

STUDIES ON THE POTENTIALS OF PROBIOTICS ON IN VITRO FERMENTATION AND PRODUCTIVE PERFORMANCE IN LACTATING DAIRY COWS

**A THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF**

**DOCTOR OF PHILOSOPHY
IN
ANIMAL NUTRITION**

**BY
TAPAS KUMAR DUTTA**

**DIVISION OF DAIRY CATTLE NUTRITION
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL-132 001 (HARYANA), INDIA**

1998

Regn. No. 91-P-AN-164

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DEDICATED TO MY

**WIFE
SHAMPA
AND
DAUGHTER
SNEHA**

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PERFORMANCE IN LACTATING DAIRY COWS**

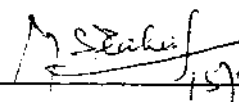
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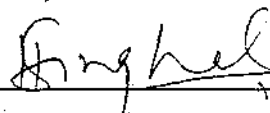

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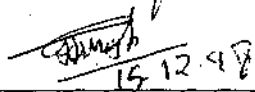

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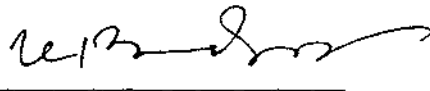
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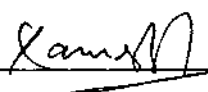
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This is to certify that the thesis entitled, "**STUDIES ON THE POTENTIALS OF PROBIOTICS ON *IN VITRO* FERMENTATION AND PRODUCTIVE PERFORMANCE IN LACTATING DAIRY COWS**" submitted by **Mr. TAPAS KUMAR DUTTA** in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL NUTRITION** of the National Dairy Research Institute (Deemed University), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance, and no part of the thesis has been submitted for any other degree or diploma.



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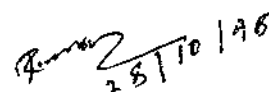
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LIST OF ABBREVIATIONS

ADF	: Acid Detergent Fibre
<i>Ad lib</i>	: <i>Ad libitum</i>
AOAC	: Association of Official Analytical Chemist
B.wt.	: Body Weight
CF	: Crude Fibre
cfu	: Colony Forming Unit
CP	: Crude Protein
CPI	: Crude Protein Intake
°C	: Degree Centigrade
d	: Day
DCP	: Digestible Crude Protein
DM	: Dry Matter
DMI	: Dry Matter Intake
DE	: Digestible Energy
DOMI	: Digestible Organic Matter Intake
EE	: Ether Extract
EF	: <i>Enterococcus faecium</i>
g	: gram
h	: hour
IVDMD	: <i>In vitro</i> Dry Matter Degradability
Kcal	: Killo calorie
KF	: Karan Fries
Kg	: Kilogram
l	: Litre
LA	: <i>Lactobacillus acidophilus</i>
LL	: <i>Lactococcus lactis</i>
LP	: <i>Lactobacillus plantarum</i>
Mcal	: Mega calorie
MCP	: Microbial Crude Protein

ME	: Metabolizable Energy
MJ	: Mega Joule
ml	: Millilitre
mg	: Milligram
mmol	: Millimole
N	: Nitrogen
NDF	: Neutral Detergent Fibre
NFE	: Nitrogen Free Extract
NH ₃	: Ammonia
No.	: Number
OM	: Organic Matter
rpm	: Rotation per Minute
SC-B	: <i>Saccharomyces cerevisiae</i> -B
SE	: Standard Error
SRL	: Strained Rumen Liquor
TCA	: Trichloro Acetic Acid
TDN	: Total Digestible Nutrient
VFA	: Volatile Fatty Acid
TVFA	: Total Volatile Fatty Acid
W ^{0.75}	: Metabolic Body Weight
wt	: Weight

ABSTRACT

Probiotics are often reported to have some potential to modulate the rumen fermentation pattern and may thereby enhance the productive performance of ruminants. In the present studies, experiments were carried out to observe the total GI tract survivability of six probiotic cultures, namely, *Saccharomyces cerevisiae*-B(SC-B), *S.cerevisiae*-522 (SC-522), *Lactobacillus acidophilus* (LA), *L.plantarum* (LP), *Lactococcus lactis* (LL), and *Enterococcus faecium* (EF), the response of these cultures on *in vitro* rumen fermentation, to evaluate the efficacy of selected promising cultures on lactation performance and nutrient digestibility in dairy cows and to prepare powder of most promising probiotic cultures. During GI tract survivability study of yeast (SC-B and SC-522), faecal yeast counts (cfu/g faeces) remained 50 and 49 percent higher in respective groups than in control group but least squares mean (LSM) values (Log_{10} cfu/g of faeces) were statistically similar during control and treatment periods. Almost similar trend was found with LL and EF cultures. Whereas, *L.acidophilus* remained more resistant and significantly higher ($P<0.01$) acid producing bacilli counts (LSM) in faeces were recorded during treatment period (6.365) over control period (6.092). But no change in faecal acid producing bacilli counts was observed in *L.plantarum* supplemented calves..

Above six cultures were evaluated *in vitro* in individual, double or triple combinations (total 22 combinations) using high roughage substrate. The supplementation of probiotic cultures increased IVDMD and total gas production (48h). Maximum response was obtained in triple culture combinations in terms of higher gas production, TVFA concentration and propionate proportion; and reduction of acetate to propionate ratio. Triple combination of SC-B, LP and EF expressed best response

on IVDMD (77.60%), total gas production (125.70 ml), TVFA concentration (9.68 mmol/100 ml incubation medium) and A/P ratio (1.28) on high roughage substrate (70%).

Selected promising probiotic cultures from above experiment, namely, SC-B, EF, SC-522+LP, SC-522+EF, SC-522+LA+LL, SC-522+LP+EF and SC-B+LP+EF also increased IVDMD, total gas production and TVFA at three levels of concentrate mixture (60, 40 and 30%). A/P ratio was depressed significantly due to promising viable cultures addition. Combined culture of SC-B, LP and EF showed maximum effect on reduction of A/P ratio and $\text{NH}_3\text{-N}$ concentration at three levels of concentrate mixture. Reduction of $\text{NH}_3\text{-N}$ could be due to incorporation of $\text{NH}_3\text{-N}$ into microbial protein which was further evident from the increased microbial counts. Increased microbial protein synthesis was also observed in terms of TCA-ppt-N and ^{35}S incorporation into microbial protein on supplementation of triple cultures (SC-B+LB+EF) in high concentrate diet. Total protozoal counts remained unaffected due to probiotics supplementation.

In lactation study, ten multiparous crossbred (KF) cows in their mid lactation yielding 9 to 11 kg milk were divided in two equal groups and fed similarly to their nutritional requirements. In experimental group, triple culture combination of SC-B, LP and EF (6:2:2) was supplemented in their ration (Total dose 10×10^9 cfu/animal/d). Due to dietary addition of triple culture milk yield increased ($P<0.01$) to 10.60 kg against 10.16 kg in control group. FCM (kg/d) yield was also increased significantly ($P<0.10$) due to supplementation of probiotics from 9.94 to 10.31 kg/d. Milk production efficiency (kg/kg DMI) was also increased in the experimental group. However, milk composition (total solid, fat, protein and ash) remained uninfluenced due to probiotics supplementation. Digestibility coefficients of NDF, ADF, hemicellulose

and EE were enhanced, whereas those of DM, OM and CP remained unaffected due to probiotics addition. Inclusion of probiotics increased TDN and DCP intake per 100 kg b.wt over control (2.29 kg VS 2.14 kg and 397.00 g VS 371.25g). Higher ($P < 0.05$) body weight gain was also observed in probiotics treated cows. Supplementation of triple combination of SC-B, LP and EF to lactating cows increased Rs.200 net return with 40 kg extra milk yield per animal during 3 months experimental period.

The same cultures SC-B, LP and EF were grown and preserved individually in the form of powder using skim milk powder and cryoprotective agents successfully. Viability study revealed that both adonitol and mono sodium glutamate were effective to enhance viability of yeast (SC-B) and *E.faecium*. Whereas mono sodium glutamate appeared effective for protecting *L.plantarum* culture during freeze drying and preservation. The data indicated possible use of probiotic cultures to improve milk yield in cattle.

सांख्यिक

प्रोबायोटिक चूँकि रूमनीय किण्वीकरण प्रक्रियाओं में फेरबदल करने हेतु समर्थ है कि प्रायः ऐसा कथन में आया है अतः ये गोपुशुओं की उत्पादन को बढ़ा सकती है । प्रस्तुत शोधकार्य में छः प्रोबायोटिक संवर्धकों जैसे सक्रोमायोसिस सरबेसी-बी (एस.सी.-बी), 50 सरबेसी-522(एससी-522), लैक्टोबैसिलस एसिडोफिल्स (एल ए), एल प्लान्टेरियम(एल पी), लैक्टोकोकस लैक्टिस(एलएल) एवं एन्ट्रोकोकस फीसीयम (ई एफ) की पाचन नाल में जीवन्तता, इन संवर्धकों का अन्तः पात्र किण्वीकरण पर प्रभाव, चुनिन्दा प्रभावशाली संवर्धकों का दूधारू गायों की उत्पादकता एवं पोषक तत्वों की पचनीयता पर परीक्षण के साथ साथ सर्वाधिक प्रभावशाली संवर्धकों की पूर्णरूप में परिरक्षित करके की विधि का विकास किया गया । पाचन जीवन्तता अध्ययन में आहारिय यीस्ट एस सी-बी, एस-522 नियन्त्रकवर्ग तुलना में क्रमशः 50 तथा 40 प्रतिशत अधिक निष्काशित हुये किन्तु औसत लीस्ट स्कायर मान (लोग 10 सी एफ यू/जी गोबर) नियन्त्रक एवं परिक्षण वर्गों में सांख्यिकी दृष्टि से समान पाये गये । अन्य संवर्धकों (एल एल तथा ई एफ) ने भी समान क्रम दर्शाया किन्तु परीक्षण अवधि में लैक्टोबैसिलस अधिक प्रतिरोधी एवं गोबर में नियन्त्रक वर्ग की तुलना में (<0.01) संख्या में अधिक पाये गये । इसके विपरित आहारिय लैक्टोबैसिलस प्लान्टेरियम वर्ग के गोबर में इनकी संख्या अप्रभावित रही।

उपरोक्त छः संवर्धकों को अन्तः पात्र मूल्यांकन हेतु अकेले, दूकेले और तिकेले मिश्रित किया गया । परिणामों द्वारा दर्शाया गया कि प्रोबायोटिक आपूर्ति से अन्तः पात्र शुष्क पदार्थ पचनीयता (अशुपा) तथा सकल गैस उत्पादन (48 घण्टे) में वृद्धि हुई । तिकेले संवर्धक मिश्रण से एकल गैस उत्पादन, टी.वी.एफ. ए. सान्द्रता एवं प्रोप्योनेट प्रतिशतता अधिकतम होने के साथ साथ एसीटेट एवं प्रोप्योनेट के अनुपात में कभी आयी। एस-बी, एल पी तथा ई एफ संवर्धकों के मिश्रण ने सर्वोत्तम अशुपा (77.60 प्रतिशत) सकल गैस उत्पादन(125.70मि.ली.) टी.वी. एफ सान्द्रता (9.68 कि.मोम/100 मि.ली. एस आर एल) एवं एसीटेट/प्रोप्योनेट अनुपात (1.28) दर्शाये जबकि कार्यद्रव्य में भूसे का स्तर उच्च (70 प्रतिशत) था ।

उपयुक्त परीक्षण में प्रयोगित संवर्धकों में से कुछ चुनी हुई प्रभावशाली संवर्धकों ने भी अशुपा, सकल गैस उत्पादन एवम् टी वी एफ ए स्तर में बढोतरी की जबकि सान्द्र मिश्रण का कार्य द्रव्य रूप में 60, 40 तथा 20 प्रतिशत स्तर पर प्रयोग किया गया । संवर्धक के प्रयोग से प्रोप्योनेट अनुपात में भी सार्थक

कमी पाई गयी विशेषकर एस.सी-बी, एल पी और इ एफ के मिश्रित संवर्धकों की आपूर्ति से मिश्रित प्रोबायोटिक आपूर्ति द्वारा अमोनिया-नाइट्रोजन स्तर में कमी इसके सुक्ष्मजीविय प्रोटीन संश्लेषण में योगदान के कारण हुई जो सुक्ष्मजीवाणुओं की संख्या में वृद्धि टीसीए-पीपीटी-एन की मात्रा में वृद्धि तथा उन के सुक्ष्मजीविय प्रोटीन में अधिक समावेशित होने से स्पष्ट होता है, हालांकि सकल प्रोटोजोआओं की संख्या मिश्रित प्रोबायोटिक आपूर्ति से अप्रभावित रही।

अन्तःपात्र परीक्षण से प्राप्त परिणामों को दृष्टिगत रखते हुए दूधारू संकर गायों के दो समान वर्गों में से एक वर्ग के आहार से सर्वोत्तम त्रिसंयोगी प्रोबायोटिक समर्थकों (कुल खुराक 10 गुणा 10^9 सी एफ यू/पशु/दिन) किया गया। परिणाम स्वरूप दूध उत्पादन परीक्षण वर्ग में (10.60 कि०ग्रा०)नियंत्रक वर्ग (10.16 कि०ग्रा०) की तुलना में सार्थक रूप (पी < 0.01) से अधिक था वसा शुद्धिकृत दूध उत्पादन भी 9.94(नियन्त्रण वर्ग) से बढ़कर 10.31(परीक्षण वर्ग) हो गया और यह वृद्धि भी सांख्यिकी दृष्टि से सार्थक(पी < 0.01) पायी गयी। परीक्षण वर्ग की गायों में दूध उत्पादन दक्षता (कि०/कि० शुष्क पदार्थ अन्तर्ग्रहण) भी अधिक थी किन्तु दूध सघटन (समक ठोस, वसा, प्रोटीन एवमं भस्म) दोनों वर्गों में समान था। इस अध्ययन के दौरान पाचकता परीक्षण में एन.डी.एफ., हैमीसल्यूलोस एवं इथरी निष्कर्ष के पाचकता गुणांक बढ़े जबकि शुष्क पदार्थ, कार्बनिक पदार्थ एवं कच्ची प्रोटीन पचनीयता प्रोबायोटिक आपूर्ति से अप्रभावित रही। आहार में प्रोबायोटिक मिश्रण के समावेश से टीडीएन अन्तर्ग्रहण (2.29 कि०) तथा डीसीपी अन्तर्ग्रहण(397.0 ग्रा०) नियंत्रक वर्ग की तुलना में अधिक थे जिसका मान क्रमशः 2.14 कि. तथा 317.25 ग्राम था। प्रोबायोटिक पूरित दूधारू गायों के शरीर भार में भी सार्थक (पी < 0.05) वृद्धि पाई गयी। अन्तोगत्वा एससी-बी, एल पी तथा ई एफ के त्रिसंयोगी संवर्धक मिश्रण के आहार में समावेश करने से तीन माह की परीक्षण अवधि में से 200 रु का शुद्ध लाभ हुआ व 40 कि० अतिरिक्त दूध प्राप्त हुआ।

सर्वाधिक सफल त्रिसंयोगी मिश्रित संवर्धक (एससी-बी, एल पी एवं इ एफ) को पूर्णरूप में परिरक्षित करने हेतु वसा रहित दुग्ध चूर्ण एवं हिमपरिजक्षक एजेन्टों का सफलतापूर्वक प्रयोग करने की विधि विकसित की गई। जीवन्तता परीक्षण द्वारा पता चला कि अडीनोटोल व मोनो सोडियम ग्लूटामेट, यीस्ट(एससी-बी) व इ एफ दोनों के लिए उपयुक्त है। परन्तु एल प्लांटअरयुम की जीवन्तता वृद्धि केवल मोनो सोडियम ग्लूटामेट से ही हुई। प्रस्तुत आकड़े दर्शाते हैं कि प्रोबायोटिकों के प्रयोग से गायों में दुध उत्पादन वृद्धि की संभावना दृढ़ है।

CHAPTER - I

INTRODUCTION

1. INTRODUCTION

India has achieved a commendable progress in milk production during last two decades. Prestigious target of 74 million tonnes of milk production to be achieved by the turn of the century is still a task before the nation. Rumen is the unique fermentor which is playing a key role for the productive efficiency of ruminants. Efforts are on to enhance the efficacy of rumen fermentation either by dietary manipulation or by supplementing additives which may result in higher growth and milk production. Recently biotechnological tools have come up for the improvement of productive performance of the dairy animals.

