestibility of hemicellulose followed the same trend as that of NDF. The digestibility values were 52.16±2.04, 57.35 ± 0.72, 62.37 ± 2.26 and 60.13 ± 1.41 for CR-1 to CR-4, respectively.

The Average digestibility value of ADF for CR-1 to CR-4 were 44.9 ± 2.72, 52.7± 1.59, 60.24 ± 2.77 and 54.04 ± 2.28 percent, respectively. Highest digestibility of ADF was observed with lambs fed CR-3 followed by CR-4 CR-2 and CR-1. Highly significant difference ($P < 0.01$) in the digestibility of ADF between was observed between CR-3 and CR-1. However, the difference between CR-1 and CR-2 or CR-4 were non significant. Similary the differences between CR-2, CR-3 or CR-4 were non-significant.

The same trend as that of ADF was observed in the digestibility of cellulose among the lambs fed complete rations 1 to 4.

The average digestibility value of cellulose for CR-1 to CR-4 was 49.38 ± 1.11, 55.89 ± 1.59, 59.53 ± 2.19 and 56.43 ± 1.49 percent, respectively.

4.5.2 Nitrogen Balance

The effect of feeding complete rations containing different probiotics on nitrogen balance in Nellore brown rams is presented in Table 9.

The average nitrogen intake expressed in g/d or $g/w_{kg}^{0.75}$ /d. for ration 1 to 4 was 22.98, 2.07; 28.11, 2.51; 28.01, 2.45 and 27.01, 23.39, respectively. There were no significant ($P > 0.05$) differences among the rations in the N intake.

The nitrogen retention expressed in g/day or $g/w_{kg}^{0.75}$ /d. for ration 1 to 4 were 9,99, 0.89; 15.54, 1.39; 16.36, 1.54 and 15.29, 1.35, respectively. The nitrogen retention expressed as g/day or $g/_{kg}^{0.75}$ /d for CR-3, CR-2 and CR-4 were significantly higher ($P < 0.05$) than that of CR-1. The difference among the complete rations CR-3, CR-2 or CR-4 was not significant.

Table 8 **Effect of Supplementing Different Probiotics (*Lacto bacillus acidophilus*, Yea-sacc^{1026}) on Nutrient Digestibility of Cell-wall Fractions of Complete Rations**

	CR-1 (Control)	CR-2 (L. acidophilus 1g)	CR-3 (yea-sacc^{1026} 10g)	CR-4 (L.acidophilus 0.5g + yea-sacc^{1026} 5g)	SEM
NDF * *	$46.32^{a} \pm 2.88$	$52.31^{a,b} \pm 1.50$	$59.96^{b} \pm 2.07$	$56.44^{b} \pm 2.43$	2.28
ADF* *	$44.9^{a} \pm 2.72$	$52.7^{a,b} \pm 1.59$	$60.24^{b} \pm 2.77$	$54.04^{a,b} \pm 2.28$	2.39
Hemicellulose * *	$52.16^{a} \pm 2.04$	$57.35^{a,b} \pm 0.72$	$62.37^{b} \pm 2.26$	$60.13^{b} \pm 1.41$	1.72
Cellulose * *	$49.38^{a} \pm 1.11$	$55.89^{a,b} \pm 1.59$	$59.53^{b} \pm 2.19$	$56.43^{a,b} \pm 1.49$	1.64

a,b Values in the rows bearing different superscripts differ significantly * * $P < 0.01$

Table 9 Effect of supplementing different probiotics (*L.acidophilus*, yea-sacc[1026]) on Nitrogen utilization by nellore brown rams

	CR-1 (Control)	CR-2 (L.acidophilus 1g)	CR-3 (yea-sacc[1026] 10g)	CR-4 (L. acidophilus 0.5g + yea-sacc[1026] 5g)	SEM
Metabolic body wt ($w_{kg}^{0.75}$) of the Means	11.15 ± 0.31	11.38 ± 0.46	11.50 ± 0.31	11.35 ± 0.42	0.37
N intake g/d	22.98 ± 1.05	28.11 ± 2.63	28.01 ± 1.43	27.01 ± 1.26	1.70
N intake g/$w_{kg}^{0.75}$/d	2.07 ± 0.11	2.51 ± 0.32	2.45 ± 0.17	2.39 ± 0.14	0.20
N excretion (g/d)					
Faecal	7.63 ± 0.37	7.52 ± 0.47	6.03 ± 0.94	7.19 ± 0.54	0.62
Urinary	5.36 ± 1.12	5.06 ± 0.64	4.42 ± 0.45	4.54 ± 0.47	0.72
Total	12.99 ± 0.74	12.57 ± 0.97	10.45 ± 1.19	11.72 ± 0.95	1.48
N retention					
g/d *	9.99^a ± 1.52	15.54^b ± 1.98	16.36^b ± 1.66	15.29^b ± 0.55	1.52
g/$w_{kg}^{0.75}$ /d *	0.89^a ± 0.14	1.39^b ± 0.23	1.54^b ± 0.11	1.35^b ± 0.05	0.14
Percent of intake *	42.95^a ± 4.93	54.84^b ±2.52	63.06^b ± 2.9	56.76^b ± 1.89	3.27
Percent of absorbed	64.62 ± 7.64	75.07 ± 2.89	79.98 ± 2.24	77.00 ± 1.85	4.33

a,b values in the rows bearing different superscripts differ significantly * $P < 0.05$

4.5.3 Calcium balance

The data on calcium intake and retention (g/d) in lambs fed complete rations is presented in Table 10. The average daily calcium intakes were 19.22 ± 0.88, 23.51 ± 2.19, 23.43 ± 1.19 and 22.59 ± 1.05 g, respectively, for CR-1 to CR-4.

The calcium retention (g/d) for CR-1 to CR-4 were 2.74 ± 0.05, 3.09 ± 0.19, 3.54 ± 0.14 and 2.89 ± 0.12, respectively. The calcium retention of CR-3 was significantly higher ($P < 0.01$) than those of rams fed CR_1, CR-2 or CR-4. The difference among CR-1, CR-2 and CR-4 fed rams were non significant.

Calcium retention expressed as percent of intake or percent of absorbed were 14.34, 77.59; 13.59, 78.60; 15.26; 79.84 and 12.97, 77.47, respectively among the rams fed CR_1 to CR_4. However the differences expressed as percent of intake or percent of absorbed were non-significant ($P > 0.05$).

4.5.4 Phosphorus balance

The data on phosphorus intake in lambs fed complete rations is presented in Table 11. The average daily phosphorus intakes (g/d) were 8.89, 10.87, 10.83 and 10.45, respectively for CR-1 to CR-4. There was no significant difference between CR-1 to CR-4 in the average daily intake of phosphorus.

Phosphorus retention expressed as g/d for CR-1 to CR-4 were 2.97 ± 0.12, 3.26 ± 0.17, 4.07 ± 0.08 and 3.56 ± 0.18, respectively. The phosphorus retention of CR-3 expressed as percent intake was significantly higher ($P < 0.01$) than those of CR-1 and CR-2. The difference among the CR-1, CR-2 and CR-4 or between CR-3 and CR - 4 were not significant.

Table 10 **Effect of Supplementing Different Probiotics (L. acidophilus, yea-sacc[1026]) on Balance of Calcium in Nellore Brown Rams fed Complete Rations**

	CR-1 Control	CR-2 (L. acidophilus 1g)	CR-3 (yea-sacc[1026] 10g)	CR-4 (L.acidophilus 0.5g + yea-sacc[1026] 5g)	SEM
Calcium intake (g/d)	19.22 ± 0.88	23.51 ± 2.19	23.43 ± 1.19	22.59 ± 1.05	1.42
Faecal Ca (g/d)	15.68 ± 0.87	19.57 ± 2.27	18.99 ± 1.21	18.85 ± 1.12	1.47
Urinary Ca (g/d)	0.79 ± 0.06	0.84 ± 0.05	0.89 ± 0.05	0.84 ± 0.07	0.05
Ca retention * * (g/d)	2.74^{a} ± 0.05	$3.09^{a,b}$ ± 0.19	3.54^{b} ± 0.14	2.89^{a} ± 0.12	0.14
Ca retention, % intake	14.34 ± 0.41	13.59 ± 1.62	15.26 ± 0.99	12.97 ± 1.05	1.1
Ca retention % absorbed	77.59 ± 1.53	78.60 ± 1.1	79.84 ± 1.4	77.47 ± 1.85	1.49

a,b values in the rows bearing different superscripts differ significantly * * ($P < 0.01$)

Phosphorus retention expressed as percent of intake and percent of absorbed were 33.64, 84.77; 30.67, 86.45; 38.01, 90.61 and 34.45, 89.18, respectively among the rams fed CR-1 to CR-4. The differences in percent of absorbed were non-significant ($P > 0.05$).

Table 11 Effect of supplementing different probitics (*L.acidophilus*, yea-sacc[1026]) on balance of Phosphorus in Nellore brown rams fed complete rations

	CR-1 Control	CR-2 (L. acidophilus 1g)	CR-3 (yea-sacc[1026] 10g)	CR-4 (L. acidophilus 0.5g + yee-sacc[1026] 5g)	SEM
P. intake (g/d)	8.89 ± 0.41	10.87 ± 1.02	10.83 ± 0.55	10.45 ± 0.48	0.66
Faecal P (g/d)	5.39 ± 0.48	7.10 ± 1.01	6.34 ± 0.63	6.45 ± 0.62	0.71
Urinary P (g/d)	0.53 ± 0.04	0.51 ± 0.04	0.42 ± 0.03	0.44 ± 0.05	0.04
P. retention (g/d)	2.97 ± 0.12	3.26 ± 0.17	4.07 ± 0.08	3.56 ± 0.18	0.14
P.retention, % intake **	33.64^{a} ± 2.21	30.67^{a} ± 2.83	38.01^{b} ± 2.79	$34.45^{a,b}$ ± 3.11	2.76
P. retention, % absorbed	84.77 ± 1.09	86.45 ± 1.01	90.61 ± 0.52	89.18 ± 1.31	1.02

a,b values in the rows bearing different superscripts differ significantly * * (P < 0.01)

4.5.5 NUTRITIVE VALUE OF COMPLETE RATIONS

Nutritive value of the four complete rations is presented in table 12. The DCP values of complete rations 1 to 4 were 9.27, 10.15, 10.93 and 10.21, respectively, and the difference among the rations were significant ($P<0.01$). The TDN content of complete rations 1 to 4 were 58.39, 62.35, 69.71 and 63.99 percent, respectively. The highest DCP($P<0.01$) and TDN values ($P<0.05$) were observed in CR-3 followed by CR-4, CR-2 and CR-1. The value for CR-1 was lower than that of CR-2 or CR-4. However, the difference between CR-2 and CR-4 was non significant.

The Digestible energy (DE) and metabolizeble energy (ME) values (Mcal/kg) for CR-1 to CR-4 were 2.57, 2.11; 2.75, 2.25; 3.07, 2.52 and 2.82, 2.31 respectively. The nutritive ratios for the complete rations were 1:5.29, 1:5.14, 1:5.38 and 1:5.27, respectively. Grams digestible protein/M.cal DE and grams digestible protein/M.cal ME were 36.07, 43.93; 36.91, 45.11; 35.60, 43.37 and 36.21, 44.19, respectively.

4.5.6 Plane of Nutrition of Experimental Animals

The data on plane of nutrition of rams fed complete rations are presented in table 13. The dry matter intake expressed as g/d and as percent of body weight of lambs fed complete rations were 1031, 4.21; 1104, 4.38; 1076, 4.16 and 1088, 4.32, respectively for CR-1 to CR-4.

The DCP intake (g/d) of rams on CR-1 to CR-4 were 95.76, 112.30, 117.70 and 110.00, respectively. Highest DCP intake was observed in CR-3 fed rams ($P<0.05$) followed by CR-2, CR-4 and CR-1. The difference in the intake of DCP by CR-2 and CR-4 fed rams was non-significant.

Intakes of DM (g) and DCP (g) per kg metabolic body weight for CR-1 to CR-4 were 93.55, 8.69; 98.20, 10.00; 93.80, 10.24 and 96.70, 9.85, respectively. The intakes of DE and ME (M.cal/$W_{kg}^{0.75}$) were 0.24, 0.19; 0.27, 0.23; 0.29, 0.24; and 0.27, 0.22, respectively for CR-1 to CR-4.

4.6 Rumen fermentation studies

Rumen liquor sample obtained from fistulated rams fed complete rations suplemented with different probiotics were analysed for Rumen pH, Ammonia Nitrogen, VFA and Total bacterial count.

4.6.1 pH of rumen fluid

The pH of rumen liquor collected at different post feeding intervals is presented in Table 14.

The average post feeding values of rumen pH for complete rations 1 to 4 were 6.01 ± 0.01, 6.02 ± 0.08, 6.19 ± 0.12 and 6.18 ± 0.13, respectively. The pH values were not significantly affected bythe supplementation of different probiotics to complete rations indicating that the probiotics had no effect on the pH of rumen fluid.

4.6.2 NH_3-N content of rumen fluid

Ammonia nitrogen values of rumen fluid collected from fistulated rams fed different probiotics is presentedin Table 15. Rumen NH_3 - N of rams fed yea-sacc[1026] and *Lactobacillus acidophilus* supplemented rations were higher than the control. Rumen NH_3-N values at 6 and 8h post feeding were significantly affected as compared to other post feeding intervals. NH_3-N values at 6 and 8h in rams fed CR-1 to CR-4 were 29.04, 29.13; 27.69, 28.25; 33.19, 33.43 and 33.98, 34.19 respectively Rumen NH^3 concentrations of in the sheep fed experimental rations (CR-3 and CR-4) were significantly higher ($P<0.05$) than those of CR_1 and CR-2. However, the difference between CR-3 and CR-4 was not significant.

4.6.3 Rumen TVFA under Probiotic feeding in fistulated rams

The TVFA values of fistulated rams fed different complete rations are presented in Table 14. The yea-sacc[1026] supplemented ration produced higher TVFA (m m/l)which were significantly ($P<0.01$) higher at 2,4 and 6h for CR-3 compared to CR-1, CR-2 or CR-4. The 2 h TVFA values were 143,153.6, 190.6 and 144.4, respectively for CR-1 to CR-4. The 4 h TVFA values for CR-1 to CR-4 were 129.5, 129.25, 157.88 and 130.75, respectively. The 6 h TVFA values for CR-1 to CR-4 were 100.0, 112.38, 135.88 and 120.38 respectively. Significantly higher ($P<0.01$) values were observed with CR-3 than those of CR-1, CR-2 or CR-4 at 2,4 and 6h post feeding intervals. There was no significant difference in TVFA among the sheep fed CR-1 Cr-2 or CR-4.

4.6.4 Total bacterial count in the rumen liquor of sheep fed differen complete rations.

The total bacterial count in the rumen liquor of fistulated rams fed different complete rations is presented in Table 17. The total bacterial count was estimated at 4h after feeding. The total bacterial count for CR_1 to CR_4 were 36.02, 54.59, 61.87 and 64.43 respectively. The bacterial counts in the rumen liquor of sheep fed CR_3, CR_4 and CR_2 were significantly ($P<0.01$) higher than the sheep fed CR-1. Higher bacterial count was observed in the rumen liquor of sheep fed ration supplement with yea-sacc[1026] and *L.acidophilus*, combination.