A wide range of materials are capable of improving ruminant productivity by manipulating the rumen environment, improving efficiency in cellulolysis and maximizing microbial protein synthesis in the rumen. One of the most exciting and useful material for supplementing the animal feed is living microflora called probiotics.

The concept of microbial manipulation in the gastro-intestinal tract was first appreciated by Metchnikoff (1907) who viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. The term "Probiotics" was first coined by Parker (1974) who described this as "microorganism or substance which contribute to the intestinal microbial balance". In 1989, Fuller defined the probiotic as "a live microbial feed supplement which beneficially affects the host animals by improving its intestinal microbial balance." The term probiotic means "for life" and has a contrast with the term antibiotic which means "against life".

At present, probiotics are classified by the US food and Drug Administration as GRAS (Generally Recognised As Safe) ingredient. Now it is generally accepted that certain viable microbial cultures beneficially affect the productive potentials of pig and poultry. The species employed in probiotic

preparation are mainly lactic acid bacteria like *Lactobacillus acidophilus*, *L.bulgaricus*, *L.lactis*, *L.plantarum*, *L.salivarius*, *Streptococcus thermophilus*, *Enterococcus faecium*, *E.faecalis*, *Bifidobacterium* species and *Bacillus subtilis*.

For ruminants, the yeast, *Saccharomyces cerevisiae* has been considered as the promising probiotic culture for efficient nutrient utilization. In contrast of earlier concept, the yeast and some lactic acid bacteria may survive and multiply in the anaerobic environment of rumen and intestine. *S.cerevisiae* may multiply and exhibit growth in the rumen or in rumen simulating continuous cultures and confer beneficial effects on cellulolysis and productive trait of the host animal (Weidmeier *et al.*, 1987; Dawson and Newman, 1988; Harris and Lobo, 1988).

Growing public disquiet over the use of antibiotics in feed additives has encouraged recent commercial interest in probiotics as an alternate therapy against harmful pathogens in the GI tract, this is due to exhibited drug resistance as a result of indiscriminate use of antibiotics. Feeding viable cells of *L.acidophilus* to young dairy calves was shown to reduce the incidence of diarrhoea (Bechman *et al.*, 1977) and increase the numbers of lactobacilli and reduce coliform counts in faeces (Bruce *et al.*, 1979). Underdahl *et al.* (1982) observed only mild diarrhoea lasting 2-4 days in gnotobiotic pig inoculated with *Streptococcus faecium* prior to artificial *E.coli* infection. Antibacterial properties of *L.acidophilus* and *Bifidobacterium* species have been demonstrated in human being against a variety of undesirable bacteria including *E.coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Streptococcus aureus* and *Vibrio* species. The inhibition may be due to a complex interaction of factors including lactic and acetic acids and antibiotic like substances.

The use of probiotics culture in large and small ruminants has been appreciated for the improvement in feed intake (Ruf *et al.*, 1953; Phillips and Von Tungela, 1985; Malcolm and Kiesling, 1986; Fallen and Harte, 1987; Gunther, 1989; Williams and Newbold, 1990b; Harris and Lobo, 1988; McLeod

et al. 1991; Adams *et al.*, 1995) and enhancement in weight gains and feed conversion efficiencies in meat producing animals (Ruf *et al.*, 1993; Phillips and Von Tungeln, 1985; Fallen and Harte, 1987; Hughes, 1988). *S.cerevisiae* has potential to improve the milk production in dairy cows (Hoyos *et al.*, 1987; Teh *et al.*, 1987; Williams and Newbold, 1990a; Harris and Lobo, 1988; Bernard *et al.*, 1992; Harris and Webb, 1990; Adams *et al.*, 1995), increase milk fat (Harris and Webb, 1990; Harris and Lobo, 1988; Dobos *et al.*, 1989; Williams, 1989b; Adams *et al.*, 1995), milk protein (Dobos *et al.*, 1989; Williams, 1989b; Kvanta and Fischler, 1989; Harris and Webb, 1990) and lactose content in milk (Kim *et al.*, 1992b).

The positive responses may be due to decreased ruminal ammonia concentration (Dawson and Newman, 1987; Harris and Lobo, 1988); altered VFA production (Teh *et al.*, 1987; Gray and Ryan, 1988; Harrison *et al.*, 1988; Martin *et al.*, 1989), stable rumen fermentation (Harrison *et al.*, 1988), increased concentration of anaerobic bacteria, more specifically cellulolytic bacteria (Weidmeier *et al.*, 1987; Harrison *et al.*, 1988; Dawson *et al.*, 1990), decreased soluble sugar and lactic acid concentration (Williams, 1990a) and altered digestive pattern (Ruf *et al.*, 1953; Gomez-Alarcon *et al.*, 1987; Weidmeier *et al.*, 1987; Williams, 1989a; Chademana and Offer, 1990).

Feeding systems in India are quite variable, therefore, use of probiotics under different ration systems should be examined for more efficient rumen fermentation of roughages. The possible synergistic response of yeast, lactobacilli and streptococci need evaluation for efficient rumen fermentation which may have added advantage over yeast alone in terms of better health and higher production. More data have to be generated before using probiotics on survivability pattern of yeast, lactobacilli and streptococci in the GI tract of animals, basic mechanism of actions of the living microflora, economics on productivity and method for preparing cultures for practical applicability etc.

Keeping in view all the above points, the project has been formulated with the following objectives:

1. To study the survivability of probiotic cultures in the digestive tract.
2. To investigate the effect of various microbial cultures on *in vitro* rumen fermentability.
3. To compare the response of probiotics on *in vitro* fibre degradability using various levels of roughages.
4. To determine the response of dietary probiotics on milk production and composition.
5. To produce low cost promising microbial cultures for field application.

CHAPTER - II

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

In proposing and promoting yoghurt and other fermented products as healthy and therapeutic, Metchnikoff (1907) implied that the ingested bacteria had a positive influence on the normal flora of the gastrointestinal tract. Yoghurt was considered as probiotic preparation which increased the life expectancy of man and animal. Indian curd, a cultured milk product, is also considered to increase health performance of human being. Contact of new born piglet with sow and the environment leads to successional colonization by a variety of microbes which is regarded as complementary to the digestive functions (Smith, 1965; Contrepois and Gouet, 1973).

The yeast, *Saccharomyces cerevisiae* and *Aspergillus oryzae* have received considerable attention for improving productive potentials of ruminants when added in small amount in their rations. Combined effect of yeast (*S.cerevisiae*) and lactic acid bacteria may have added advantage for better productivity as well as controlling the pathogenic intestinal flora.

2.1 LACTIC ACID BACTERIA - MODE OF ACTION AS PROBIOTICS AND IMPACT ON THE INTESTINAL ECOLOGY

2.1.1 Colonization and Survivability of Lactic Acid Bacteria in the GI Tract:-

The probiotic bacteria possess specific characteristics for successful colonization in the gastrointestinal (GI) tract. The main features include an ability to adhere to epithelial cells and rapid growth in the GI tract having continuously varying composition of digesta. Some portion of organisms taken in the diet survive in the digestive tract (Sissions, 1989; Renner, 1991). Greater number of lactobacilli in the jejunum of calves is the indication of bile tolerance of *L. acidophilus* (Gilliland *et al.*, 1984). In new born calves, supplementation of bile resistant strain of *L.acidophilus* increased population of facultative lactobacilli in the upper small intestine compared to a strain exhibiting lower

resistance to bile salts (Renner, 1991).

Diet and digesta may have some significant influence on growth and colonization characteristics of bacteria. Long chain unsaturated fatty acids are recognized as having inhibitory effect on bacterial growth, whereas, milk fat do not have inhibitory effect on the lactobacilli number in the digesta collected from different regions of GI tract (Brockert and Tannock, 1981). However, addition of 32 mM propionic acid to milk feed depressed lactobacilli counts by nearly 1000 fold. Dietary protein rich in lectins may compete with bacteria for binding sites at the brush border (Firon *et al.*, 1984) which could be a disadvantage to the gut colonization by probiotic organisms.

Relative changes in faecal counts of lactobacilli and coliforms are often used to assess probiotic efficiency. Although faecal measurements are not a satisfactory indicator of the behaviour of these organisms in different parts of the GI tract always (Pollmann *et al.*, 1980; Ward and Nelson, 1982). It has been demonstrated in different species of animals that lactic acid bacteria survive gastroenteric passage and are found in the faeces (Garvie *et al.*, 1984; Ushe and Nagy, 1985; Jonsson, 1986; Ratcliffe *et al.*, 1986; Conway *et al.*, 1987; Bianchi-Salvadori, 1986; Bianchi-Salvadori *et al.*, 1988). In calves, faecal lactobacilli increased when fed lactobacilli (Ellinger *et al.*, 1978; Gilliland *et al.*, 1980; Jenny *et al.*, 1991; Abu-Tarboush *et al.*, 1996). Calves fed *L.acidophilus* 27SC had higher faecal lactobacilli count than calves fed mixed lactobacilli (*L.acidophilus* and *L.plantarum*) (Abu-Tarboush *et al.*, 1996). Jenny *et al.* (1991) noted that counts of lactobacilli tended to remain higher than those of coliforms in calves given mixed concentrates containing lactobacilli for 4 weeks. Same trend was observed in healthy pig (Mitchell and Kenworthy, 1976; Muralidhara *et al.*, 1977).

2.1.2 Mode of Action:-

Several mechanisms of lactic acid bacteria have been proposed which include adhesion to the digestive tract to prevent colonization of pathogens,

neutralization of toxins, bacteriocidal activity, prevention of amine synthesis and enhanced immuno competence.

2.1.2.1 Competitive Attachment:-

It has been demonstrated that lactobacilli compete with coliform and other pathogenic organisms for the sites of adherence on the intestinal surface. The attachment is believed to support proliferation and reduce peristaltic removal of organisms. For enterotoxin producing strains of *E.coli* to induce diarrhoea, it is essential to perform surface action through attachment to the intestinal wall (Jones and Rutter, 1972). Muralidhara *et al.*(1977) have reported the competitive attachment between lactic acid bacteria and coliform in the intestinal tract. The ability of bacteria to adhere to squamous epithelial cells appears to depend on attraction between an acidic mucopolysaccharide forming the outer layer of bacterial cell wall and a similar coating on epithelial cells (Fuller and Brooker, 1974). Generally fibrils are found on this type of adhering bacteria, which may reinforce attachment, although adhesion can also occur *in vitro* in the absence of fibrils. Lactic acid bacteria are known to be associated with the gut wall of chicken (Fuller and Turvey, 1971) and pigs (Barrow *et al.*, 1980) and that adhesion varies between strains of the same species (Barrow *et al.*, 1980).

2.1.2.2 Antimicrobial Activity:-

Lactic acid bacteria have the potential in limiting the growth of pathogenic microorganisms in calves (Bechman *et al.*, 1977; Ellinger *et al.*, 1978; Gilliland *et al.*, 1980; Abu-Tarboush *et al.*, 1996), pig (Ratcliffe *et al.*, 1986; Fuller, 1986; Cole *et al.*, 1987), and in man (Prajapati *et al.*, 1986). The protective effect of indigenous gut flora have been confirmed against *Salmonella* (Snoeyenbos *et al.*, 1978), *E.coli* (Weinack *et al.*, 1981), *Campylobacter foetus* sub sp. *Jejuni* (Soerjadi *et al.*, 1982; Soerjadi Liem *et al.*, 1984a), *Clostridium botulinum* (Snoeyenbos *et al.*, 1983) and *Mersinia enterocolitica* (Soerjadi Liem *et al.*, 1984b). *Streptococcus thermophilus* suppressed the growth of pathogenic bacterial, *Clostridium perfringens* and *Salmonella entridides* by 73.3 and 51.8 per

cent respectively (Sikes and Hilton, 1987). Following possible mechanisms have been suggested to explain the anti-microbial activity of lactobacilli and streptococci.

- Adhesion to the intestinal wall preventing colonisation by pathogens	VanDer <i>et al.</i> (1971), Tannock <i>et al.</i> (1982), Fuller (1989)
- Lowering of intestinal pH (due to production of H ₂ O ₂ or organic acid)	Ratcliffe <i>et al.</i> (1985,1986), Fuller <i>et al.</i> (1986), Renner (1991)
- Competition of nutrients	Fredrickson (1977). Wilson and Perini (1988)
- Production of antibacterial substances	Bloksma <i>et al.</i> (1979), Renner (1991)
- Increase in phagocytic activity and immunoglobulin levels	Saito <i>et al.</i> (1981), Wade <i>et al.</i> (1984), Bealmear <i>et al.</i> (1984)

2.1.2.3 Enterotoxins Neutralization:-

Investigations revealed that *L.bulgaricus* in pigs produces a metabolite which is thought to neutralise the effect of enterotoxins released from coliforms (Mitchell and Kenworthy, 1976). Although the neutralising substance has yet to be identified, further support for antienterotoxin activity has also been obtained from experiments with rats and calves (Stuart *et al.*, 1978; Schwab *et al.*, 1980). Coliform and certain other bacteria in the pig gut have the ability to decarboxylate amino acids and would yield toxic amines (Hill *et al.*, 1970b), lactic acid bacteria inhibit the growth of coliforms and prevent the synthesis of toxic amines.

2.1.2.4 Enhancement of Immunity:-

Lactic acid bacteria have the capability to enhance the immunity by the

animals. Phagocytic activity and immunoglobulin levels have been increased by probiotics (Bealmear *et al.*, 1984; Raibaud and Raynaud, 1991). *L.casei* and *L.plantarum* given parenterally stimulate phagocytic activity (Saito *et al.*, 1981). Yoghurt has been shown to increase antibody levels when fed to germ free mice (Wade *et al.*, 1984) and lactobacilli are also involved in the stimulation of phagocytic activity. Pollmann *et al.* (1980) observed elevated levels of total serum protein, apparently globulin rather than albumin and WBC counts when the animal fed *L.acidophilus*.

Lactobacilli could be important in the development of immunity in young animals, particularly during weaning when protection must be acquired against antigens likely to cause gut inflammatory reactions (Miller *et al.*, 1985). A strain of *E. faecium* was established to reduce the *S.typhimurium* in the spleen of germ free mice implying a systematic effect (Roach and Tannock, 1980).

2.1.3 Translocation of Micro-organisms from the Gut:-

Lactobacilli can translocate (Berg, 1983) and can survive for many days in the spleen, liver, lungs and lymph (Bianchi-Salvadori *et al.*, 1988). In the animals pre-treated with lactic acid bacteria (*L.bulgaricus* and *S.thermophilus*), *E.coli* were absent in the lymph nodes and stimulate the immunity (Bourliouse, 1986). In mice feeding of *L.bulgaricus* and *S.thermophilus* gave a reduction in the translocation of *E.coli* into the mesenteric lymph nodes of 70 and 50 per cent respectively (Bianchi-Salvadori *et al.*, 1988).