Table 12 Nutritive Value of complete rations containing different probiotics (*L.acidphilus*, Yea-Sacc[1026])

Item	CR-1 (control)	CR-2 (L.acidophilus) 1g	CR-3 (Yea-Sacc[1026] 10g	CR-4 (L.acidpohilus 0.5 g + Yea-Sace 5g)
Digestible Crude Protein (DCP) Kg/100 kg **	9.27 ± 0.244[a]	10.15 ± 0.13[b]	10.93 ± 0.35[c]	10.21 ± 0.13[b]
Total Digestive Nutrients * (TDN) Kg/100kg	58.39 ± 1.78[a]	62.35 ± 1.12[b]	69.7 ± 2.09[c]	63.99 ± 3.21[b]
Nutritive Ratio	1:5.29	1:5.14	1:5.38	1:5.27
Digestible Energy (DE) (M.cal/kg)	2.57	2.75	3.07	2.82
Metabolizable Energy (ME) (M.cal/kg)	2.11	2.25	2.52	2.31
Gram Digestible Protein/M.cal DE	36.07	36.91	35.60	36.21
Gram Digestible Protein/M.cal ME	43.93	45.11	43.37	44.19

a value of 4.409 Mcal/Kg TDN was used.

b value of 3.616 Mcal/Kg TDN was used.

a,b,c values in the rows bearing different superscripts, differ significantly.** (P<0.01) and * (P<0.05)

Table 13 Plane of Nutrition of rams fed complete rations containing different probiotics.

Complete rations	Body wt.	Metabolic Body wt.	Intake						Intake $W_{kg}^{0.75}$			
			DM		DCP	TDN	DE	ME	DM	DCP	DE	ME
			g	% Bodywt								
CR-1 *	24.68	11.05	1031.23	4.21	95.76[a]	603.93[a]	2.66	2.18	93.55	8.69	0.24	0.19
CR-2 *	25.63	11.38	1104.25	4.38	112.29[b]	688.21[b]	3.03	2.56	98.20	10.00	0.27	0.23
CR-3 *	25.98	11.50	1076.87	4.16	117.68[c]	750.71[c]	3.31	2.71	93.81	10.24	0.29	0.24
CR-4 *	25.53	11.35	1088.28	4.32	110.93[b]	690.85[b]	3.04	2.49	96.68	9.85	0.27	0.22
ICAR (1985)	25.00	11.18	800.00	3.20	65.00	500.00	2.20	1.81	71.56	5.81	0.20	0.16
Kearl (1982)	25.00	11.18	780.00	3.12	36.00	420.00	1.85	1.56	69.77	3.22	0.17	0.14

a,b,c Values in the columns bearing different superscripts differ significantly * $P<0.05$

Table 14 Rumen pH at different post feeding intervals in sheep fed complete rations

Post feeding collection intervals (hr)	CR_1 (Control)	CR_2 (L.acidoplhilus 1g)	CR_3 (Yea-Sacc[1026] 10g)	CR_4 (L.acidophilus 0.5g+Yea-Sacc 5g)	SE Means
0	6.04 ± 0.12	6.19 ± 0.07	6.71 ± 0.12	6.72 ± 0.07	0.09
2	6.30 ± 0.07	6.34 ± 0.12	6.35 ± 0.02	6.41 ± 0.08	0.09
4	6.15 ± 0.11	5.97 ± 0.13	6.06 ± 0.14	6.13 ± 0.12	0.01
6	5.70 ± 0.14	5.86 ± 0.16	5.97 ± 0.15	6.06 ± 0.06	0.09
8	6.01 ± 0.19	5.88 ± 0.11	6.01 ± 0.12	5.92 ± 0.04	0.091
12	6.01 ± 0.25	5.86 ± 0.09	6.08 ± 0.20	5.85 ± 0.15	0.12
Average values	6.10 ± 0.10	6.02 ± 0.08	6.19 ± 0.12	6.18 ± 0.13	-

Table 15 Rumen NH_3-N (mg/100ml) at different post feeding intervals in sheep fed complete rations

Post feeding collection period	CR_1 (Control)	CR_2 (L.acidoplhilus 1g)	CR_3 (Yea-Sacc^{1026} 10g)	CR_4 (L.acidophilus+Yea-Sacc) 0.5g + 5g	SE Means
0	27.13 ± 1.23	28.88 ± 0.73	29.31 ± 1.07	29.86 ± 2.00	0.68
2	30.59 ± 1.39	29.81 ± 1.43	32.96 ± 0.24	32.00 ± 2.34	1.54
4	28.10 ± 1.16	29.04 ± 1.43	31.73 ± 1.91	30.60 ± 2.59	0.96
6*	29.04^a ± 0.90	27.69^a ± 2.03	33.19^b ± 1.63	33.98^b ± 2.52	1.18
8*	29.13^a ± 1.55	28.25^a ± 1.27	33.43^b ± 1.32	34.19^b ± 2.79	0.98
12	28.98 ± 1.80	27.19 ± 1.51	30.44 ± 2.05	28.70 ± 1.74	1.19
Average values	28.83 ± 0.47	28.48 ± 0.39	31.84 ± 0.68	31.56 ± 0.91	-

a,b values in the rows bearing different superscripts differ significantly * $P < 0.05$

Table 16 Rumen TVFA (mm/l)under probiotic feeding in fistulated rams

Post feeding collection period	CR_1 (Control)	CR_2 (L.acidoplhilus 1g)	CR_3 (Yea-Sacc[1026] 10g)	CR_4 (L.acidophilus+Yea-Sacc) 0.5g + 5g	SE Means
0	114.13 ± 3.75	126.00 ± 2.79	127.25 ± 5.89	111.38 ± 8.84	4.76
2 **	143.00^a ± 6.76	153.63^a ± 5.19	190.63^b ± 6.47	144.38^a ± 8.69	3.59
4 **	129.50^a ± 5.01	129.25^a ± 4.023	157.88^b ± 6.48	130.75^a ± 5.4	2.83
6 **	100.00^a ± 7.78	112.38^a ± 5.48	135.88^b ± 4.88	120.38^a ± 10.39	5.06
8	102.75 ± 3.49	111.63 ± 10.41	125.13 ± 7.12	105.38 ± 7.81	6.31
12	113.13 ± 4.34	119.50 ± 6.76	127.88 ± 4.88	119.63 ± 6.26	4.45
	117.09 ± 6.7	125.4 ± 6.34	144.10 ± 10.54	121.98 ± 5.70	-

a,b values in the rows bearing different superscript differ significantly * * $P<0.01$

Table 17 The Total Bacterial count in the rumen liquor of fistulated rams fed different complete rations

Post feeding collection period	CR_1 (Control)	CR_2 (L.acidoplhilus 1g)	CR_3 (Yea-Sacc[1026] 10g)	CR_4 (L.acidophilus+Yea-Sacc) 0.5g + 5g	SE Means
Total bacteria(x 10^9/ ml)**	36.02^a ± 0.58	54.59^b ± 1.83	61.87^b ± 1.16	64.45^b ± 1.58	3.22

a,b values in the rows bearing different superscripts differ significantly ** $P<0.01$

CHAPTER - V

5. DISCUSSION

Many microorganisms are used in the form of *pro* or *prebiotics* in animal feeding. For instance, *Lactobacillus acidophilus* when fed, pass through the stomach and into the lower gut where they help to establish a normal microbial population in the intestines (Higgin Bothams *et al* 1993). With the increasing ban of antimicrobial feed additives, yeast cultures are employed more and more as "*probiotics*". These are by definition microbial food/feed suplements that beneficially affect the host animal by improving its intestinal microbial balance (Gibson and Robert froid, 1994). *Saccharomyces cerevisiae* are increasingly finding favour for manipulation of rumen fermentation as a means of improving the nutrition of ruminant animals. There are reports indicating changes in rumen fermentation and a stimulation of ruminal digestion in goats, cattle and sheep given yeast culture supplements (Kumar *et al*, 1994). Therefore it is desirable to confirm these results by further trials so that microorganisms can be used as pro or prebiotics with advantage in livestock production.

5.1 CHEMICAL COMPOSITION OF FEED INGREDIENTS

The chemical composition of the feed ingredients used in the present study is presented in table 1. The crude protein content of groundnut haulms used in the present experiment was 9.3% while earlier reports showed 12.8 (Shukla *et al*, 1985) and 8.6% (Madhavilatha 1996). The CF, Hemicellulose, cellulose and ADL contents of the G.N. haulms observed at present were 31.6, 8.8, 27.7 and 12.4% respectively. (Table 1 and 2). Crude fibre, hemicellulose, cellulose and ADL contents ranging from 22.5 to 35.1, 5.1 to 19.2, 24.0 to 31.1, 7.0 to 15.8% were reported in literature. (Ramprasad *et al.*, 1988; Dhanunjayudu 1996. Wanapat and Devendra, 1985; Madhavilatha, 1996.)

The Ca (1.5%) and P (0.5%) contents of groundnut haulms observed in the present study are with in the range of 1.0 to 2.4%, for Ca (Madhavilatha 1996, Shukla *et al.*, 1985) and 0.2 to 0.42% for P (Madhavilatha 1996, Dhanunjayudu 1996).

The CP, EE, NFE, Ca and P content of maize used in the present study were 10.3, 0.7, 81.0, 0.6 and 0.3%, respectively (table 1). These values are with in the range of values reported for CP, 9.08 to 11.7; EE, 2.99 to 4.10; NFE, 80.0 to 82.3; Ca, 0.2 to 0.8 and P, 0.3 to 0.4% (Ganesh, 1995, Madhavilatha 1996; Varaprasad *et al.*, 1995).

For Groundnut cake the range of values reported were, for, CP, 40.1 to 46.0; EE, 2.4 to 7.6; NFE, 32.3 to 40.3; Ca, 0.3 to 0.4 and P, 0.8 to 1.1% (Varaprasad 1997, Ganesh 1995, Madhavilatha 1996, Padmaja (1996). The values observed in the present study are within the range for CP (42.7), EE (5.7), NFE (37.1), Ca (0.3) and P (0.9%) (Table 1).

The CP, CF, NFE, Ca and P contents of deoiled rice bran reported in the literature were 14.5 to 15.8, 14.7 to 18.3, 47.8 to 54.0, 0.39 to 0.44 and 1.2 to 1.9%, respectively (Madhavilatha 1996, Prakash 1995; Padmaja, 1996; Geetapriya 1996). The values observed in the present study were CP, 15.6; C.F., 16.4; NFE, 51.0; Ca 0.4 and P 1.6% (Table 1), which are with in the range of values cited above.

The ingredient composition of the experimental complete rations is presented in table 3. In addition to the feed ingredients used in the (control) complete ration (CR-1), the complete rations 2 to 4 were supplemented with 1 g of *lactobacillus acidophilus* (CR-2), 10 g of Yea-sacc^{1026} (CR-3) and 0.5 g of *Lactobacilli acidophilus* + 5 g of Yea-sacc ^{1026} (CR-4).The chemical composition and cell wall constituents of the complete rations are presented in tables 4 and 5 respectively. The OM, CP, NFE, CF, Ca, P, Hemicellulose, Cellulose and ADL contents for complete ration used were 79.4, 13.9, 49.9, 21.9, 1.9, 0.9, 11.3, 22.1 and 9.8%, respectively.

5.2 GROWTH STUDY WITH NELLORE BROWN LAMBS

The growth performance of lambs fed complete rations without or with the supplementation of *probiotics* is presented in table 6. Highest ($P < 0.05$) total weight gain (Kg) and ADG (g) ($P < 0.05$) were observed in the lambs fed complete ration supplemented with yea-sacc^{1026} (11.0 kg, 157 g) followed by the lambs fed rations supplemented with L. acidophilus + yea-sac^{1026} (9.44 Kg, 135 g), L. acidophilus (8.4 Kg, 120 g) and control (7.1 Kg, 101 g) ration. These observations are in agreement with Rauzbehan *et al* (1994) who observed higher growth rate and feed conversion in lambs fed yea-sacc supplemented barley. Kmet *et al* (1993) reported improved growth rate in lambs fed rations containing fungal *probiotics* based on *Saccharomyces cerevisiae.* Birch *et al* (1994) has also reported that feeder lambs given *probiotics* were more feed efficient than control. In the present experiment higher feed efficiency in the lambs fed *probiotics* compared to those fed control ration was observed. Mir and Mir (1994) attributed this to the increased nutrient digestibility.

The higher growth in yea-sacc supplemented ration might be due to increased flow of microbial protein leaving the rumen and an enhanced supply of amino acids entering the small intestine (Erasmus *et al.*, 1992; Caton *et al.*, 1993). Williams *et al* (1990) measured an increased uptake of non-ammonia N at the duodenum in sheep fed yeast culture.

In the present experiment the total weight gain or the ADG of the lambs fed rations supplemented with yea-sacc^{1026} (5 g) + L. acidophilus (0.5 g) was next to the lambs fed rations supplemented with yea-sacc^{1026} alone. Although there was no significant difference in total weight gain or ADG between CR-1, CR-2 and CR-4 the feed conversion rate of CR-4 (8.13) was significantly ($P < 0.05$) higher than the control ration fed lambs (11.32). Addition of individual organisms (*S. cerevisiae* and *L. acidophilus*) increased growth rate of kids, over the control but combination of both exhibited better results (Mudgal *et al.*, 1995). Further higher daily weight gain, high feed conversion efficiency and lower cost of feeding were reported by the same authors in probiotic supplemented (*L. acidophilus + S. cerevisiae*) group compared to control.

Holstein dairy bulls weighing about 120 Kg when fed on concentrate diet during growing and finishing period without or with 0.25% live yeast culture with 0.1%. Clostridium butyricum or 0.15%. *L.acidophilus* + enzyme + yeast culture resulted in higher ($P < 0.05$) daily gain and nutrient intake per Kg live weight than for controls for the first finishing period (Kg) and higher ($P < 0.05$) than control and C. butyricum group for the second finishing period (Kang *et al.*, 1992). The results of the present experiment is in confirmity with the results of Mudgal *et al.* (1995) and Kang *et al.* (1992) with regard to the growth rate of the lambs.

The growth rate of lambs fed complete ration supplemented with L. acidophilus (Table 6) was not superior to the lambs fed control ration. However, better ($P < 0.05$) feed efficiency (9.47) was observed with the group supplemented with *L.acidophilus* compared to control (11.32) group.

Grudkov *et al.* (1985) reported that when whey enriched by fermentation with *L.acidophilus* was used in feed preparation for calves and lambs it helped to control gastrointestinal disorder and increased live weight gain. Hussain (1991) reported accelerating effect on time of puberty in buffalo calves with *L.acidophilus.*

Podshibyakin *et al.* (1991) while feeding propionibacterium species and *L.acidophilus* preparations to ewes during winter reported improvement in milk yield and weight gain of the ewe lambs in comparison to untreated ewes.