2.1.4 Prophylactic and Therapeutic Effect of Lactic Acid Bacteria:-

2.1.4.1 Against Diarrhoea:-

Neonates of all species can suffer from a number of adverse environmental conditions soon after birth, including dampness, chilling, drought, inadequate milk and periods of separation from their dams. These conditions favour for non-beneficial bacteria to colonise the digestive tract and result in increased neonatal morbidity and mortality. In neonatal piglets and calves, probiotics aided in the prompt establishment of an animal-microbes relationship

beneficial to the new born's intestinal tract. These studies have demonstrated improved animal health and performance (Collington *et al.*, 1988). Since Metchnikoff's early study data in several species have shown the ability of lactobacilli to suppress coliform growth. Viable lactobacilli feeding was shown to reduce the incidence of diarrhoea in young dairy calves (Bechman *et al.*, 1977; Fettman *et al.*, 1987; Sissons, 1988; Kopeeny *et al.*, 1989; Abu-Tarboush *et al.*, 1996), steers (Walter *et al.*, 1988), and in bulls (Libersa, 1991). Diarrhoea symptoms disappeared at the rate of 95.8 per cent when lactobacilli was added in the diet of cattle (Guo *et al.*, 1990). These findings contrast with those of other workers who have not observed benefits from feeding either *L.acidophilus* (Hatch *et al.*, 1973; Ellinger *et al.*, 1978) or milk cultured with *L.acidophilus* or *L.lactis* (Morrill *et al.*, 1977).

Muralidhara *et al.* (1977) have observed that piglets given on *L.lactis* concentrate for upto 8 weeks after birth showed a progressive decline in coliform counts in faecal samples. Mild diarrhoea was observed lasting 2-4 days in gnotobiotic pigs when inoculated with *Streptococcus faecium* prior to artificial *E.coli* infection (Underdahl *et al.*, 1982). Havrevoll *et al.* (1988) also found that diarrhoea was less pronounced in calves supplemented with *S.faecium*.

2.1.4.2 Against Mastitis:-

Greene *et al.* (1991) have attempted to treat clinical mastitis in dairy cattle with intramammary infusion of lactobacillus. They observed that lactobacillus cured 35.5% of infected quarters but milk somatic cell counts remained unchanged, and concluded that Lactobacillus product was not effective as an intramammary treatment for mastitis.

2.1.4.3 Anticarcinogenic Activity:-

A number of available reports in the literature suggest that certain lactic acid bacteria, including *L.acidophilus* and *Bifidobacterium* species may have an anti-cancer role, the specific evidence for this is not yet compelling. Epidemiological evidence and dietary studies have shown that supplementation

of lactic acid bacteria may reduce the risk of colon cancer in both animals and herdman (Friend and Shahani, 1984). Feeding of lactic acid bacteria inhibited the growth of tumor in mice when inoculated with tumor cells intraperitoneally (Shackelford *et al.*, 1983; Takano *et al.*, 1985). They found similar results with *L.bulgaricus* and *S.thermophilus*.

The anticarcinogenic properties of lactic acid bacteria may be possibly due to:-

- Stimulation of immuno system of the host FriendandShahani(1984)
- Assimilate and detoxify dietary, endogenous and Keating (1985)
intestinal flora generated toxic compounds
- *L.acidophilus* may reduce the level of enzymes Shackelford *et al.* (1983),
such B-glucosidase, B-glucoronidase, nitroreduc- Cole *et al.* (1984), Goldin
tase and azo-reductase which are the factors to be and Gorbach (1984a),
carcinogenic Cole and Fuller (1987)

2.1.5 Digestive and Metabolic Activity as Influenced by Lactic Acid Bacteria:-

Feeding of bacterial preparations increased feed conversion efficiency and live weight gain which may be due to improved digestion performance or indirectly due to the suppression of gut pathogens.

2.1.5.1 Carbohydrates:-

It is feasible that extracellular enzymes of gut microflora supplement endogenous secretions, particularly in neonates when the digestive system is immature (Kiddar and Manners, 1978). Szabo (1979) observed estimated activities of lactase and lypase in the large intestine of conventional piglet compared with germ-free animals, but at the same time inactivation of some endogenous enzymes was also found by gut microflora. For example, higher levels of peptidase and disaccharidase activities were found in the small and large intestines of germ-free than in conventional piglets.

2.1.5.2 Lactose Tolerance:-

Lactase deficiency is generally observed in human being which leads to a digestive malfunctions such as gastro-distension, abdominal cramps and watery diarrhoea. The symptoms are thought to relate to the metabolism of excess lactose by bacteria. Fermentation products, notably short chain VFAs, H₂ and CO₂ accumulate in the lower gut and colon where they cause hyperosmosis and increase intraruminal pressure (Gray, 1984). The symptoms experienced by lactase-deficient subjects after ingesting lactose do not occur when they consume yoghurt (Savaiano *et al.*, 1984). This apparent benefit has been attributed to bacterial β -galactosidase activity in the yoghurt (Speck, 1983; Kolars *et al.*, 1984). Microbial enzymes or some other factors may be involved in alleviation of the disorder.

2.1.5.3 Protein Deamination:-

Microbial deamination of amino acids is not a favourable process for the host animals. The extent of deamination by gut microflora of pigs was studied *in vitro* (Buraczewska and Buraczewska, 1985) by incubating casein hydrolysate (as a source of amino acids) with digesta collected from the ileum or caecum and found that total amino acids decreased by 8% and 24% respectively. Marked depression was noted in the concentration of aspartic and glutamic acids, and also that of serine, lysine, histidine and arginine in ileal digesta, but all of the added amino acids were deaminated to some extent during incubation with caecal digesta. Hill *et al.* (1970a,b) observed similar deamination activity in piglet and apparent breakdown of amino acids was enhanced by adding lactobacilli to the diet. However, comparisons of net nitrogen absorption of different proteins in germ free and conventional chicks suggest that intestinal microflora do not compete with the host for amino acid nitrogen (Salter *et al.*, 1974).

2.1.5.4 Fats and Cholesterol Metabolism:-

Improved fat digestibility in growing pigs was observed by Mason and

Just (1976) under the suppression of gut bacteria by antibiotics. This suggested that fat metabolising bacteria reduce the availability of dietary lipid to the host. Ratcliffe *et al.* (1985) proposed that microbial hydrogenation could increase the amount of stearic acid, which is less well absorbed than unsaturated fatty acids. Impaired lipid absorption is also thought to occur as a result of deconjugation of bile acids by gut flora (Eyssen and de Somer, 1967). This action on bile acids was suggested as a reason for the growth-depressing effect of *S.faecium* on germ-free chicks (Cole *et al.*, 1981). However, deconjugation of bile acids was also shown with strains of lactobacilli (*L.acidophilus* and *L.fermentor*) but these organisms did not inhibit the growth of chickens (Cole and Fuller, 1984). A number of studies suggested that lactic acid bacteria produced hypocholesteromic effects in pigs (Gilliland *et al.*, 1985), rabbit (Kiyosawa *et al.*, 1984), and in human (Bazzarre *et al.*, 1983; Jaspers *et al.*, 1984; Gorbach *et al.*, 1988). Hypocholesteromic effects were reported on yoghurt culture with *S.thermophilus* and *S.bulgaricus* (Jaspers *et al.*, 1984).

2.1.5.5 Mineral Metabolism:-

Supplementation of lactic acid bacteria increased the bioavailability of calcium, phosphorus, magnesium and zinc from all diets (Schaafsma *et al.*, 1988). Balasubramanya *et al.* (1984) observed 7 and 11 per cent higher availability of Ca and P respectively in rats when fed yoghurt. During ageing of rats, loss of bone weight, ash and Ca content was noticed in control diet, but not so in lactic acid bacteria supplemented animals (Dupis *et al.*, 1985). Consumption of lactic acid bacteria resulted into increased bone calcium and improved bone formation (Kaup, 1988). From the available literature it can be concluded that lactic acid bacteria adhere to the intestinal wall and prevent colonization of pathogens, control neonate diarrhoea, having anticarcinogenic activity and influence digestive and metabolic process. Thereby, lactic acid bacteria improve health and immune status of the animals and human beings.

2.2 YEAST CULTURE:MODE OF ACTION AND EFFECT ON RUMINANT PRODUCTIVITY

2.2.1 Yeasts and Moulds in the Bovine Rumen:-

Yeasts are widely distributed unicellular fungi, belonging mainly to Ascomycetes, which multiply typically by a budding process. Yeast is one of the member of mixed rumen microbial population. Lund (1974) has shown that rumen fluid contains 3500 yeast colonies/ml, with similar number of mould colonies. Nine species of yeast were isolated belonging to *Candida* (including corresponding species of *Pichia*), *Trichosporon*, *Torulopsis*, *Kluyveromyces*, *Saccharomycopsis* and *Hansenula*. The predominating species of *Candida* *krusoei*, *Trichosporon cutaneus* and *Trichosporon capitatum*. The most common moulds were numbers of the Mucoraceal, of which *Absidia corymbifera*, *A.ramosa* and *Mucor pusillus* were identified. *Aspergillus fumigatus* was isolated frequently. The predominating *Candida krusei* and also the rarely isolated species *Torulopsis pintolopesii* and *Kluyveromyces bulgaricus* could reproduce under anaerobic conditions *in vitro*, but another commonly occurring yeast, *Trichosporon capitatum*, were unable to grow under same conditions. The majority of yeast cells were obviously destroyed during passage through the elementary tract, whereas large quantities of moulds could be excreted in a viable state.

2.2.2 Survivability in the GI Tract:-

Certain yeasts and aerobic fungi are normal inhabitants of the rumen but most species isolates were considered to be transient and non-functional, entering the rumen via the feed (Orpin *et al.*, 1988). Dawson (1988) reported that in fermentors fed a ration consisting of 0.75 forage and 0.25 concentrate, the concentration of yeast cells for Yea-Sacc was consistently 3-6 times greater than could be accounted for by the yeast present in the supplements suggesting that some yeast replication was occurring. They (Dawson *et al.*, 1990) also observed the growth of yeast and lactobacilli in the rumen, whereas streptococcus

remained constant when the steers fed with mixed microbes (yeast, lactobacilli and streptococci). The concentration of rumen viable yeast increased when yeast culture was added to the diet of steers (McLeod *et al.*, 1990). In another study by Newbold *et al.* (1990) it has been observed that there was increased counts of yeasts in rumen fluid 1 h after yeast culture addition. Numbers then fell by 61% after 6h, but remained two orders of magnitude higher than that of control animals. Viable yeast persisted in the duodenum and ileum of treated animals, at values 6.5 and 6.8 times higher than control. Earlier Arambel and Rung-Syin Tung (1987) concluded that *Saccharomyces cerevisiae* was unable to maintain a productive population within the rumen ecosystem, and the same type of observation was reported by Harrison *et al.* (1987), that supplemental yeast did not affect yeast count in rumen fistulated Holstein cows.

The viability of yeast cells in the rumen has been considered crucial because El Hassan *et al.* (1993) reported that yeast cultures need to be both viable and metabolically active to have a full stimulatory effect on ruminal fermentation. Similar observation was registered by Girard and Dawson (1994) who reported that the maximum stimulation of growth of several ruminal bacteria was dependent on viable yeast cells and that part of the stimulatory effect was lost when yeast cells were autoclaved. In Rusitech fermentors viable cells were present when received the yeast supplement for upto 14 hours after feeding (Dawson and Newman, 1987). Kung *et al.* (1997) reported the viability of yeast that the number of colonies remained constant after anaerobic incubation in ruminal fluid for 24h. After 24h, the number of yeast colonies declined. It is also suggested that the yeast might be more beneficial in high concentrate diets because such diets would supply nutrients that most closely resemble the malt extract addition.

2.2.3 Mode of Action of Yeast Culture:-

The mechanism of action of yeast culture has been proposed as stabilization of rumen pH in high carbohydrate diet, increased number of

anaerobic and cellulolytic bacteria as well as enhanced microbial protein synthesis. The mode of action of yeast culture, and more recently called "Direct fed microbial" (DFM) (Martin and Nisbet, 1992) has been shown by different workers.

2.2.3.1 pH and Carbohydrate:-

An important factor in the stimulation of forage digestion by yeast culture may be the stabilizing effect of yeast culture on pH and the metabolism of readily fermentable carbohydrate (RFC). Cereal grains in ruminant rations provide substrates for rapidly growing ruminal bacteria (*Streptococcus bovis*) that produce large quantities of lactate (Slyter, 1976). It is reported that rumen pH is mainly associated with concentration of lactic acid in the rumen (Williams, 1990a; Williams *et al.*, 1991). Diets having RFC depressed ruminal pH (Schneider and Flatt, 1975; Offer, 1990; Williams and Newbold, 1990b; Williams *et al.*, 1991) lead to reduction in number of cellulolytic bacteria (Stewart, 1977; Thomas and Rook, 1981), impaired forage degradation (Williams, 1989a,b,c,d) and feed intake (Orskov *et al.*, 1978).

Supplementation of *S.cerevisiae* decreased the lactic acid concentration which ultimately help in elevation of ruminal pH (Williams, 1989a, 1990a) and sufficient to rise the pH by 0.2 to 0.5 units (Dawson, 1987; Williams *et al.*, 1991), pH changes in order of 0.2 units may have considerable effect on cellulolysis when the mean pH level are low or in the region of 6.0 (Williams, 1989b,1990a). This direct reduction of lactic acid concentration could be due to utilization of lactic acid precursor by *S.cerevisiae* or by indirect stimulation of lactic acid utilising, bacteria in the rumen (Dawson, 1990; Martin and Nisbet, 1992; Williams *et al.*, 1991). However, lactic acid is not a substrate used by *S.cerevisiae* (Panchal *et al.*, 1984) but reduce the concentration of starch degradation products. Cartwright *et al.* (1986) also reported that yeast culture addition increased the pH which is due to more utilization of lactate precursors by the yeast.

Recent research (Nisbet and Martin, 1990, 1991) has demonstrated that soluble components in *A.oryzae* and *S.cerevisiae* culture filtrate stimulated lactate uptake by *Selenomonas ruminantium* and *Megasphaera elsdenii*. For this reason in concentrate diet there is more cellulolytic activity due to addition of yeast culture (Carro *et al.*, 1992a).

2.2.3.2 Production of Growth Stimulating Factors:-

Yeast cells in the rumen may supply a chemical growth factor to the cellulolytic microorganisms (Offer, 1990). *S.cerevisiae* produces specific factors like B-vitamins or branched chain fatty acids to stimulate the growth of cellulolytic bacteria (Weidmeier and Arambel, 1985; Dawson, 1987; Chademana and Offer, 1990). Callaway and Martin (1997) also reported that yeast culture provided soluble growth factor (i.e., organic acids, B-vitamins, and amino acids) that stimulated growth of ruminal bacteria that utilize lactate and digest cellulose. The same mechanism has been explained for higher protozoal number due to *S.cerevisiae* treatment (Chung *et al.*, 1989; Carro *et al.*, 1992a). However, there is conflicting evidence as to the microbial need for such preformed nutrients (Redman *et al.*, 1980; Sriskandarajah *et al.*, 1982).

2.2.3.3 Increased Microbial Population:-

Production potential of yeast culture can be associated to their effect on the increased microbial population in the rumen. Addition of yeast culture resulted into increase in concentration of total anaerobic bacteria, but the increase was associated with fibre digesting and lactic acid utilising bacteria (Dawson, 1992). Yeast culture supplementation stimulate the growth of cellulolytic bacteria in the rumen (Harrison *et al.*, 1987, 1988; Weidmeier *et al.*, 1987; Dawson, 1990; Newbold *et al.*, 1995). It is particularly pronounced when forage containing diets are fed (Dawson, 1990). Combined culture of yeast (*S.cerevisiae*), lactobacilli and enterococci supplementation to the diet of steers enhanced the concentration of cellulolytic bacteria in rumen (5 to 40 times). Enhanced growth of rumen bacteria could result in enhanced microbial protein

synthesis and increased contribution of the microbial population to the digestive process.