The grams drymatter intake (DMI) of lambs fed, yea-sacc[1026] supplemented ration (CR-3) was highest (1183) followed by control (1021), Yea-sacc[1026] + *L.acidophilus* (998) and *L.acidophilus* (996) supplemented rations (Table 6). However, there was no significant difference in DMI from among the rations. These results confirm the observation of Cole *et al* (1992) that lambs fed yeast culture had greater DMI. Williams and Newbold (1990) speculated that in many studies the increase in production observed when fungal *probiotics* were added to the diet could be explained largely by an increase in drymatter intake. Putnam *et al* (1997) observed a tendency for increased drymatter intake in cows fed yeast culture. Increased DM degradability when yeast culture was added to high concentrate diet for dairy cows was reported (Carro *et al.*, 1992b). The observation in the present study confirm the earlier findings with regard to increased drymatter intake as the sheep fed ration supplemented with Yea-sacc[1026] consumed higher DM compared to the control. The cost per kg live weight gain is least (Rs.28.10) with the lambs fed ration supplemented with Yea-sacc[1026] + *L.acidophilus*. Although the cost per kg live weight is highest (Rs.35.65) with lambs fed control ration, the cost per kg gain in lambs fed CR-3 (30.25) or CR-2 (30.87) are not much different from the Yea-sacc[1026] supplemented ration.

5.3 NUTRIENT DIGESTIBILITIES

The digestibility of drymatter and nitrogen free extract in the lambs fed yea-sacc supplemented ration (CR-3) was highest (70.7, 80.4) followed by CR-4 (69.6, 74.8), CR-2 (60.1, 73.4) and CR-1 (66.9, 70.3) (except for DM in the case of CR-1). However the differences among the rations were non significant (Table 7).

The crude protein digestibility was highest in CR-3 fed lambs (78.6%) and the difference between CR-3 and CR-1 was highly significant ($P < 0.01$) while the differences between CR-2 and CR-3 or CR-4 were non significant. The crude fibre digestibility was highest (59.8%) and significantly different ($P < 0.01$) in CR-3 compared to CR-1 (44.7%). The differences between CR-1, CR-2 and CR-4 or CR-2, CR-3 and CR-4 were non-significant. Similarly the ether extract digestibility was highest (86.08%) and significantly different ($P < 0.01$) in CR-3 fed lambs from those fed CR-1 (77.3%). The differences in digestibilities of EE between CR-3 and CR-4 or between CR-1, CR-2, CR-3 were non-significant. Fisher *et al* (1984) observed higher N digestibility in sheep when *L.acidophilus* was added as additive to silage of cocks foot grass. Improvement in the performance of dairy cattle with increased digestibility of protein in cows fed YC was reported (Wohlt *et al.*, 1991).

Moloney and Drennan (1994) concluded that dietary inclusion of yeast culture had a small influence on rumen fermentation parameters and *in vivo* digestibility but that its effect on N metabolism appeared to be dependent on N content of the basal diets.

Arambel (1988) reported effectiveness of Yea-sacc and fungal cultures in ruminant-rations to improve the rumen fibre digestion and concluded that such cultures could influence rumen fermentation but the mode of action was not clearly understood. However, Chademan and Offer (1990) reported that yeast culture appeared to increase the initial rate of forage digestion in the rumen without altering over all food digestibility.

The theory that yeast culture inclusion stimulates appetite has been enthusiastically pursued. Williams (1989) suggested a direct stimulation of cellulolysis and increase in the rate of fibre digestion.

Nisbet and Martin (1991) suggested that some of the stimulatory activities of yeast preparations for rumen function could be related to the presence of dicarboxylic acids in aqueous extracts from cultures of *S.cerevisiae*. The mechanism is based on the observation that malic acid constitute the growth and activities of one of the predominant lactic acid utilizing bacteria from the rumen, *Selenomonas ruminantium*. Similarly, Rossi *et al* (1995) observed that extracts from yeast culture preparations could enhance the growth of a second lactic acid utilizing ruminal organism, *Megasphore elsdenii*. These two organisms play an important role in a process which prevent lactic acid accumulation in the rumen. Selective stimulation of these organisms could moderate ruminal pH and prevent ruminal dysfunction. The metabolic basis for this stimulatory mechanism may be related to a biochemical role for malate as an electron sink for lactate metabolism in these anaerobic organisms (Martin and Park, 1996). Studies of Chaucheyras *et al* (1996) described a nutritional role for specific components in yeast culture preparations which can stabilize the physicochemical conditions in the rumen and provide for improved fermentation efficiencies by moderating ruminal pH. The ability of yeast to stimulate the growth of ruminal bacteria by removing trace amounts of oxygen from the environment has also been proposed as a basic mechanism which can be used to explain its stimulatory activities (Newbold *et al* 1996).

5.4 NUTRIENT DIGESTIBILITIES OF CELL WALL FRACTIONS

Nutrient digestibility of cell wall fractions presented in Table 8 indicated higher ($P < 0.01$) NDF, ADF, hemicellulose and cellulose digestibility when the complete ration was supplemented with Yea-sacc[1026] followed by *L.acidophilus* + Yea-sacc and *L.acidophilus*. Least digestibility of the cell wall fractions was observed with the control ration.

Fisher *et al* (1984) reported higher ADF digestibility in sheep fed with cocks foot grass supplemented with dried culture of *L.acidophilus*. Arambel (1988) concluded that yeast and fungal culture could influence rumen fermentation thereby increasing fiber digestion.

Improved NDF digestibility and propionate molar percentage were observed by Plate *et al* (1994) when saccharomyces cerevisiae was added to three mixed diets for steers. Further they opined that the increased ruminal protozoal population could be responsible for NDF digestibility changes. Roa *et al* (1997) studying the effect of three fiber sources with or without yeast culture on *in situ* digestion and rumen environment of cattle, reported increased potentially digestible NDF when Yea-sacc was added to complete diet containing alfalfa hay. However, Kamalamma *et al* (1996) did not observe differences in the digestibility of NDF and ADF between Yea-sacc adapted and Yea-sacc unadapted rumen inoculum for finger millet straw or commercial cattle feed.

Several authors have noted increases in the cellulolytic bacterial populations in response to *S.cerevisiae* (Harison *et al.*, 1988; Dawson *et al* 1990, Kim *et al.*, 1992b). It has been suggested that an increase in the population of cellulolytic bacteria in the rumen could account for the increases in total tract digestibility of drymatter and ADF which have been reported in animals fed *S.cerevisiae* (Van Horn *et al.*, 1984; Wiedmeir *et al.*, 1987). Williams *et al* (1991) suggested that the effect of *S.cerevisiae* on the fiber digestion in the rumen might be mediated via the effect on rumen pH. However, Chademana and Offer (1990) found that *S.cerevisiae* stimulated DM degradation over a range of forage: Concentrate ratios, with little effect on rumen pH. Thus it appears that the stimulation of fiber degradation in the rumen by *S.cerevisiae* is mediated by a more general mechanism than simply an increase in rumen pH, such as an increase in the numbers of cellulolytic bacteria. Williams and Newbold (1990) speculated that in many studies the increase in production observed when fungal *probiotics* were added to the diet could be explained largely by an increase in DM intake. This trend has also been observed in the present experiment confirm the observation of Fisher *et al* (1984); Plate *et al* (1994) and Roa *et al* (1997) but differs with the observation of Kamalamma *et al* (1996).

5.3.2 N BALANCE

Cole *et al* reported greater ($P < 0.08$) N balance in lambs fed Yeast culture. Jassim-Ram *et al* (1985) in an experiment on 42 merino weathers weighing 35 kg given 920 g DM from roughage daily without or with 24, 48, 72g yeast *saccharomyces cerevisiae* and 90g CP.Supplemented more Nretention then the other groups. The results of the present experiment confirm the above observation as the lambs fed yea-sacc[1026], supplemented rations (CR-3) reported retention of higher ($P < 0.05$) quantity of N (16.36g) compared to the lambs fed control ration (9.99). The N retention in lambs fed CR-4 (15.29g) or CR-3 (15.56 g) in the present study were higher ($P < 0.05$) than the lambs fed CR-1 but similar to those fed CR-3. Nitrogen retention expressed as $g/w_{kg}^{0.75}$ or percent of intake followed the same trend as N retention (g/d). However, there was no significant difference from among the treatments when N retention was expressed as per cent of absorbed. Stimulated proteolytic bacterial count with treatments containing yeast culture was reported by Yoon and Stern (1996). Increased nitrogen by ruminants would benefit the nutrition by the animal by retaining more energy and N in the body (Flint and Wallace, 1991).

5.3.3 BALANCE OF CALCIUM

The calcium intake (g/d) in rams increased in the order of *L.acidophilus* (CR-2) Yea-sacc[1026] (CR-3), *L.acidophilus* + Yea-sacc[1026] (CR-4) supplemented rations than in the control, (CR-1), but the differences were not significant among the different treatments (Table 10). Similar trend as that of intake was also observed with faecal and urinary calcium excretion in rams fed CR-1 to CR-4.

Calcium retention expressed as g/d was highest ($P < 0.01$) in yea-sacc fed (CR-3) rams followed by *L.acidophilus* (CR-2), *L.acidophilus* + yea-sacc[1026] (CR-4) supplemented and control (CR-1) ration fed rams. The differences between CR-1 and CR-3 were significant ($P < 0.01$) while the differences between CR-1 and CR-2 or between CR-1, CR-2 and CR-4 were non-significant. Calcium retention expressed as percent of intake or percent of absorbed did not show any significant difference among the different treatments. Madhavilatha (1996), feeding complete ration to sheep similar to the one used as control in the present study observed Ca retention of 2.21 (g/d), 14.91 (as percent intake) and 81.02 (as percent absorbed). The results observed in the present study are in agreement with those observed above.

5.3.4 BALANCE OF PHOSPHORUS

The phosphorus intake (g/d) in rams increased in the order of *L.acidophilus*(CR-2), yea-sacc[1026](CR-3), *L.acidophilus* + yea-sacc[1026] (CR-4) supplemented rations compared to control (CR-1), but the differences were not significant among the different treatments (Table 11). Similar trend as that of intake was also observed with faecal and urinary phosphorus excretion in rams fed CR-1 to CR-4. Phosphorus retention expressed as g/d was higher ($P < 0.01$) in yea-sacc[1026] fed groups than *L.acidophilus* and control ration fed groups. Phosphorus retention when expressed as percent of intake , significantly higher ($P < 0.01$) value was observed in CR-3 fed (yea-sacc) rams than those fed other rations. However, the differences between CR-1, CR-2 and CR-4 or between CR-3 and CR-4 were non-significant. The results of P balance for control ration (without any *probiotic* supplement) containing 60% G.N.haulms in the present study are similar to the observation of Madhavilatha (1996) in P retention g/d (2.73). However, P retention when expressed as percent of intake (38.0) or % of absorbed (97.2) were higher compared to the results obtained in the present study. The difference might be due to the difference in body weight and total feed consumed by the rams in these experiments.

The results of Ca and P balance of the rams fed CR-1 to CR-4 indicate that *probiotics* had no marked influence on Ca and P balance. The differences reported might be due to higher quantity of feed consumed.

5.3.5 NUTRITIVE VALUE OF COMPLETE RATIONS CONTAINING DIFFERENT *PROBIOTICS*:

The nutritive value of the different complete rations was evaluated using 16 Nellore brown rams divided into 4 groups in a completely randomised design experiment. The Digestible crude protein (DCP) value of CR-3 was highest (10.93). The differences between CR-1 (9.27) and CR-2 (10.15) or CR-4 (10.21) or CR-3 and CR-2 or CR-4 were significantly different ($P < 0.01$) (Table 12). The total Digestible nutrients (TDN) of the different rations followed the same trend as that of DCP. The differences in TDN value between CR-1 (58.39) and CR-2 (62.35) or CR-4 (63.99) or CR-3 (69.7) and CR-2 or CR-4 were significantly different ($P < 0.05$). There was no difference from among the complete rations in nutritive ratio (NR), Digestible energy (DE), Metabolizable energy (ME), grams DCP/M.cal DE or grams DCP/M.cal ME. Madhavilatha (1996) observed 8.89, 57.35, 1:6:45, 2.52, 2.07, 35.28 and 42.95 for DCP, TDN, NR, DE, ME respectively for complete rations containing 60% G.N. haulms with sheep. The results of the present study (control ration) are comparable to the observation of Madhavilatha (1996).

5.3.6 PLANE OF NUTRITION OF EXPERIMENTAL ANIMALS

Nutrient intake of complete rations is presented in Table 13. As per the nutrient requirements of live stock published by ICAR (1985), the requirements suggested for growing lambs weighing 25kg body weight are DM 800g, DCP 65g and TDN 500g. Kearl (1982) has recommended 780g, of DM, 36g of DCP and 420g of TDN for growing sheep of 25 kg body weight. In the present study DMI of CR-1 to CR-4 were 1031, 1104, 1076, and 1088g respectively. DCP intake of CR-1 to CR-4 were 95, 112, 117 and 110g respectively. The TDN intake of CR-1 to CR-4 were 603, 688, 750 and 690g respectively. In present study the nutrient intakes of the rams fed CR-1 to CR-4 were higher than the recommended levels of ICAR (1985) and Kearl (1982) which might be due to good quality ingredients and better utilization.

5.4.1 RUMEN pH

Rumen pH at different post feeding intervals in fistulated rams fed complete rations supplemented with different *probiotics* is presented in table 14. There was no significant difference from among the rams fed different rations in pH at any post feeding time interval studied, and in accordance with Chademana and Offer (1990) who observed that rumen pH was not significantly affected by inclusion of yeast culture supplement. Gray (1989) reported that the effect of yea-sacc in the sheep rumen is to keep the acidity near to neutral which is the optimum for microbial fermentation of fibrous type of foods. Dawson *et al* (1990) reported effect of two microbial feed supplements containing yeast and lacto bacilli on roughage fed ruminal microbial activities. The pH tended to be greater ($P < 0.13$) in continuous culture receiving yeast culture supplement than in culture receiving the unsupplemented diet. The pH in the rumen is influenced by the diet composition and by feeding practice. The mean rumen pH is depressed by giving diet containing high proportions of rapidly fermentable material. It has been suggested that the presence of viable yeast act as modulator of rumen pH (Williams *et al.*, 1991). Gambos *et al* (1995) reported increased PH in rumen cannulated cows fed yea-sacc[1026]. In the present study differences in pH could not be observed in the rams fed different *probiotic* supplemented rations as complete rations were constituted with 40% concentrated mixture and 60% roughage.

5.4.2 RUMEN AMMONIA NITROGEN

Rumen NH_3-N (mg/100m/) at different post feeding intervals is presented in table 15. The results showed that there was no influence of supplementing either *L.acidophilus*, yea-sacc or combination of both *probiotics* on the Rumen NH_3-N production. At 6 and 8 hour post feeding there was high NH_3-N production ($P < 0.05$) in CR-3 and CR-4 compared to CR-1 or CR-2. Dawson *et al* (1990) reported that neither yeast nor lactobacilli supplement consistently altered the relative concentration of ammonia in continuous cultures and in the rumen of steers. Chademana and Offer (1990) also observed that rumen ammonia concentration was not significantly affected by the inclusion of yeast culture supplement. Gombos *et al* (1995) reported that yea-sacc[1026] added to the diet of rumen cannulated cows at 10g/head daily decreased the concentration of ammonia in rumen.