2.2.4. Influence of Yeast Culture on Rumen Fermentation:-

2.2.4.1 pH and Lactic Acid Concentration:-

An important factor in the stimulation of forage digestion by yeast culture may be the stabilizing effect of yeast culture on rumen pH and on the metabolism of RFC. The details have been discussed earlier [No.2.2.3.1].

2.2.4.2 Bacterial and Protozoal Population:-

Many of the responses to yeast culture supplementation in ruminants can be related to the effects of the viable yeast cells on microbial activities in the rumen. One of the common features in many studies of yeast culture supplementation is its ability to stimulate the growth of certain types of anaerobic bacteria in the rumen (Dawson and Newman, 1987; Harrison *et al.*, 1987; Weidmeier *et al.*, 1987; Dawson, 1990; Newbold *et al.*, 1995). Increased concentration of anaerobic bacteria appears to be closely related to the presence of live yeast cells in the supplemented group and was not observed when supplements were heat treated to kill the yeast (Dawson *et al.*, 1990). The stimulation of fibre degrading bacteria has been observed in both rumen simulating fermentor cultures (Dawson and Newman, 1987) and in the rumen of dairy cattle (Harrison *et al.*, 1987; Weidmeier *et al.*, 1987). It is particularly pronounced when forage containing diets were fed (Dawson *et al.*, 1990). Addition of yeast culture in dairy cow increased total viable bacteria and cellulolytic bacteria 1.3 and 1.5 fold (Weidmeier *et al.*, 1987). Dawson *et al.* (1990) observed that concentration of cellulolytic bacteria in continuous cultures and the rumen of steers receiving supplements containing only yeast were from 5 to 40 times greater than those observed in mixed cultures (yeast, lactobacilli and enterococci) or steers receiving unsupplemented diet. Earlier also, Dawson and Newman (1988) reported that commercial probiotic preparation containing live yeast and lactic acid bacteria had no influence on the concentrations of

anaerobic and cellulolytic bacteria in the Rusitech system. But non-significant improvement of total anaerobic and cellulolytic bacteria was observed in Holstein cows when fed yeast or mixed culture of yeast and *Aspergillus oryzae* (Kim *et al.*, 1992a).

Dawson (1989) indicated that different cellulolytic bacteria responded differently to yeast culture. The yeast strains that stimulate *Bacteriodes succinogenes* in pure cultures did not stimulate *Ruminicola alba* to the same extent. In pure culture media lag times were decreased by 20 to 40% in the cultures of *Prevotella ruminicola*-GA-33, *Butyrivibrio fibrisolvens*-D-1, *Ruminococcus albus*-7, *R.flavefaciens*-FD-1 and *Megasphaera elsdenii*-T-81 treated with yeast (Girard and Dawson, 1994). Cell yields were increased by 7 to 63% when cultures of *Ruminobactor amylophilus*-1024 and *Fibrobactor succinogenes*-S-85 were treated with yeast. Behaviour of *S.cerevisiae* as probiotic preparation varies with the strains. *S.cerevisiae* NCYC240, NCYC1026 and Yea-Sacc stimulated total and cellulolytic bacteria by more than 35%, whereas, *S.cerevisiae* NCYC694 and NCYC1088 failed to stimulate either total or cellulolytic bacterial numbers (Newbold *et al.*, 1995). It has been suggested that an increased bacterial flora in animals fed *S.cerevisiae* is central to the action of yeast in the rumen (Wallace and Newbold, 1992). The effect of yeast on rumen microbes has been summarised in table 1.

Recent investigations revealed that *S.cerevisiae* showed mixed response on rumen protozoal population. Plata *et al.* (1994) demonstrated increased rumen protozoal population in steers fed oat straw based diet with *S.cerevisiae*. Whereas, Kim *et al.* (1992a) observed no response of yeast with or without *A.oryzae* on rumen protozoa in non-lactating Holstein cows. There was a reduction in Entodiniomorphid count in Holstein heifer due to *S.cerevisiae* addition (Miranda *et al.*, 1994). Strains variability of yeast was investigated by Newbold *et al.* (1995) who found that *S.cerevisiae* NCYC-1026 and NCYC-1088 improved rumen protozoal population, whereas NCYC-240, NCYC-694

and Yea-Sacc depressed protozoa population. However, the difference was found non-significant.

2.2.4.3 Ammonia Production and Microbial Protein Synthesis:-

One of the common observations associated with the addition of yeast culture to ruminants and in rumen simulating fermentors has been the reduction of rumen ammonia concentration (Dawson and Newman, 1988; Harrison *et al.*, 1988; Newbold *et al.*, 1990; Williams and Newbold, 1990a,b; Edwards, 1991; Sohn and Song, 1996). The concentration of ammonia was decreased by 10 to 35 per cent *in vitro* (Dawson and Newman, 1987, 1988; Carro *et al.*, 1992a). Similar results have been reported by Harrison *et al.* (1988), Newbold *et al.* (1990) and Chademana and Offer (1990) *in vivo*, suggesting an improved microbial capture of ammonia (Chademana and Offer, 1990). In steers, supplementation of yeast culture slightly reduced ammonia concentration in rumen (18.1 Vs 20.3 mg/100 ml) (Adams *et al.*, 1981). Moloney and Drennan (1994) also reported that rumen ammonia concentration was not affected when yeast was included in low concentrate diet of steer, but was reduced when yeast was added to the diet having high concentrate. In non-lactating Holstein cows addition of yeast culture improved rumen ammonia concentration (199.24 Vs 154.34 $\mu\text{g/ml}$) (Kim *et al.*, 1992a). Whereas, cows supplemented with yeast culture showed a significant reduction of ammonia concentration (87.5 Vs 12.6 mg/l) (Harrison *et al.*, 1987).

Reduced ammonia levels have not been associated with decreased protein degradation or deamination (Williams and Newbold, 1990a). Incorporation of ammonia into microbial protein was enhanced due to supplementation of yeast (Dawson, 1987; Weidmeier *et al.*, 1987; Chademana and Offer, 1990; Carro *et al.*, 1992a; Olson *et al.*, 1994), which was confirmed by greater microbial yield and microbial true protein reaching the duodenum (Gomez-Alarcon *et al.*, 1987; Erasmus, 1991).

The duodenal flow of UDN was significantly increased by the addition

of yeast culture (Carro *et al.*, 1992b). Putnam *et al.* (1997) also observed higher flow of duodenal non-microbial, non-ammonia-N in cows when fed yeast culture. Recently it has been revealed that yeast increased the ammonia N content of culture fluid (Kung *et al.*, 1997).

Erasmus *et al.* (1992) reported higher flow of NAN, microbial-N and dietary-N to the duodenum by addition of yeast culture in the diet of lactating dairy cows. The flow of methionine increased significantly by yeast culture supplementation whereas there were non-significant improvements in the flow of lysine, phenylalanine, threonine and histidine to the duodenum of dairy cows.

2.2.4.4 Total Volatile Fatty Acid and Their Proportions:-

Much of the work on the effects of microbial cultures in the rumen has involved the measurement of concentrations and proportions of volatile fatty acids (VFA) as indicators of microbial activity. Many workers have reported that VFA production is improved due to addition of yeast culture in ruminants. Yeast culture may alter the pattern of VFA production (Teh *et al.*, 1987; Weidmeier *et al.*, 1987; Martin *et al.*, 1989). The TVFA concentration was increased from 172.2 to 184.5 mmol/d (Harrison *et al.*, 1987). Similarly improved TVFA was observed by Martin *et al.* (1989) who have used commercial yeast culture. In lactating cow, Harrison *et al.* (1988) observed that there was higher production of propionate and reduction of acetate to propionate ratio due to supplementation of yeast culture. Similarly, enhanced production of propionate and reduction of acetate to propionate was reported by Martin *et al.* (1989), Dawson *et al.* (1990), Plata *et al.* (1994) and Moloney and Drennan (1994). Recently, enhanced production of TVFA was revealed by Kim *et al.* (1992a), Moloney and Drennan (1994) and Rouzbehan *et al.* (1994) who showed higher TVFA in rumen due to addition of yeast culture. Addition of yeast culture to the diets based on barley grain given to growing bulls increased concentrations of VFA (Mutsvangwa *et al.*, 1992).

Supplementation of commercial yeast to the high concentrate diet of steer

reduced acetate and acetate to propionate ratio; molar proportion of butyrate and iso-acids were higher (Moloney and Drennan, 1994). McLeod *et al.* (1991) and Putnam *et al.* (1997) also observed higher iso-acids; isobutyrate and isovalerate in cattle when fed with yeast culture. In sheep, yeast culture addition increased TVFA non-significantly and acetate:propionate ratio was decreased from 5.01 to 3.81 mainly due to increase in propionate (Newbold *et al.*, 1990).

Another type of observation was shown by Piva *et al.* (1993) who investigated higher tendency of acetate production and increased acetate to propionate ratio in dairy cow fed 30% corn silage, 22% alfalfa hay and 48% concentrate. Addition of *S.cerevisiae* improved acetate *in vitro* (Martin *et al.*, 1989) and *in vivo* (Rouzbehan *et al.*, 1994). In lamb, yeast culture addition alongwith sugar-beet pulp and rolled barley (0.8:0.2) and 60g soyabean meal increased total VFA and acetate (Rouzbehan *et al.*, 1994).

Under Rusitech system, rates of VFA production for the high-concentrate diet were higher in the supplemented vessels than in the control ones, although the molar proportions were unchanged. Carro *et al.* (1992a), Flachowsky *et al.* (1993), Newbold *et al.* (1995) and Kung *et al.* (1997) have observed no positive response of yeast culture supplementation on total VFA production in rumen either *in vitro* or *in vivo*. Dawson *et al.* (1990) have reported that neither the yeast culture supplement nor the mixed microbial (yeast, lactobacilli and enterococci) supplement consistently alter the patterns of VFA production in the continuous cultures or in the rumen of steers. Earlier, several authors (Dawson and Newman, 1988; Martin and Nisbet, 1989; Chademana and Offer, 1990) have reported either small or no change in VFA when yeast culture supplemented to a high roughage diet.

Table 2.1: Response of yeast culture on rumen bacteria

Diet	Bacterial group	Condition	Conc. in control (CFUx10 ⁹ /ml SRL)	Conc. in yeast (CFUx10 ⁹ /mlSRL)	Increase	Reference
40%hay,60% concentrate	Total anaerobic cellulolytic	<i>in vitro</i> <i>in vitro</i>	104.000 13.400	157.000 16.000	50%* 19.4%	Newman & Dawson (1987)
77% hay	Total anaerobic cellulolytic	<i>In vitro</i> <i>In vitro</i>	3.800 0.105	43.600 3.980	11 fold* 38 fold*	Dawson <i>et al.</i> (1990)
50% grass hay		<i>In vitro</i>				
50% concentrate	Total anaerobic cellulolytic	NCYC-240	0.270 0.0043	0.390 0.0059	* *	Newbold <i>et al.</i> (1995)
	Total anaerobic cellulolytic	NCYC-1026	0.270 0.0043	0.370 0.0069	* *	
	Total anaerobic cellulolytic	Yea-Sacc	0.270 0.0043	0.430 0.0059	* *	
50% concentrate	Total anaerobic cellulolytic	<i>In vivo</i> <i>In vivo</i>	19.600 2.500	25.500 3.980	30% 56%*	Weidmeier <i>et al.</i> (1987)
40% silage	Total anaerobic	<i>In vivo</i>	7.760	12.200	58%	Harrison <i>et al.</i> (1988)
60% concentrate	Cellulolytic	<i>in vivo</i>	0.066	0.120	81%	Dawson <i>et al.</i> (1990)
77% hay	Total anaerobic cellulolytic	<i>In vivo</i> <i>In vivo</i>	0.708 0.019	7.41 0.158	10-fold 8-fold	
Barley based finished diet	Total anaerobic Cellulolytic Lactate utilizer	<i>In vivo</i> <i>In vivo</i> <i>In vivo</i>	0.641 0.018 0.0083	1.390 0.035 0.028	116% 89% 3-fold*	
Basal ration	Total anaerobic Cellulolytic	<i>in vivo</i> <i>in vivo</i>	20.83(ln/ml) 11.77(ln/ml)	21.08 12.39		Kim <i>et al.</i> (1992a)

2.2.4.5 Total gas and Methane Production:-

The issue of global warming by CO, CO₂, and CH₄ is of increasing concern to the general public. Out of total global emission of CH₄ from all sources ruminants (cattle, buffalo, sheep, goat and deer) are thought to contribute 12 to 15 per cent (Leng, 1991). Grutzen *et al.* (1986) calculated that cattle in developed countries emitted 55 kg of CH₄/year per animal in contrast to cattle in developing countries, where CH₄ emission is 35 kg/year per animal. Therefore, methane reduction strategies offer an effective means of slowing global warming.

Methane emissions from lactating Holstein cows have been reported to vary from 1.7 to 14.7% of gross energy intake, equivalent to 245 and 419 L of CH₄/day per cow (Holter and Young, 1992). The removal of hydrogen by methanogenesis has been reported to increase the production of acetate (Wolin and Miller, 1983). Both propionate and CH₄ formation compete for the available hydrogen in the rumen as a result of fermentation. Yeast culture supplementation resulted into reduction of methane production in steers by 28% (Williams, 1989a). The addition of yeast culture resulted in a lower methane production for medium concentrate diet, but in a higher for the high concentrate diet (Carro *et al.*, 1992a). Harrison *et al.* (1988) have shown that *in vitro* gas production was not affected due to yeast supplementation.

The effect of probiotic addition on total gas and methane production has not been studied precisely. This area requires work to confirm the effect of probiotics on methane and animal production.

2.2.5 Forage Degradation in Rumen:-

The theory that yeast culture inclusion stimulates appetite has been enthusiastically pursued. Chademana and Offer (1990) reported that addition of Yea-Sacc to concentrate/forage diets in which the proportion of concentrate ranged from 20 to 60 per cent of the diet consistently increased degradability of hay upto 24h (37.3 to 42.2 per cent), but had no effect on the 48h degradability.

Many workers have reported improvement in degradability patterns of nutrients either *in vitro* or *in sacco* due to supplementation of yeast culture (Williams *et al.*, 1991; Kim *et al.*, 1992c; Carro *et al.*, 1992a,b; Mpofu and Ndlovu, 1994; Malik, 1993; Newbold *et al.*, 1995). The extent of DM degradability of hay incubated in the rumen of steers fed the hay and rolled barley diet was increased in the presence of YC at 12h of incubation, but degradation was similar in all treatment groups after 24 h of incubation (Williams *et al.*, 1991). In fistulated Holstein cows rumen DM disappearance was lower for the control (1.66%/h) when compared with two treatments (yeast culture 1.90%/h and yeast culture + *Aspergillus oryzae* 1.74%/h (Kim *et al.*, 1992c).

Supplementing the high concentrate diet with yeast culture resulted in significantly higher DM and NDF degradabilities (Carro *et al.*, 1992a). Moreover, filter paper (cellulose) degradability (24h) tended to be higher for the vessels supplemented with yeast culture than for the control (33% Vs 27%). These results might suggest that yeast culture increased the population of fibre-degrading bacteria and/ or their activity. However, yeast culture had no significant effect on DM and NDF degradabilities with medium or low concentrate diet (Carro *et al.*, 1992a).