However, Roa *et al* (1997) observed increased ($P < 0.05$) ruminal NH_3-N concentration (mg / 100 ml) for alfalfahay (36.3. vs 27.7), coffee hulls (36.4 vs 26.9) and corn stalk (36.5 vs 28.2%) by the addition of yea-sacc.

5.4.3 TOTAL VOLATILE FATTY ACIDS

Total volatile fatty acid production in the rumen has been studied in fistulated rams fed complete rations supplemented with different *probiotics* (Table 16). At 2 (190.6), 4 (157,8) and 6h (135.9 mm/l) post feeding intervals significantly higher ($P < 0.01$) TVFA production was observed in CR-3 (yeast supplemented) fed rams, while the differences between CR-1 (143.0, 129.5, 100.0), CR-2 (153.6, 129.3, 112.4) and CR-4 (144.4, 130.8, 120.4 mm/l) were nonsignificant. These results are in agrement with the observations of Gray and Rayan (1989), and Nisbet *et al* (1990) that there was marked increase in VFA production during yea-sacc administration in sheep. Similarly Calleway and Martin (1997) observed increase in acetate, propionate and TVFA when *saccharomyces cerevisiae* culture was added to ruminal bacteria. Many papers on the effect of yeast culture include data on rumen pH , ammonia and VFA concentrations. Although yeast culture has been found to affect one or more of these parameters in many studies (Wallace, 1996), and the effects can be statistically significant, most lack biological significance. Changes in VFA proportions are small, and in any case vary from study to study: mostly the acetate: propionate ratio decreases. Sometimes it increases (Wallace and Newbold, 1992). However, None of the changes would affect the animal, (Wallace, 1996).

5.4.4 TOTAL BACTERIAL COUNT IN THE RUMEN LIQUOR

Total bacterial count in the rumen liquor of fistulated rams fed complete rations supplemented with different *probiotics* is presented in table 17. Highest cell count was observed (64.5×10^9) in sheep fed ration supplemented with *L.acidophilus* + yea-sacc followed by yea-sacc (61.9×10^9), *L.acidophilus* (55×10^9) and control (36×10^9). The difference between the control and probiotic supplemented rations was significant ($P < 0.01$) although from among the probiotic supplemented rations the differences were non-significant.

Supplementation of Yea-sacc significantly increased the number of total bacteria and marginally increased the number of amylolytic bacteria and protozoa in rumen fluid (Kumar *et al.*, 1994). Since facilities for identification of different types of ruminal microorganisms were not available in our laboratory total bacterial count only was made. Our present work is in confirmity with the observation of Kumar *et al* (1994) as significantly higher bacterial count was observed with yea-sacc or yea-sacc + *L.acidophilus* supplemented rations. In CR_2 the increased bacterial count might be due to *L.acidophilus* itself. There is no literature to support stimulation of cell count in rumen fluid by *L.acidophilus*. Weidmeir *et al.*, 1987 and Dawson and Newman, 1988, reported increase in rumen total bacteria and cellulolytic bacteria when live yeast culture was used as food supplement. The viable yeast culture itself (Dawson *et al.*, 1990; Dawson 1990) or some heat labile nutrients in the yeast (El Hassan *et al* 1993., Girard 1996) may be responsible for the stimulation of bacterial numbers.

Williams (1989) suggested that addition of yea-sacc to the rumen increased the number of bacteria especially cellulolytic bacteria in the rumen possibly by assisting in H_2 transfer than by allowing increased cellulolysis and reducing losses of H_2 as methane. It has been suggested that the ability of the yeast cells to stimulate bacterial numbers in the rumen may also be related to their ability to decrease potentially inhibitory concentrations of rumen fluid oxygen (Newbold *et al.*, 1993). Yoon and Stern (1996) reported that yeast culture stimulated proteolytic bacterial count. Newbold *et al* (1995) reported that increased bacterial number were associated with an increase in the rate of straw degradation. Callaway and Martine (1997) observed that yeast culture provides soluble growth factors (ie organic acids, B.Vitamins and amine acids) that stimulate growth of ruminal bacteria that utilize lactate and digest cellulose.

According to the model suggested by Wallace and Newbold (1992) increased bacterial viability which is associated with improved pH stability, decreased lactate production, altered methanogensis and changed VFA proportions lead to increased rate of cellulolysis and increased flow of microbial protein resulting in increased food intake. This ultimately benefits improved production. The results of growth trial in the present investigation showed increased food intake and growth in sheep fed complete rations supplemented with yea-sacc. Similarly the results of the metabolism trial has shown higher CF, ADF and cellulose digestibility in yea-sacc supplemented rations. The total bacterial count was also higher in the probiotic supplemented rations compared to the control ration.

CHAPTER - VI

6. SUMMARY

For getting high productivity in the livestock at economical rate it is essential to enhance the feeding value of animal feed resources. Recently the use of probiotics in the ration of ruminants has attracted the attention of many research workers. The use of lactic acid producing bacteria are known to create an environment to enhance digestion and to stimulate immunity. The ability of specific yeast preparations to increase the concentrations and activities of the beneficial bacteria in the rumen is believed to be key to their overall effect on ruminal production.

In the present investigation, an attempt was made to study the effect of supplementing *Loctobacillus acidophilus, Saccharomyces cerevisiae* (yea-sacc^{1026}) or a combination of *L. acidophilus* + yea-sacc^{1026} to complete rations for sheep on voluntary feed intake, growth rate, feed efficiency, rumen environment, nutrient digestibility and N balance.

A complete ration with 60:40 roughage to concentrate ratio was prepared by blending groundnut haulms, 60; maize grain, 20; groundnut cake 13.5; deoiled rice bran, 3.5; mineral mixture, 2.0 and salt, 1.0% Rovimix (A B_2 D_3) was added at 25g/100 kg of the feed. This ration was used as control (CR-1). Three more such rations were prepared and each one was supplemented with one of the probiotics *L.acidophilus* 1g (CR-2), yea-sacc^{1026} 10g (CR-3)(or) *L.acidophilus*, 0.5g+ yea-sacc^{1026}, 5g (CR-4).

The chemical analysis of the complete ration was DM 91.26; OM,79.39; CP, 13.9; CF, 21.86; EE, 2.46; TA, 11.87; NFE, 49.91; AIA, 7.93; Ca, 1.86; and P, 0.86%. The cell wall constituents of the complete ration were NDF, 43.26; ADF, 31.92; ADL, 9.82; Hemicellulose, 11.34 and cellulose (ADF-ADL), 22.1%. For evaluation of the complete rations three experiments, a growth study, a metabolism study and rumen metabolic profile study were conducted using Nellore brown sheep.

Growth Study: In a completely randomized experiment twenty Nellore brown weaner lambs (14.11 kg ± 0.53) were divided into 4 groups of 5 animals each and were allotted at random to one of the experimental complete rations and fed for 70 d. The ADG (g),DMI (g) EFU were 101, 1021, 11; 199, 996, 9.5; 157, 1183, 8.3 and 135, 998, 8.1 for CR-1 to CR-4, respectively.

Highest ADG was observed in the lambs fed CR-3 containing yea-sacc. The difference between CR-1 and CR-3 was significant ($P < 0.05$). Highest feed efficiency was recorded in the lambs fed CR-4. However, the difference between -2,CR-3 and CR-4 was non-significant. The feed efficiency of CR-1 fed lambs was the least and differed significantly ($P < 0.05$) from CR-2, CR-3 or CR-4. Higher growth rate in CR-3 might be due to increased DMI, increased flow of microbial protein leaving the rumen and enhanced supply of amino acids entering small intesestine. The feed cost per kg live weight gain were Rs. 35.65, 30.87, 30.25 and *28.10 for CR-1 to CR-4, respectively.*

Metabolism Study : In a completely randomised design experiment 16 Nellore brown rams (25.06 kg ± 0.57) were devided into 4 groups of 4 animals each. Each group was allotted at random to one of the complete rations described in experiment 1. A metabolism trial with 15 day preliminary and 7 day collection periods was conducted. The dry matter digestibility of the complete rations 1-4 were 66.9, 60.1, 70.7 and 69.6% respectively, and the difference from among the rations was non-significant. The digestibility of CP in CR-1 fed rams (60.7) was significantly lower (P<0.01) than CR-2 (72.9), CR-3 (78.6) or CR-4 (73.5) fed rams. The crude fibre digestibility in CR-3 (59.8) was higher (P < 0.01) than CR-1 (44.7%) followed by CR-4 fed rams (54.3%) Higher digestion of CF in CR-3 might be due to increase in the rate of fibre digestion. Yeast culture preparations could have stabilized the physicochemical conditions in the rumen and provided for improved fermentation efficiencies.

The ether extract digestibility of CR-1 to CR-4 fed rations were 77.3, 80.9, 86.1 and 82.7% respectively. The digestibility of EE in CR-3 was higher (P< 0.01) than CR-1 or CR-2. The NFE digestibility of the different rations were 70.3 (CR-1), 73.4 (CR-2), 80.4 (CR-3) and 74.8% (CR-4). There was no significant difference among the complete rations in NFE digestibility.

The nutrient digestibility of cell wall fractions were NDF, 46.3, 52.3, 60.0, 56.4;ADF,44.9, 52.7, 60.2, 50.0; Hemicellulose, 52.2, 57.4, 62.4, 60.1 and cellulose 49.4, 55.9, 59.5, 56.4%, respectively in CR-1 to CR-4 fed rams. The increase in cell wall fractions by CR-3 (P<0.01) might be due to increase in number of cellulolytic bacteria.

The Nitrogen retention (g/d, g/w $_{kg}^{0.75}$ /d or percent of intake) was significantly lower (P<0.05) in CR-1 (9.9,0.89,42.9) compared to CR-2 (15.5,1.4,54.8), CR-3 (16.4, 1.5, 63.1,) or CR-4 (15.3, 1.4, 56.8). The difference between CR-2 to CR-4 were non-significant. Higher N retention in probiotic supplemented rations might be due to stimulated proteolytic bacterial count in the rumen.

Calcium intake and retention (g/d) in rams fed complete rations 1 to 4 were 19.2,2.74;23.5, 3.1; 23.4, 3.5 and 22.6, 2.9 respectively. Phosphorus intake and retention (g/d) in rams fed CR-1 to CR-4 were. 8.9, 2.97, ; 10.9, 3.3; 10.8, 4.1 and 10.5, 3.6, respectively. Highly significant (P<0.01) difference was observed between CR-3 and CR-1 or CR-4 in calcium retention (g/d). Higher (P<0.01) P retention as % of intake in CR-3 compared CR-1 or CR-2 was observed. The differences might be due to higher quantity of DMI rather than due to influence by yea-sacc [1026] or *L.acidophilus.*

The nutritive value of the complete rations in terms of DCP (%), TDN (%) DE and ME (M.cal/kg) were 9.3,58.4, 2.6, 2.1; 10.2, 62.4, 2.8, 2.3; 10.9,69.7, 3.1, 2.5 and 10.2, 64.0, 2.8, 2.3, respectively, for CR-1 to CR-4. The daily total DMI expressed in g/d, percent of live weight or $g/w_{kg}^{0.75}$ /d were 1031, 4.2, 94; 1104, 4.4, 98; 1076, 4.2, 94 and 1088, 4.3, 97, respectively, for CR-1 to CR-4 fed rams. The values were higher than those suggested by ICAR,(1985) or Kearl (1982). .

Rumen metabolic profile study: The rumen pH at different post feeding intervals (0,2,4,6,8, 12 h) revealed that there was no difference in the rumen pH among the cannulated rams fed complete rations 1 to 4. The average post feeding values of rumen pH for the complete ration 1 to 4 were 6.01±0.01, 6.02±0.08, 6.19±0.12 and 6.18±0.13, respectively. similarly the differences in Rumen NH_3 - N at different post feeding intervals among the rations was non-significant except at 6 and 8 h. significantly higher ($P< 0.05$) values (mg/100 ml) were observed in CR-3 (33.2, 33.4) and CR-4 (34.0, 39.2) compared to CR-1 (29.0, 29.1) or CR-2 (27.7, 28.3). Yea-sacc[1026] supplementation might have kept the acidity in the rumen near to neutral which is the optimum for microbial fermentation of fibrous foods.

Significantly higher ($P< 0.01$) TVFA production (mm/l) was observed in CR-3 fed rams at 2 (190.6), 4 (157.9) and 6 h (135.9) compared to CR-1 (143.0,129.5, 100.0) CR-2 (153.6, 129.3, 112.4) or CR-4 (144.4, 130.8,120.4),respectively. The increase in CR-3 or CR - 4 might be due to administration of yea-sacc[1026].

The total bacterial count in CR-4 (64.5 X 10^9) was significantly higher ($P< 0.01$) than CR-1, (36.0 X 10^9), while the bacterial count of CR-2 (54.6 X 10^9) or CR-3 (61.9 X 10^9) were not different from that of CR-4 ($P>0.10$). Supplementation of yea-sacc[1026] or yea-sacc[1026] + *L. acidophilus* might have significantly increased number of total bacteria. The viable yeast culture itself or some heat labile nutrients in the yeast might be responsible for the stimulation of bacterial numbers.

The following conclusions are drawn from the results obtained in the present study.

1. The DMI and ADG were higher in yea-sacc[1026] fed lambs while EFU was highest in yea-sacc[1026] +*L. acidophilus* fed animals. The cost per kg live weight gain was Rs. 35.65 ± 2.56, 30.87 ± 3.04, 30.25 ± 0.68 and 28.1 ± 1.0 for lambs fed complete rations 1 to 4, respectively.

2 The DMI intakes of rams fed ration supplemented with yea-sacc[1026] + *L. acidophilus* or yea-sacc [1026] were higher. The digestibility of CP, CF, EE, NDF, ADF, Hemicellulose and cellulose were higher in yea-sacc[1026] supplemented rations. The nutritive value in terms of DCP, TDN, DE and ME were higher in CR-3 followed by CR-4, CR-2 and CR-1.

3. The rumen pH in cannulated rams fed complete rations 1 to 4 on an average were 6.10 ± 0.10, 6.02 ± 0.08, 6.19 ± 0.12 and 6.18 ± 0.13 respectively and did not differ significantly among the rations. Rumen NH_3-N values except at 6 h and 8 h post feeding were similar in all the rations. The TVFA in yea-sacc[1026] supplemented rams was significantly higher at 2,4, and 6 h post feeding compared to control. The rumen bacterial count was higher in probiotic particularly (yea-sacc [1026]) supplemented rams.

The results of the present experiment showed that supplementation of yea-sacc[1026] or yea-sacc[1026] + *L. acidophilus* are advantageous as they have improved dry matter intake, nutrient utilization and growth rate leading to lower cost of production in Nellore brown lambs.

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