Williams and Newbold (1990b) suggested that the effects on digestion may be very subtle and can not easily be identified in studies of total tract digestion. DM degradability was higher in yeast treatment group at 6h and 12h of incubation in rumen (Malik, 1993), similarly DM degradability was higher in mixed culture of *S.cerevisiae* and *L.acidophilus* by 16.55, 11.66 and 4.97% at 6, 12 and 24h.

In vitro digestibility of NDF was increased by yeast or yeast + white rot fungi (*Armillaria heimii*) treatment, with the yeast + fungi combination showed highest values in hay based ration. Strain variability of *S.cerevisiae* was observed on nylon bag degradability of straw and hay (Newbold *et al.*, 1995).

Degradability of straw in the rumen tended to be increased by yeast addition from 16h onward, and this increase was significant for NCYC-1026 at 72 and 96h and just failed to reach significance at 24h and 48h.

Flachowsky *et al.* (1993) have shown negative impact of commercial preparation of yeast (*S.cerevisiae*) on nylon bag degradability (DMD) in fistulated wether.

Table 2.2: Some effects of yeast culture on rumen fermentation

Effect	References
1. Moderated ruminal pH	Malcolm and Kiesling, 1986; Teh <i>et al.</i> 1987; Williams, 1987; Williams <i>et al.</i> 1991
2. Stabilized fermentation	Harrison <i>et al.</i> , 1988
3. Altered VFA production	Teh <i>et al.</i> 1987; Harrison <i>et al.</i> , 1988; Williams, 1989a; Martin <i>et al.</i> 1989; Kim <i>et al.</i> , 1992a; Rouzbehan <i>et al.</i> , 1994
4. Decreased ammonia concentration	Dawson and Newman, 1987; Harrison <i>et al.</i> , 1988; Carro <i>et al.</i> , 1992a
5. Increased concentrations of anaerobic bacteria and cellulolytic bacteria	Weidmeier <i>et al.</i> 1987; Harrison <i>et al.</i> 1988; Dawson <i>et al.</i> , 1990; Kim <i>et al.</i> , 1992a; Newbold <i>et al.</i> 1995
6. Enhanced microbial protein synthesis	Dawson, 1987; Weidmeier <i>et al.</i> , 1987; Chademana and Offer, 1990; Carro <i>et al.</i> , 1992a; Olson <i>et al.</i> , 1994b.
7. Reduced methane production	Williams, 1989a
8. Increased concentrations of yeast in the populations	Dawson <i>et al.</i> , 1990; Newbold <i>et al.</i> 1990
9. Decreased lactic acid concentration	Williams, 1989a, 1990a

10. Altered digestive patterns	Ruf <i>et al.</i> , 1953; Chademana and Offer, 1990; Malik, 1993; Mpofu and Ndlovu, 1994; Newbold <i>et al.</i> , 1995
11. Decreased soluble sugar concentrations	Williams, 1989a
12. Increased ethanol concentrations	Ingledew and Jones 1982; Bruning and Yokoyama, 1988

2.2.6 Feed Intake and Nutrient Utilization as Influenced by *S.cerevisiae*:-

2.2.6.1 Feed Intake:-

Nutrient supply to the animals has been improved due to yeast culture supplement at a fixed intake (Williams *et al.*, 1990a), but in farm trials, its effect on intake appears the most important cause of improved performance. In several studies it has been observed that yeast culture addition increased the feed intake in calves (Bonaldi *et al.*, 1986; Fallon and Harte, 1987; Hughes, 1988; Hancock *et al.*, 1994). Steers (Adams *et al.*, 1981; Williams *et al.*, 1991; Hancock *et al.*, 1994; Mpofu and Ndlovu, 1994; Olson *et al.*, 1994a) and lactating cows (Malcolm and Kiesling, 1986; Quinonez *et al.*, 1988, 1989; Williams, 1990a; Williams *et al.*, 1991; Wohlt *et al.*, 1991; Piva *et al.*, 1993; Scott *et al.*, 1994)

In lactating cows yeast culture supplementation significantly increased the DM intake by 1.2 to 1.6 kg/day with a higher milk production (Williams, 1990a; Williams *et al.*, 1991). The increased milk yield was due to enhanced nutrient supply to the mammary gland rather than mobilization of body reserves. The additional DM intake provided 11.1 MJ of ME/d extra for cows given yeast culture, which is used for milk synthesis and weight gain (Williams *et al.*, 1991). Greater DMI during first 6 weeks of lactation was observed due to yeast supplementation in dairy cattle when fed with silage:grain (1:1, DM basis) and hay (0.9 kg/d) (Wohlt *et al.*, 1991). Whereas, Gomez-Alarcon (1987); Harrison *et al.* (1988), Piva *et al.* (1993) and Scott *et al.* (1994) have observed non-

significant improvement of DMI by lactating cows fed with yeast adjunct. However, some studies indicated no added advantage of yeast culture supplementation to lactating cows on DMI (Erdman and Sharma, 1989; Arambel and Kent, 1990; Wohlt *et al.*, 1991; Quinonez *et al.*, 1988; Harris and Webb, 1990; Swartz *et al.*, 1994; Kung *et al.*, 1997). Differences in response to added yeast might have been due to interactions among yeast, diet and stage of lactation.

2.2.6.2 Digestibility of Nutrients:-

The opportunities for microbial degradation of dietary nutrients occur in the stomach where simple sugars are known to be fermented to more lactic acid (Ratcliffe *et al.*, 1985) and in the rumen where a variety of organisms, mainly anaerobic gram-negative species are capable of fermenting complex carbohydrates and yielding VFAs. In studies of digesta collected from the small intestine of piglets, Hill *et al.* (1970a,b) observed increased deamination activity to occur within 48h of weaning. Apparent breakdown of amino acids was enhanced by adding lactobacilli to the diet.

A number of studies provided evidence indicating that live yeast cells enhances the digestive process in the gastrointestinal tract. Supplementation of *S.cerevisiae* increased the digestibility of protein (Wohlt *et al.*, 1991; Kim *et al.*, 1992b), cellulose (Gomez-Alarcon *et al.*, 1987; Wohlt *et al.*, 1991), fibre (Weidmeier *et al.*, 1987; Gomez-Alarcon *et al.*, 1990), NDF (Kim *et al.*, 1992b; Plata *et al.*, 1994) and ADF (Kim *et al.*, 1992b). Similarly, Weidmeier *et al.* (1987) observed that supplementation of yeast culture increased hemicellulose and CP digestibility in ruminants. DM digestion in the rumen of dairy cows was increased receiving yeast culture (Gomez-Alarcon *et al.*, 1987). Carro *et al.* (1992a) reported that effect on digestibility is dependent on the forage to concentrate ratio, supplementation of yeast culture with high concentrate diet resulted in significantly higher DM and NDF digestibilities in rumen (Rusitech). However, on high forage diet yeast culture had no effect on DM, NDF and

cellulose digestibility. In lactating Holstein cows addition of yeast culture had similar effect on DM, NDF, ADF and hemicellulose digestibilities but CP and cellulose digestibility tended to be improved (Wohlt *et al.*, 1991). In another study by Kim *et al.* (1992b), CF, ADF and NDF digestibilities were significantly improved due to supplementation of yeast to lactating cows. Bhoi (1992) has shown that the fibre digestion was better in the combined culture of yeast and *L.acidophilus* in goats as compared to individual ones. Supplementation of *S.cerevisiae* alone or alongwith *L.acidophilus* increased the *in vivo* DM and CF degradability (Malik, 1993).

Some studies (Harrison *et al.*, 1987; Arambel and Kent, 1988; Williams, 1989a,b; Chademana and Offer, 1990) revealed that DM digestibility was not changed by addition of yeast culture, suggesting that the effects of yeast on digestion may be very subtle and can not easily be identified in studies of total tract digestibility (Williams, 1989a, Gomez-Alarcon *et al.*, 1990) but influences the initial digestion rates of fibrous substrate in the rumen (Dawson, 1992).

Few reports have indicated that addition of yeast culture to ruminants had no added advantage on the digestibility of DM (Harrison *et al.*, 1988), OM (Chademana and Offer, 1990; Carro *et al.*, 1992b), NDF (Harrison *et al.*, 1988; Chademana and Offer, 1990; Carro *et al.*, 1992b; Kim *et al.*, 1994), ADF (Harrison *et al.*, 1988; Carro *et al.*, 1992b; Kim *et al.*, 1994), CP (Kim *et al.*, 1994), cellulose (Carro *et al.*, 1992b), hemicellulose (Harrison *et al.*, 1988) and starch (Harrison *et al.*, 1988). Jung and Varel (1987) noted that increased number of cellulolytic bacteria did not correspond to increases in digestion of cell wall, cellulose or hemicellulose. Kobayashi *et al.* (1995) have observed that concentrations of free methionine, arginine, lysine and isoleucine in blood plasma were significantly higher in early lactation cows fed the yeast culture, suggesting a greater rate of essential amino acid absorption in these cows.

2.2.7 Response of probiotics on productive performance in dairy cows:-

Addition of yeast culture to lactating dairy cows can improve the

lactational performance, though mixed responses have been obtained by some workers.

2.2.7.1 Yield:-

Several workers have reported that supplementation of yeast culture to the lactating cows improved milk yield or FCM yield (Hoyos *et al.*, 1987; Williams, 1989c; Dobos *et al.*, 1989; Huber *et al.*, 1989; Gunther, 1989; Wohlt *et al.*, 1991; Williams *et al.*, 1991; Piva *et al.*, 1993; Swartz *et al.*, 1994; Scott *et al.*, 1994; Kobayashi *et al.*, 1995; Adams *et al.*, 1995; Putnam *et al.*, 1997; Kung *et al.*, 1997). European commercial farm trials demonstrating the effect of including Yea-Sacc in the diet of dairy cows on milk yield revealed that there was increase in milk yield in maximum cases. However, in some cases the effect was not so prominent (Williams, 1989c). Dobos *et al.* (1989) showed that addition of Yea-Sacc increased milk yield and DMI. Lactating animals supplemented with yeast culture tended to loss body weight as lactation progressed. On several occasions when cows were in poor condition at the start of the trial, it was observed that animals gained in body condition over a period of few weeks before responses in terms of milk yield were observed. Using the same commercial preparation, Huber *et al.* (1989) also reported higher milk yield and FCM. Gunther (1989) has shown the same trend in dairy cows indicating that the inclusion of Yea-Sacc increased FCM from 30.10 kg/day to 35.35 kg/day. Milk production by primiparous Holstein cows fed corn silage:grain (1:1, DM basis) and hay (0.9 kg/d) improved by the addition of yeast culture (Wohlt *et al.*, 1991).

In early lactating cows, it has been observed that there was increase in milk yield (Scott *et al.*, 1994) and FCM (Harris and Lobo, 1988; Swartz *et al.*, 1994; Putnam *et al.*, 1997; Kung *et al.*, 1997) due to supplementation of yeast culture in their diet. Whereas, in midlactating cows, more milk yield (Piva *et al.*, 1993; Kobayashi *et al.*, 1995; Adams *et al.*, 1995) and FCM (Piva *et al.*, 1993) were recorded due to addition of yeast culture.

Teh *et al.* (1987) tested the effect of feeding yeast culture with or without added NaHCO₃ on lactating goats and found that both yeast culture and NaHCO₃ increased milk yield without an increase in DM intake. In the high producing cows, there was a 6% increase in milk production due to supplementation of viable mixed microbial feed additives (*S.cerevisiae*, *Streptococcus faecium* and *Lactobacillus acidophilus*), whereas there was no treatment effect in the low productive cows (Hoyos *et al.*, 1987). Williams *et al.* (1991) have shown that there was interaction between diet composition and yeast culture regarding the DMI and milk yield, effects of yeast culture were greatest in diets containing 60:40 (concentrate:forage) ratio.

Some workers reported that milk yield was unaffected by the addition of yeast culture to the diet of cows (Malcolm and Kiesling, 1986; Quinonez *et al.*, 1988; Arambel and Kent, 1990; Erdman and Sharma, 1989; Kim *et al.*, 1992b; Higginbotham *et al.*, 1994). Dawson (1993) stated that yeast culture supplements did not have equal beneficial effects with all types of diets, and it is currently not possible to define the dietary conditions that provide optimum response to yeast culture supplementation.

2.2.7.2 Butterfat:-

Under farm trials, it has been observed that there was positive response on total butterfat production due to addition of Yea-Sacc in dairy cows (Williams, 1989c). Same commercial culture (Yea-Sacc) has been used by Huber *et al.* (1989) and Gunther (1989) who recorded mixed responses on milk fat per centage. Gunther (1989) have observed significant improvement in butterfat per centage in dairy cows; whereas, Huber *et al.* (1989) showed no positive advantage on milk fat. Teh *et al.* (1987) found that both yeast culture and NaHCO₃ increased milk fat per centage in early lactation goats. Other authors have also observed significant improvement in milk fat per centage of lactating cows due to supplementation of yeast culture (Harris and Lobo, 1988; Dobos *et al.*, 1989; Piva *et al.*, 1993; Putnam *et al.*, 1997). Addition of mixed

microbial culture to the lactating cows improved significantly milk fat percentage in the top producers (averaging 19.4% higher) and in the lower producing cows (averaging 14% higher) (Hoyos *et al.*, 1987). Slightly higher milk fat test has been observed by some workers in lactating cows when supplemented yeast culture as feed additive (Quinonez *et al.*, 1988; Williams *et al.*, 1991; Kung *et al.*, 1997). Williams *et al.* (1991) have noticed that milk fat percentage slightly improved only in high concentrate diet (60%). Whereas other authors did not record any difference in milk fat percentage due to yeast culture supplementation (Erdman and Sharma, 1989; Arambel and Kent, 1990; Wohlt *et al.*, 1991; Scott *et al.*, 1994; Swartz *et al.*, 1994; Higginbotham *et al.*, 1994).

2.2.7.3 Milk Protein:-

The results of on-farm trials revealed that there was increase in total milk protein after using Yea-Sacc in dairy cows' rations (Williams, 1989c). Gunther (1989) also observed significant improvement in milk protein in dairy cows using same culture (Yea-Sacc). Higher milk protein was reported by Erdman and Sharma (1989), Dobos *et al.* (1989); Williams *et al.* (1991), Kobayashi *et al.* (1995), Putnam *et al.* (1997). Daily milk protein increased only in cows given high concentrate diet plus yeast culture (Williams *et al.*, 1991) and altered by a significant diet treatment interaction. Yeast culture supplement in early and mid lactation cows increased milk protein by a mean of 5% and 8% respectively (Kobayashi *et al.*, 1995). Similarly, milk protein enhanced in early lactating cows supplemented with yeast culture on low CP (16.1%) diet (Putnam *et al.*, 1997). A culture of one or more viable non-pathogenic lactic acid producing bacteria (*Lactobacillus*, *Streptotoccus*, *Pediococcus*, *Leuconostoc*), amylase and proteinase-producing bacteria (*Bacillus subtilis* or *B.toyoi*) is blended with a protein carrier (dried milk, amino acids) or carbohydrate carriers (Starch, cellulose, sugar) and given to the milk producing ruminants at a daily rate of 10^3 - 10^5 cfu/animal. It is claimed that this facilitated optimum growth of the bacteria

in the stomach/ intestine system of the animal and increased the protein content of the milk (Kvanta and Fischier, 1989).

However, some researchers revealed no added advantage of yeast culture treatment to lactating cow on milk protein per cent (Quinonez *et al.*, 1988; Huber *et al.*, 1989; Arambel and Kent, 1990; Piva *et al.*, 1993; Scott *et al.*, 1994; Swartz *et al.*, 1994; Higginbotham *et al.*, 1994; Kung *et al.*, 1997). Few workers reported reduction in milk protein per cent in cows supplemented with yeast culture additive in its diet (Harris and Lobo, 1988; Wohlt *et al.*, 1991; Kim *et al.*, 1992b; Adams *et al.*, 1995).

This variability of results may be due to behaviour of yeast culture, nature of ration, stage of lactation or interaction of these factors.

2.2.7.4 Lactose:-

Very limited reports are available on the effect of yeast culture influencing milk lactose per centage in lactating cows. Kim *et al.* (1992b) have reported significant improvement of milk lactose per centage in early to mid lactating dairy cows when supplemented with yeast culture. Commercial preparation of yeast culture (Yea-Sacc) had no positive response on lactose per cent in milk of dairy cows (Huber *et al.*, 1989). Similar type of observation was recorded by Arambel and Kent (1990), Scott *et al.* (1994) and Kim *et al.* (1994) who have also shown no added effect of yeast culture on milk lactose per centage in dairy cows. Whereas, per centage of lactose was lower for cows fed yeast culture plus *Aspergillus oryzae* (Higginbotham *et al.*, 1994).

The positive responses reported in literature for the increase in milk yield, butterfat and protein may be the result of increased nutrient supply to the mammary gland, probably as a result of increased nutrients intake rather than mobilization of body resources. The response may be due to the increase in feed intake, increase in the metabolizability of the diet, improvement in the digestibility of the ration and/or from a reduction in some of the obligatory losses such as methane production.

Table2. 3: Yeast culture influencing milk yield and composition in lactating cows

Effect	References
Increased milk yield	Hoyos <i>et al.</i> , 1987; Williams, 1989c; Dobos <i>et al.</i> , 1989; Huber <i>et al.</i> , 1989; Wohlt <i>et al.</i> , 1991; Williams <i>et al.</i> , 1991; Piva <i>et al.</i> , 1993; Scott <i>et al.</i> , 1994; Kobayashi <i>et al.</i> , 1995; Adams <i>et al.</i> , 1995.
Enhanced fat corrected milk (FCM) production	Harris and Lobo, 1988; Huber <i>et al.</i> , 1989; Gunther, 1989; Piva <i>et al.</i> , 1993; Swartz <i>et al.</i> , 1994; Putnam <i>et al.</i> , 1997; Kung <i>et al.</i> , 1997.
Improved butterfat per centage	Hoyos <i>et al.</i> , 1987; Teh <i>et al.</i> , 1987; Harris and Lobo, 1988; Williams, 1989c; Gunther, 1989; Dobos <i>et al.</i> , 1989; Piva <i>et al.</i> , 1993; Putnam <i>et al.</i> , 1997
Increased milk protein per centage	Williams, 1989c; Gunther, 1989; Erdman and Sharma, 1989; Dobos <i>et al.</i> , 1989; Williams <i>et al.</i> , 1991; Kobayashi <i>et al.</i> , 1995; Putnam <i>et al.</i> , 1997.
Altered lactose per centage	Kim <i>et al.</i> , 1992b; Higginbotham <i>et al.</i> , 1994

2.3 PREPARATION AND STORAGE OF YEAST AND LACTIC CULTURES POWDER

The maintenance and use of pure, well characterised probiotic cultures is of paramount importance for the improvement of productive performance of dairy animals. Formerly, dairies often obtained their cultures in a freeze-dried form and then transferred them to obtain bulk cultures. Freeze-drying as a method of preserving bacterial cultures is still utilized extensively. Also during recent years, several freeze dried preparations of lactobacilli have appeared in the market for use in therapy of gastric intestinal disorders. Their efficiency appears

to be related to the extent of viability of the freeze dried preparations (Gordon *et al.*, 1957). A simplified liquid-drying method used for the successful preservation of microorganisms sensitive to freezing and freeze-drying (Malik, 1990) has now been adopted for the preservation of yeasts, using various protective agents.

Feeding form of probiotics culture could be either in powder or tablet (pellet). Available literature indicated that dried form of probiotics cultures could be prepared using skim milk powder as a basal material and cryoprotective agents. However, if the lyophilized cultures are to be used as starter cultures or for therapeutic applications, the survival per cent and hence the number of viable cells have to be much higher. Numerous cryoprotective agents have been used to improve the survival of freeze-dried culture cells (Hecklay, 1961; Lamprech and Foster, 1963). With *L.bulgaricus* the use of malt extract and milk solids as the cryoprotective agents resulted in the survival ratios of 58 and 80 per cent respectively. However, with *L.acidophilus* use of the same two cryoprotective agents showed 89 and 48 per cent survival. With *L.bulgaricus* only two agents, namely milk solids and whey powder, were effective in producing more than 80 per cent survival (Kilara *et al.*, 1976). The same authors found that monosodium glutamate afforded greater protection to *L.acidophilus* with 36 per cent survival after 48 weeks storage. For the streptococci, malt extract was the protective agent of choice resulting in 30 and 22 per cent survivals for *S.thermophilus* and *S.lactis*, respectively. Also, a direct relationship was found between the initial bacterial load and the survival ratio after freeze drying. Malik (1992) observed that at elevated temperatures losses in viability of dried cultures stored without sealing under vacuum are higher than those stored under vacuum. For long term preservation, ampules should be stored at low temperatures (<0°C preferably -30°C) if they are not sealed under vacuum (Malik, 1992). Malik and Hoffmann (1993) showed that long-term survival of yeasts that have been successfully liquid dried is an attractive advantage. Since cultures so preserved can be stored

until required without further attention.

Valdez *et al.* (1983) demonstrated that adonitol has a strong protective effect on lactic acid bacteria during freeze-drying. *L.plantarum* and *S.faecium* were adequately protected by adonitol. Sodium glutamate was also found effective protectant for *L.plantarum* (Pereda-Alardin *et al.*, 1990; Dave *et al.*, 1992). The viability rate of *S.cerevisiae* cells is increased from 30 per cent to 96-98 per cent by using an appropriate protective medium containing 10% skimmed milk with 2 compounds among honey, sodium glutamate, trehalose or raffinose in the freeze-drying process carried out at a 3°C/min cooling rate (Berny and Hennebert, 1991). The literature indicated that viability of probiotic cultures increased when the cultures were freeze-dried and kept under low temperature.

CHAPTER - III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Investigations were carried out in five phases to evaluate the efficacy of probiotics for lactating cows. Six cultures namely, SC-B, SC-522, LA, LP, LL and EF were evaluated for survivability in GI tract of crossbred calves (phase-I). These cultures were then taken in single, double, and triple combination for screening of suitable culture based on *in vitro* rumen fermentation (phase-II). The promising cultures from phase-II were employed for further evaluation in *in vitro* system (Phase-III) and detailed studies including energy and protein fermentation were undertaken. A combination of triple culture namely SC-B+LP+EF, which gave most encouraging results in Phase-III, was fed to lactating cows to elucidate the effect on milk production aspects (Phase-IV). Lastly the triple culture was preserved in powder form using basal material (Phase-V) for field application.

3.1 PRESERVATION AND PRODUCTION OF PROBIOTIC SUPPLEMENTS

3.1.1 Source and Type of Probiotics:-

Six different cultures were obtained from National Collection of Dairy Cultures (NCDC), DM Division of NDRI, Karnal as listed under:-

- | | | |
|----|--|------------|
| 1. | <i>Saccharomyces cerevisiae</i> - Strain-B | (NCDC-47) |
| 2. | <i>S.cerevisiae</i> - Strain-522 | (NCDC-45) |
| 3. | <i>Lactobacillus acidophilus</i> | (NCDC-15) |
| 4. | <i>L.plantarum</i> | (NCDC-25) |
| 5. | <i>Lactococcus lactis</i> | (NCDC-94) |
| 6. | <i>Enterococcus faecium</i> (<i>Streptococcus faecium</i>) | (NCDC-124) |

3.1.2 Maintenance of standard cultures:-

The pure cultures of *S.cerevisiae* of two strains (B and 522) were maintained on the malt extract agar slants and four lactic acid production

bacteria, i.e., *L.acidophilus*, *L.plantarum*, *L.lactis* and *E.faecium* were maintained on the litmus milk. The cultures were inoculated in their respective medium and incubated for 24 to 48h depending upon the growth (*L.acidophilus*, *L.plantarum*, *L.lactis* and *E.faecium* at 37°C and *S.cerevisiae* at 25°C). After optimum growth mouth of each test tube was wrapped in aluminium foil and kept at 4°C upto a fortnight for further use.

3.1.3 Preparation of Litmus Milk:-

About 10-12 ml of litmus milk and 1 to 2 g of CaCO₃ were added in each test tube, and sterilized at 121°C/15 psi for 30 minutes and incubated at 37°C for 24 to 48h depending upon the development of fade or redish colour. Test tubes having fade or reddish colour were discarded. The mouth of each test tube was wrapped with aluminium foil and kept at 4°C for further use upto a period of 30 days.

Composition of litmus milk

Fresh skim milk	100 ml
Litmus solution (5% soln in distilled water)	1.0 to 1.5 ml/100 ml of skim milk
CaCO ₃	1.0 to 2.0 g/test tube

3.1.4 Preparation of Malt Extract Slants:-

About 1.5 to 1.8 per cent agar agar powder was added in the malt extract broth and dissolved by heating. And 10 to 12 ml of this solution was transferred to a test tube and sterilized. The test tubes were kept at 4°C and used within 30 days.

3.1.5 Production and preservation of probiotics:-

For harvesting the live cells of *L.acidophilus* and *L.plantarum*, *L.lactis* and *S.cerevisiae*, the cultures were grown in MRS, LYP and malt extract broth respectively.

Ingredients	g/l
<u>MRS Media</u>	
Peptone	10.00
Meat extract	10.00
Yeast extract	5.00
K ₂ HPO ₄	2.00
Tri-ammonium citrate	2.00
MgSO ₄	0.25
MnSO ₄	0.04
Dextrose	20.00
Tween 80	1.00
pH	6.2-6.6
<u>LYPE Media</u>	
Peptone	5.00
Yeast extract	5.00
Beef extract	10.00
Na ₂ HPO ₄	3.00
Lactose	20.00
pH	No need to adjust
<u>Malt Extract</u>	
Malt extract	2.00
Glucose	10.00
Yeast extract	3.00
Peptone	5.00
pH	5.4

3.1.6 Preparation of Broth and Agar

(i) Broth:- The broths were prepared by dissolving all the ingredients of respective media in one litre of distilled water and autoclaved.

(ii) Agar:- 1.8g of agar-agar powder was dissolved in 100 ml of respective broth by heating and distributed in test tubes and autoclaved.

3.1.7 Procedure for Inoculation and Preservation:-

For each microorganism inoculation was made from the parent culture in 250 ml conical flask having about 150 ml broth. After incubation and having optimum growth, the above inoculum (@1%) was used for further inoculation in a 3 litre flask having about 1 litre of broth. This was incubated at specific temperatures for 24 to 48h depending upon the growth. After having final growth, the biomass was centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and cells were washed with normal saline solution (0.9% NaCl) and again centrifuged at 3000 rpm for 15 minutes. The cells were dispersed in 10 per cent sterilized glycerol solution which acts as a cryoprotecting agent (Satadholders *et al.*, 1979). These cells were preserved in sterilized small tubes placing in the deep freeze for further use within one month. At the time of using the culture for feeding animals, the tubes were thawed in luke warm water.

3.1.8 Purity of cultures:-

The purity of these cultures were confirmed by microscopic examination using Gram's staining techniques and standard plate count.

3.1.9 Counting of live cells:-

For estimation of microbial population, standard plate count technique was used before and one month after keeping in deep freeze.

Standard plate count:-

Procedure:-

Culture tube was vigorously shaken to dispense the cells uniformly. The culture was diluted serially using sterilised saline water. Sixth, 7th and 8th dilution of culture was taken for plating in the agar medium. The petri-dishes having 30 to 250 clearly visible colonies in a similar dilution were considered for counting. The average of two plate counts of the same dilutions was used for accumulating numbers of viable micro-organisms.

PHASE-I

3.2 SURVIVABILITY STUDY OF PROBIOTIC CULTURES

3.2.1 Yeast (*Saccharomyces cerevisiae*) Feeding to Growing Calves:-

Six crossbred (KF) calves (age 7-8 months) were randomly divided into two groups based on their body weight. (Table 3.1).

3.2.1.1 Feeding schedule and yeast feeding:-

Animals of both groups were offered 1.5 kg concentrate mixture (Table 3.2) plus maize fodder *ad lib* for 20 days. Thereafter, total tract survivability of yeast culture was determined by collecting and counting faecal yeast. For consecutive three days, faecal samples (about 50g) were collected from rectum and cultured in agar plate to take the control observations. Subsequently, *S.cerevisiae*-strain B (SC-B) and *S.cerevisiae*-strain 522 (SC-522) were given @ 1.08×10^9 cfu/kg of conc.mixture/animal for 5 days in group-I and group-II respectively. Yeast suspension was mixed in about 250g concentrate mixture and offered to each animal at 9 A.M. followed by remaining amount of concentrate. The faecal observations for treatment period were recorded for 5 days after starting of feeding. After 5 days of yeast feeding, faecal samples were collected from rectum (9 A.M.) for 3 days and survivability was tested again as discussed earlier.

3.2.1.2 Culturing of Faecal Samples:-

Malt extract agar (at 3.5 pH) was used for culturing of faecal samples. The pH of the media was adjusted by adding 10% tartaric acid to inhibit bacterial growth.

3.2.1.3 Plating of Faecal Samples:-

The faecal samples were thoroughly mixed under aseptic condition, and one gram representative sample was taken and diluted with sterile saline water in the ratio of 1:50 and 1:100. After thoroughly mixing 0.5 ml of diluted sample was added in the culture plate, and incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 36 to 40h.

The number of colonies were counted and viable colonies per gram of faeces were calculated.

3.2.14 Microscopic Examination:-

Colonies in the malt extract agar plate were smeared and stained with gentian violet. The slide was examined by compound microscope under oil immersion lense.

Table-3.1. Distribution of crossbred calves (Tharparker x Holstein Friesian) for survivability study of yeast culture

Treatment	Animal No.	Date of birth	Body wt.(kg)
Group-I			
1.5 kg conc.mix + <i>ad lib</i>	KF-5890	10.04.96	98.00
maize fodder + <i>S.cerevisiae</i> -B	KF-5895	19.04.96	121.00
@1.08 x 10 ⁹ cfu/kg of conc.mix.	KF-5887	27.03.96	114.00
		Av.	111.07±6.82
Group-II			
1.5 kg conc.mix + <i>ad lib</i>	KF-5885	26.03.96	115.50
maize fodder + <i>S.cerevisiae</i> -522	KF-5889	08.04.96	108.00
@1.08 x 10 ⁹ cfu/kg of conc.mix.	KF-5893	16.04.96	111.50
		Av.	111.67±2.17

Table-3.2. Ingredients of concentrate mixture used for probiotic survivability experiment

Ingredients	Parts (kg)	CP(g)	TDN(g)
Maize	40	4.40	36.00
Groundnut cake	25	11.25	19.75
Mustard cake	10	3.60	7.50
Wheat bran	22	2.86	14.30
Mineral mixture	2	-	-
Common salt	1	-	-
	100	22.11	77.55

3.2.2 Lactobacilli Feeding to Growing Calves:-

Six crossbred calves were divided into two groups based on their body weight (Table 3.3).

3.2.2.1 Feeding Schedule and Lactobacilli Feeding :-

As detailed in Table 3.3. Faecal samples were collected as mentioned in [No.3.2.1.1].

3.2.2.2 Culturing of Faecal Samples:-

Lactobacilli selective MRS agar media was taken for culturing of faecal samples.

3.2.2.3 Plating of Faecal Samples:-

About 50g of faecal sample was collected directly from rectum of experimental animals. The faeces was thoroughly mixed under aseptic condition. Out of this one gram representative sample was taken in a beaker and diluted with sterile saline water in the ratio of 1:10⁵. After thorough mixing of faecal sample with normal saline 0.5 ml of diluted sample was added in the culture plate. The culture plates were then incubated at 37°C for 24h. The

number of colonies were counted and viable colonies per gram of faeces were calculated by multiplying the dilution factor.

Table-3.3: Distribution of crossbred calves during survivability study of Lactobacilli

Treatment	Animal No.	Date of birth	Body wt.(kg)
Group-I			
1.5 kg conc.mix + <i>ad lib</i>	KF-5885	26.03.96	134.30
oat fodder + <i>L.acidophilus</i>	KF-5887	27.03.96	119.00
@1.08 x 10 ⁹ cfu/kg of conc.mix.	KF-5889	08.04.96	128.30
		Av.	127.20±4.46
Group-II			
1.5 kg conc.mix + <i>ad lib</i>	KF-5890	10.04.96	107.50
oat fodder + <i>L.plantarum</i>	KF-5893	16.04.96	134.50
@1.08 x 10 ⁹ cfu/kg of conc.mix.	KF-5895	19.04.96	131.00
		Av.	124.33±8.49

3.2.2.4 Identification of Acid Producing Bacilli:-

5 ml of diluted milk was taken in the test tube, to it 2 drops of litmus solution was added. After adding 0.2 g CaCO₃ in each tube, it was plugged with cotton and autoclaved. Colony developed in the MRS agar plate was then added in the litmus milk and incubated for 30h at 37°C alongwith control tube. It was observed that there was curd formation and development of red colour in the tubes within 18 to 30h of incubation when added with faecal isolate from MRS agar plate, whereas in the control tubes there was no curd formation and blue litmus colour remained unchanged.

3.2.2.5 Microscopic Examination:-

Faecal isolate from MRS agar plate was smeared on the slide and stained with gentian violet to observe the acid producing bacilli. The stained slide was seen under compound microscope.

3.2.3 Streptococcus Feeding to Growing Calves:-

Six crossbred calves were randomly divided into two groups based on body weight (Table 4). Feeding schedule and Streptococci feeding were detailed in Table 3.4. The experimental procedure was as described in Saccharomyces experiment [No.3.2.1.1].

3.2.3.1 Culturing of Faecal Samples:-

Media:- Streptococci selective LYPA agar media was used for culturing faecal samples.

3.2.3.2 Plating of Faecal Samples:-

It was done as described in Lactobacilli experiment[No.3.2.2.3]

3.2.3.3 Identification of Acid Producing Cocci:-

The test has been conducted as described in lactobacilli feeding [No.3.2.2.4].

3.2.3.4 Microscopic Examination:-

Faecal isolate from LYPA agar plate was smeared on the slide and stained with gentian violet to observe the acid producing cocci. The stained slide was seen under compound microscope.

Table-3.4: Distribution of crossbred calves during survivability study of Streptococci

Treatment	Animal No.	Date of birth	Body wt.(kg)
Group-I			
1.5 kg conc.mix + <i>ad lib</i>	KF-5885	26.03.96	140.40
oat fodder + <i>Lactococcus lactis</i>	KF-5889	08.04.96	121.00
@ 1.08×10^9 cfu/kg of conc.mix.	KF-5893	16.04.96	132.40
		Av.	131.30 $\pm$ 5.64
Group-II			
1.5 kg conc.mix + <i>ad lib</i>	KF-5890	10.04.96	111.50
oat fodder + <i>Enterococcus</i>	KF-5895	19.04.96	138.70
<i>faecium</i> @ 1.08×10^9 cfu/kg of conc.mix.	KF-5887	27.03.96	137.40
		Av.	129.20 $\pm$ 8.87

PHASE-II

3.3 *IN VITRO* EVALUATION OF PROBIOTICS CULTURE

Six species, namely, *Saccharomyces cerevisiae*-B, *S.cerevisiae*-522, *Lactobacillus acidophilus*, *L.plantarum*, *Lactococcus lactis* and *E.faecium* were taken in single, double or triple combination and supplemented in the *in vitro* system using oven dried oat fodder and concentrate mixture (70:30 on DM basis). Total dose of probiotics supplemented in each bottle was 10^9 cfu in 60:40 ratio of yeast and lactic acid bacteria in double combination and 60:20:20 ratio of yeast, lactobacilli and streptococci in triple combination. The *in vitro* bottles were incubated for 48 h at $39^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and following observations were taken:-

1. Total gas production at 4 hourly interval
2. DM degradability
3. Total VFA production
4. Fractional VFA, i.e., Acetate, propionate and butyrate

3.3.1 Feeding of Donor Animals:-

Two rumen fistulated crossbred calves were fed 2.5 kg concentrate mixture plus *ad lib* roughage comprising maize fodder and wheat straw. The concentrate mixture contained groundnut cake 25%, mustard cake 10%, maize 40%, wheat bran 22%, mineral mixture 2%, and common salt 1% (Table - 3.5). The feed was offered to the animals in the morning at 9 A.M. and the animals were given water at 12 A.M. and 4 P.M.

3.3.2 Collection of Rumen Liquor:-

Rumen liquor was collected from the donor animals from all parts of the rumen into a clean thermoflask. The rumen liquor was strained through four layers of muslin cloth. All precautions were taken to ensure the maintenance of optimum temperature while collecting and handling of rumen liquor.

3.3.3 In vitro Technique:-

The substrates containing probiotic cultures were incubated in triplicate according to the procedure described by Tilley and Terry (1963). The buffer was prepared as per the method of McDougall's (1948). The samples were incubated for 48h at $39^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Treatment combinations of probiotic cultures:-

Treatment	Probiotic culture
T ₁	<i>Saccharomyces cerevisiae</i> -Strain B
T ₂	<i>S.cerevisiae</i> -Strain-522
T ₃	<i>Lactobacillus acidophilus</i>
T ₄	<i>L.plantarum</i>
T ₅	<i>Lactococcus lactis</i>
T ₆	<i>Enterococcus faecium</i>
T ₇	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i>
T ₈	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i>
T ₉	<i>S.cerevisiae</i> -B+ <i>E.faecium</i>
T ₁₀	<i>S.cerevisiae</i> -B+ <i>L.lactis</i>
T ₁₁	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i>
T ₁₂	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i>
T ₁₃	<i>S.cerevisiae</i> -522+ <i>E.faecium</i>
T ₁₄	<i>S.cerevisiae</i> -522+ <i>L.lactis</i>
T ₁₅	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i> + <i>E.faecium</i>
T ₁₆	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i> + <i>L.lactis</i>
T ₁₇	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i> + <i>E.faecium</i>
T ₁₈	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i> + <i>L.lactis</i>
T ₁₉	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i> + <i>E.faecium</i>
T ₂₀	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i> + <i>L.lactis</i>
T ₂₁	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i> + <i>E.faecium</i>
T ₂₂	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i> + <i>L.lactis</i>

3.3.4 Feed and Probiotics Supplementation:-

In each flask 0.5g (DM) of substrate (proportion of roughage:concentrate, 70:30) consisting of oat fodder and wheat straw in the ratio of 60:40 was incubated. The ingredient composition of concentrate mixture is detailed in table-3.5. A total dose 10^9 cfu culture was in 1 ml of suspension and added in each flask except control.

Table-3.5: Composition of concentrate used for substrate for *in vitro* studies

<i>Ingredients</i>	<i>Parts</i>	<i>CP(g)</i>	<i>TDN(g)</i>
Maize	40	4.00	36.00
Groundnut cake	25	10.00	19.75
Mustard cake	10	3.70	7.50
Wheat bran	22	3.50	14.30
Min.mix.	2	-	-
Common salt	1	-	-
	100	21.20	77.55

3.3.5 Estimation of *in vitro* Dry Matter (DM) Degradability:-

The contents of the flask after 48h of incubation were filtered through G-I crucibles. The DM was estimated according to AOAC (1984) in the samples of substrates as well as the residues.

3.3.6 Estimation of Total Gas Production:-

Total gas production was measured according to Prins (1987) using artificial saliva as per McDougalls (1948). To each 100 ml bottle 0.5g substrate plus 10 ml SRL plus 40 ml McDougall's buffer were added. Probiotic culture was added in the treatment bottles. Total gas production (ml) was measured at 4h interval upto 48h using pressure transducer (Bailey and Mackey Ltd., UK). The pressure created in the bottle was measured by transducer and sucked to

zero by 50 ml Syringe. The reading shown in the syringe was the gas (ml) produced at that hour.

3.3.7 Estimation of Total Volatile Fatty Acids:-

Total volatile fatty acid (TVFA) concentration in incubation medium was estimated according to Barnet and Reid (1957).

3.3.8 Estimation of Proportion of Volatile Fatty Acids:-

The TVFA was fractioned in accordance with the method of Ervin *et al.* (1961) using Nucon gas chromatograph series 5500 fitted with dual flame ionization detector and a pair of stainless steel columns packed with chromosorb 101 to serve as stationary phase.

PHASE-III

3.4 *IN VITRO* EVALUATION OF SELECTED PROMISING PROBIOTIC CULTURES

Seven promising probiotic cultures or culture combinations were selected based on the IInd phase *in vitro* study. The cultures used were as follows:

- P₁ *Saccharomyces cerevisiae*-Strain B (SC-B)
- P₂ *Enterococcus faecium* (EF)
- P₃ *S.cerevisiae*-Strain-522 (SC-522) and *L.plantarum* (LP) combination
- P₄ *S.cerevisiae*-522 (SC-522) and *E.faecium* (EF) combination
- P₅ *S.cerevisiae*-522 (SC-522), *L.acidophilus* (LA) and *L.lactis* (LL) combination.
- P₆ *S.cerevisiae*-522(SC-522), *L.plantarum*(LP) and *E.faecium* (EF) combination
- P₇ *S.cerevisiae*-B(SC-B), *L.plantarum*(LP) and *E.faecium* (EF) combination

The following observations were recorded in *in vitro* study:-

- i) Total gas and methane production of 18h and 24h of incubation.
- ii) DM degradability
- iii) TVFA and VFA proportions
- iv) $\text{NH}_3\text{-N}$, Total bacteria and Protozoal count

3.4.1 Feeding of the Donor animals, collection of rumen liquor and other details are similar to Phase-II *in vitro* experiment.

3.4.2. Feed and Probiotic Culture Supplementation:-

In each flask 0.5g of substrate consisting 40:60, 60:40 and 70:30 roughage:concentrate were taken. Out of total roughage, oven dried maize fodder and wheat straw were taken in the ratio of 60:40. The ingredients of concentrate mixture were same as in Phase-II and mentioned in the table 3.5. The dose of probiotic culture was kept same as mentioned earlier(3.3.4) in Phase-II. Each treatment was done in triplicate.

3.4.3 Estimation of total gas production was done as detailed earlier(3.3.6).

3.4.4 Estimation of Methane:-

The gas sample was taken from each bottle with the help of gas tight Hamilton syringe and the same was analysed for methane using gas chromatograph (Nucon, Series 5700). Gas chromatograph was fitted with a stainless steel column (2m x 2mm) which was filled with porapack as the stationary phase. The flow of carrier gas (H_2) was adjusted to 40 ml/min. The temperature of injection part, oven and detector (TCD) was adjusted to 40°C. The known amount of standard mixture of CO_2 and CH_4 (EDT Research, London), containing 27.4 per cent methane and 2.2 per cent CO_2 , was injected into the column with the help of gas tight Hamilton syringe. Peaks were identified and the peak area of each peak of standard mixture was compared with those of unknown samples to calculate the gas quantitatively.

3.4.5 DM Degradability, TVFA and VFA proportions were determined as described in earlier sections.

3.4.6 Ammonia-N:-

Ammonia-N was estimated according to Conway (1962).

3.4.7 Counting of Bacterial Population:-

Rumen bacterial population was counted according to Gall *et al.* (1949) using nigrosin stain. The rumen liquor samples were diluted with distilled water, so that there were about 10 to 40 microorganisms per field. The dilution usually was 1:1000. Firstly 1:10 or 1:100 dilution was made and shaken for 3 minutes. Further dilution were made from this and shaken by hand vigorously. After shaking, 0.01 ml of the properly diluted sample was transferred by pipette to the centre of 1 sq.cm round area of the slide. The diluted sample was mixed with loopful of nigrosin stain and the material was spread over the 1 sq.cm area of the slide. This slide was dried quickly on a very hot electric plate. Then 10 fields from all parts of the slide were counted. The bacteria appeared white against a black background, while debris takes the darker colour and was not confused with the bacteria.

$$\text{Number of bacteria/ml} = \text{Dilution factor} \times \text{microscopic factor} \times \text{average number per field} \times 100$$

Where, microscopic factor is the number of microscopic fields per square centimeter.

3.4.8 Counting of Rumen Protozoa:-

Sub-sampling and fixation of rumen protozoa for counting was done as per the method described by Dehority (1984). One ml strained liquor from *in vitro* fermentation bottles was taken with a wide orifice pipette and transferred to a 5 ml stoppered vial. To this 1 ml of formal saline (physiological saline having 10% formaldehyde) was added. Finally 2 drops of Brilliant green dye (2g Brilliant green dye and 2 ml of glacial acetic acid diluted to 100 ml with distilled water) was added. The mixture was allowed to stand overnight at room

temperature. If required further dilution was done in 30% glycerol. The number of total protozoa, holotrichs and spirotrichs were counted as described by Kamra *et al.* (1991). The number was calculated as follows:

$$\text{No. of protozoa/ml of liquor} = \frac{\text{No. of protozoa} \times \text{dilution factor} \times 1000}{\text{Volume of sample taken}}$$

3.4.9 In vitro Estimation of Total-N, NPN, TCA-ppt-N and ³⁵S Incorporation into Microbial Protein:-

Two most promising treatments were taken to study the ³⁵S incorporation into microbial protein. The culture treatments were as follow:-

S₁ = *S.cerevisiae*-B

S₂ = *S.cerevisiae*-B+*L.plantarum*+*E.faecium*

Following observations were recorded under *in vitro* system:

- i) ³⁵S incorporation into microbial protein
- ii) Total-N, NPN and TCA-ppt-N

3.4.9.1 ³⁵S incorporation into Microbial Protein:-

In vitro ³⁵S incorporation into microbial protein was determined as per Gupta and Singh (1985). For the determination of *in vitro* ³⁵S incorporation in TCA precipitable protein. 0.5g substrate, 40 ml McDougall's buffer, 50 μ Ci Na₂ ³⁵SO₄ and 10 ml rumen liquor were incubated for 24h. After incubation, protein was precipitated using 10 ml of 30% TCA. The content was centrifuged at 3500 rpm and washed several times with 10 ml TCA and absolute alcohol to remove free radio-activity. This washed sediment was then dissolved in 0.1N NaOH and volume was made upto 100 ml. Subsequently 0.2 ml of this solution was taken, alongwith 10ml of scintillation fluid (Dioxane- 250 ml + Toluene 250 ml + Ethanol 150 ml + Naphthalene 65g + PPO 3.25 g), in a vial and radioactivity counted in a liquid Scintillation Counter.

3.4.9.2 Total-N, NPN and TCA-ppt-N:-

Total-N, NPN and TCA-ppt-N in the *in vitro* incubation medium were estimated using standard micro-Kjeldahl (AOAC, 1984).

PHASE-IV

3.5 EFFECT OF PROBIOTICS ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND COST OF MILK PRODUCTION

3.5.1 Selection, Distribution and Management of Animals:-

Ten crossbred (Holstein Friesian x Tharparker) lactating cows were selected from National Dairy Research Institute, Karnal herd for lactation trial. Animals were divided into two groups based on the milk yield and stage of lactation. The observations on the animals were recorded after one week of adjustment.

3.5.2 Management:-

Animals were kept in the open byre having concrete flooring and sheds on mangers as well as sitting place of the animals. The animals were milked after brushing, washing and cleaning of animals and udders thrice a day (5 A.M., 12 A.M. and 6 P.M.). Concentrate mixture was offered just before milking for immediate let down of milk..

Period of Experiment:- The lactation trial was started from the 4th week of Oct'97 and completed by the 4th week of Jan'98. During the trial period meteorological observations were recorded from the Meteorological Observatory of Central Soil Salinity Research Institute, Karnal.

3.5.3 Allotment of Treatments:-

Animals in group-I served as control and in group-II were offered probiotic culture with the feed. The treatment group was provided a dose of 10×10^9 cfu/animal/day of probiotic culture in triple combination (*S.cerevisiae*-B, *L.plantarum* and *E.faecium* in the ratio of 6:2:2) alongwith concentrate mixture at the time of noon milking.

3.5.4 Feeding of Lactating Cows:-

Animals in both the groups were maintained on similar feeding schedule following NRC (1989). All the animals were offered green fodder and concentrate mixture at the ratio of 60:40. Depending on availability of green

fodder the animals were offered *ad lib* maize fodder at the initial stage of lactation trial, in the middle of the trial maize fodder plus berseem fodder (60:40 ratio) was given to each animal and at the last phase of trial maize fodder was replaced with oat fodder. One third of the total concentrate mixture was offered to each animal before milking (Table-3.7). Fresh water was offered immediately after each milking.

3.5.5 Daily Feed Intake:-

The weight of feed offered and residue left were recorded daily and their DM was determined ($100^{\circ}\text{C}\pm 4^{\circ}\text{C}$). Feed samples pooled for four days in each fortnight were subjected for CP estimation and OM determination as per AOAC (1984).

3.5.6 Milk Yield and Composition:-

Daily milk yield was recorded and representative samples were collected (50 ml each) at each milking time at two consecutive days in each fortnight. The representative sample was used for analysis of total solid, total-N, fat, lactose and ash contents.

3.5.7 Total Solid in Milk:-

Known weight (10-15 g) prewarmed milk sample (38°C) was taken into preweighed crucible. The crucible was kept in hot air oven at 85 to 90°C for 24-30 h, and weighed.

3.5.8 Total-N and Ash:-

Total-N and ash per cent of milk samples were estimated as per AOAC (1984).

3.5.9 Milk Fat:-

The samples of milk stored at refrigeration were tested for fat percentage using Milco Tester (Reit, Jaipur). No preservative was added and the bottles were thoroughly stirred and warmed by dipping in lukewarm water to ensure homogeneous distribution of fat globules, before analysing the samples.

3.5.10 Lactose:-

Lactose per centage in milk was calculated as follows:-

$$\text{Lactose \%} = \text{Total solid \%} - \text{Fat \%} - \text{Protein\%} - \text{Ash\%}$$

3.5.11 Fat corrected Milk (FCM) Yield:-

It was calculated according to ARC (1980) using following formula:-

$$\text{FCM} = 0.4M + 15F$$

FCM = Fat corrected milk at 4% fat

M = Milk yield in kg

F = Weight of fat contained in it

3.5.12 Efficiency of Milk Production:-

Milk yield (kg)/kg DMI and FCM(kg)/kg DMI were calculated using standard procedures.

3.5.13 Estimation of Economics of Milk Production:-

Variable cost and fixed cost were considered during the estimation of economics of milk production (Kumar, 1997).

3.5.13.1 Variable Cost:-

The variable cost comprised of the expenses on green fodder, concentrate, probiotics, human labour, veterinary aid and miscellaneous expenses such as ropes, chains, electricity, water charges and minor repair charges on cattle shed.

3.5.13.2 Fixed Cost:-

Fixed cost refers to those costs which remain unchanged over the short period of time. Fixed costs computed in the present study included depreciation on fixed assets like animal, cattle shed and stores, dairy equipments and interest on fixed capital.

3.5.13.3 Gross Cost:-

It included all the expenditure incurred on the milch animals. Thus gross cost was estimated by adding the fixed and variable costs together.

3.5.13.4 Gross Income:-

The value of milk and dung was added to estimate the gross income.

3.5.13.5 Net income:-

This was calculated by subtracting the gross cost from that of gross income.

Cost of Dietary Component and Milk

Dietary component	Cost(Rs)/quintal
Concentrate mixture	512.25
Maize fodder	55.00
Oat fodder	73.80
Berseem	78.80
Probiotics(single dose/animal)	1.44
Milk/Kg	10.00

Table-3.6. Details of experimental animals and their distribution in two groups for milch trial

Treatment	Animal No.	Date of birth	Body wt (kg)	No. of lactation completed	Days in lactation	Per day yield of present lactation(Kg)
Group-I(Control)						
	KF-5217	29.07.90	375.00	3	108	11.00
	KF-5171	26.01.90	372.00	3	75	10.58
<i>Ad lib</i> green fodder + conc. mix.(60:40 ratio)	KF-5286	11.04.91	372.00	3	118	9.40
	KF-5525	10.10.93	330.00	1	68	8.70
	KF-5397	27.06.92	373.00	2	93	7.25
			364.40±8.62		92.40±9.48	9.39±0.67
Group-II						
<i>Ad lib</i> green fodder + conc.mixture(60:40 ratio) + Probiotics culture (SC-B+LP+EF)	KF-5301	29.05.91	302.00	3	108	12.02
	KF-5326	12.10.92	335.00	2	48	10.00
	KF-5231	13.09.90	350.00	3	117	9.73
	KF-5471	20.03.92	390.00	1	98	8.13
	KF-5132	16.09.89	380.00	5	71	6.90
			351.40±15.84		88.40±12.71	9.36±0.45

Table 3.7: Ingredients of concentrate mixture used for lactation trial

Ingredients	Parts	CP(Kg)	TDN(Kg)
Maize	15	1.65	13.50
Barley	18	1.82	15.48
Groundnut cake	18	8.10	14.22
Mustard cake(oiled)	15	5.40	11.25
Wheat bran	20	2.60	13.00
Rice bran	11	1.05	7.04
Min.mix.	2	-	-
Common salt	1	-	-
	100	20.62	74.49

3.5.14 Effect of Probiotics on Feed Utilization:-

Conventional digestibility trial was conducted on all ten lactating crossbred cows during the last phase of lactation trial. Animals were adapted for two days to the environments of metabolism shed followed by 6 days collection period. The animals were weighed on two consecutive days before offering feed and water prior and at the end of digestibility trial.

3.5.14.1 Aliquoting of Dung:-

For estimation of nitrogen, 1/200 aliquot of the total dung voided each day was preserved with 25% H₂SO₄ in pre-weighed plastic bottles. At the end of collection period, the bottles were again weighed, mixed thoroughly and 10g sample was taken for nitrogen estimation following Kjeldahl method. For the estimation of proximate principles and cell wall components 1/100 part of the total dung was dried daily at 100°C in the oven and pooled for 6 days for each animal. At the end of collection period, the dried pooled dung samples were ground in a Willey mill for the estimation of proximate principles and cell wall components.

3.5.14.2 Estimation of Proximate Principles of Feeds and Dung Samples:-

Samples of feed and dung were analysed for proximate principles, i.e., moisture, total ash, nitrogen, EE, CF and NFE as described in AOAC (1984).

3.5.14.3 Estimation of cell wall components:-

NDF component of feeds and faeces was analysed as per method described by Robertson and VanSoest (1981). ADF of feeds and faeces were estimated in accordance with modified method of Clancey and Wilson (1976). Cellulose and lignin were estimated as per the method of Goering and VanSoest (1970).

STATISTICAL ANALYSIS

Statistical analysis of data was carried out as per Snedecor and Cochran (1980).

PHASE-V

3.6 PREPARATION OF PROBIOTIC CULTURE POWDER

Based on *in vitro* studies and lactation trial, following promising cultures were preserved in powder form, so that field application of the technology become possible.

- i) *Saccharomyces cerevisiae* - Strain-B (SC-B)
- ii) *Lactobacillus plantarum* (LP)
- iii) *Enterococcus faecium* (EF)

Two basal materials as well as two cryprotective agents were employed in different combinations along with individual probiotic culture.

Two basal materials were:-

- i) Skim milk powder - 15 per cent in the suspension.
- ii) Sucrose - 5 per cent in the suspension.

Following two cryprotective agents were used:

- 1) Adonitol -3 per cent of the skim milk based suspension.
- 2) Mono-sodium glutamate - 3 per cent of the skim milk based suspension.

Following treatment combinations were made in order to evaluate the basal material alongwith protective agents which can preserve the above mentioned cultures in viable form for longer duration.

Table 3.8: Details of preservatives used during preparation of culture powder

Treatment	Dose infused (cfu)/ml of suspension	Total dose/vial (cfu)
1. Skim milk powder + SC-B	1.78x10 ⁹	17.8x10 ⁹
2. Skim milk powder + LP	1.73x10 ⁹	17.3x10 ⁹
3. Skim milk powder + EF	1.70x10 ⁹	17.0x10 ⁹
4. Skim milk powder + Adonitol + SC-B	1.78x10 ⁹	17.8x10 ⁹
5. Skim milk powder + Adonitol + LP	1.73x10 ⁹	17.3x10 ⁹
6. Skim milk powder + Adonitol + EF	1.70x10 ⁹	17.0x10 ⁹
7. Skim milk powder + Mono-sodium glutamate + SC-B	1.78x10 ⁹	17.8x10 ⁹
8. Skim milk powder + Mono-sodium glutamate + LP	1.73x10 ⁹	17.3x10 ⁹
9. Skim milk powder + Mono-sodium glutamate + EF	1.70x10 ⁹	17.0x10 ⁹
10. Skim milk powder + Sucrose + SC-B	1.78x10 ⁹	17.8x10 ⁹
11. Skim milk powder + Sucrose + LP	1.73x10 ⁹	17.3x10 ⁹
12. Skim milk powder + Sucrose + EF	1.70x10 ⁹	17.0x10 ⁹
13. Skim milk powder + Sucrose + Adonitol + SC-B	1.78x10 ⁹	17.8x10 ⁹
14. Skim milk powder + Sucrose + Adonitol + LP	1.73x10 ⁹	17.3x10 ⁹
15. Skim milk powder + Sucrose + Adonitol + EF	1.70x10 ⁹	17.0x10 ⁹
16. Skim milk powder + Sucrose + Mono-sodium glutamate + SC-B	1.78x10 ⁹	17.8x10 ⁹
17. Skim milk powder + Sucrose + Mono-sodium glutamate + LP	1.73x10 ⁹	17.3x10 ⁹
18. Skim milk powder + Sucrose + Mono-sodium glutamate + EF	1.70x10 ⁹	17.0x10 ⁹

3.6.1 Preparation of Probiotics Culture:-

Culturing and processing of the above mentioned cultures were done as per method mentioned in earlier chapter (No.3.1). The cultures were preserved in 1 per cent glycerol suspension and kept in deep freezer(-15°C to -20°C) for further use.

3.6.2 Preparation of Culture Powder:-

The required dose of culture was added in the skim milk based suspension as mentioned under each treatment group. The suspension was added in the sterilised petridish and freeze dried under vacuum. Complete drying of the culture suspension took about 12h. The dried culture powder were then kept in the sterilized glass vial and sealed under vacuum. The sealed vials were preserved in the refrigerator (4°C to 6°C) for testing of viability of cultures at fortnight intervals.

3.6.3 Viability Study of Probiotic Cultures:-

To estimate the viability of above preserved cultures in the powder form it was cultured at fortnight intervals. The culture powder was dissolved in 10 ml of autoclaved saline water. The culture suspension was diluted serially upto 10^8 . For plating 10^7 and 10^8 dilution were taken. The media used for plating of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* cultures were Malt extract, MRS and LYP A agar, respectively. After incubation period of 36-40h, the colonies were counted for viability study of the cultures.

CHAPTER - IV

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

PHASE-I

4.1 TOTAL GASTROINTESTINAL TRACT(GI) SURVIVABILITY OF YEAST, LACTOBACILLI AND STREPTOCOCCI:-

Total GI tract survivability of Yeast, Lactobacilli and Streptococci was studied in crossbred calves. The species taken for the study were *S.cerevisiae* (Strain-B and 522) (Plate 1 and 2), *L.acidophilus* (Plate 5), *L.plantarum* (Plate 6), *L.lactis* (Plate 9) and *E.faecium* (Plate 10). A total dose of 1.08×10^9 cfu/kg conc. mix.of above cultures were supplemented to three animals in each group for 5 days. Faecal counts in the respective groups were recorded during control (pretreatment), treatment period of 5 d and post treatment period to observe the survivability status of the above cultures.

4.1.1 Total Gastrointestinal Tract Survivability of Yeast:-

4.1.1.1 Composition of Feed During Yeast Culture Feeding:-

Animals were fed maize fodder and concentrate during the experiment. The OM, CP, CF, EE, NFE and ash content in concentrate mixture were 89.89, 20.65, 8.45, 4.41, 56.38 and 10.11 per cent. For maize fodder the respective values were 84.90, 11.69, 25.16, 1.88, 46.17 and 15.10 per cent (Table 4.1A).

4.1.1.2 Feed Intake During Yeast Feeding:-

The DM intake (kg/day) was 4.06 and 3.88 in T_1 (SC-B) and T_2 (SC-522). The DMI (kg)/100kg b.wt and DMI (g)/ $W^{0.75}$ kg were 3.67 and 3.44; 119.09 and 112.21 in T_1 and T_2 , respectively (Table 4.5). The OMI also followed the similar trend. The total CP intake (g/day) was 598.42 and 578.20 in T_1 and T_2 . The value of CPI (kg)/100 kg b.wt and CPI(g)/ $W^{0.75}$ kg were

540.48 and 513.20; 17.52 and 16.59 in respective groups. No significant difference was observed on DMI, OMI and CPI per unit body weight between these two treatment groups. The intake was comparable with NRC (1989) standard.

4.1.1.3 Effect of Yeast Feeding on its Total GI Tract Survivability:-

Viable yeast colonies were counted in the faecal samples (per gram) using malt-extract agar media. Colonies were stained and examined under microscope. It was ascertained that only yeast growth occurred in the agar plate and no bacterial growth took place due to lowering of pH (3.5) (Plate 3 and 4). Observations were recorded during control period (before start of yeast feeding), treatment period (during yeast feeding) as well as during post treatment period. It was observed that least square mean (LSM) value ($\log_{10}$ cfu/g of faeces) of yeast culture was higher in treatment period (2.919) than control period (2.791) during *S.cerevisiae*-B feeding. But the difference was not significant. The LSM value during post treatment period was 2.803 and comparable to pretreatment period (Table 4.2). In *S.cerevisiae*-522 fed group also, LSM was higher during treatment period (2.944). The values in control (2.752) and post treatment period (2.892) were comparable. Therefore, in the treatment group of *S.cerevisiae*-522, same trend was observed as in *S.cerevisiae*-B fed group.

The faecal colony counts (LSM) of *S.cerevisiae*-B and *S.cerevisiae*-522 during treatment period were 2.919 and 2.944 and there was no significant difference between the two yeasts (Table 4.4). The results indicated that a fraction of yeast which was fed to the animals remained viable and appeared in the faeces in viable state, during treatment period. Probably yeast fed to the animals could not multiply for long in GI tract and hence, faecal yeast counts in the post-treatment period were similar with that of control.

Increased counts of yeasts were observed in rumen fluid, 1h after yeast culture addition. Number then decreased by 61% after 6 h, but remained higher

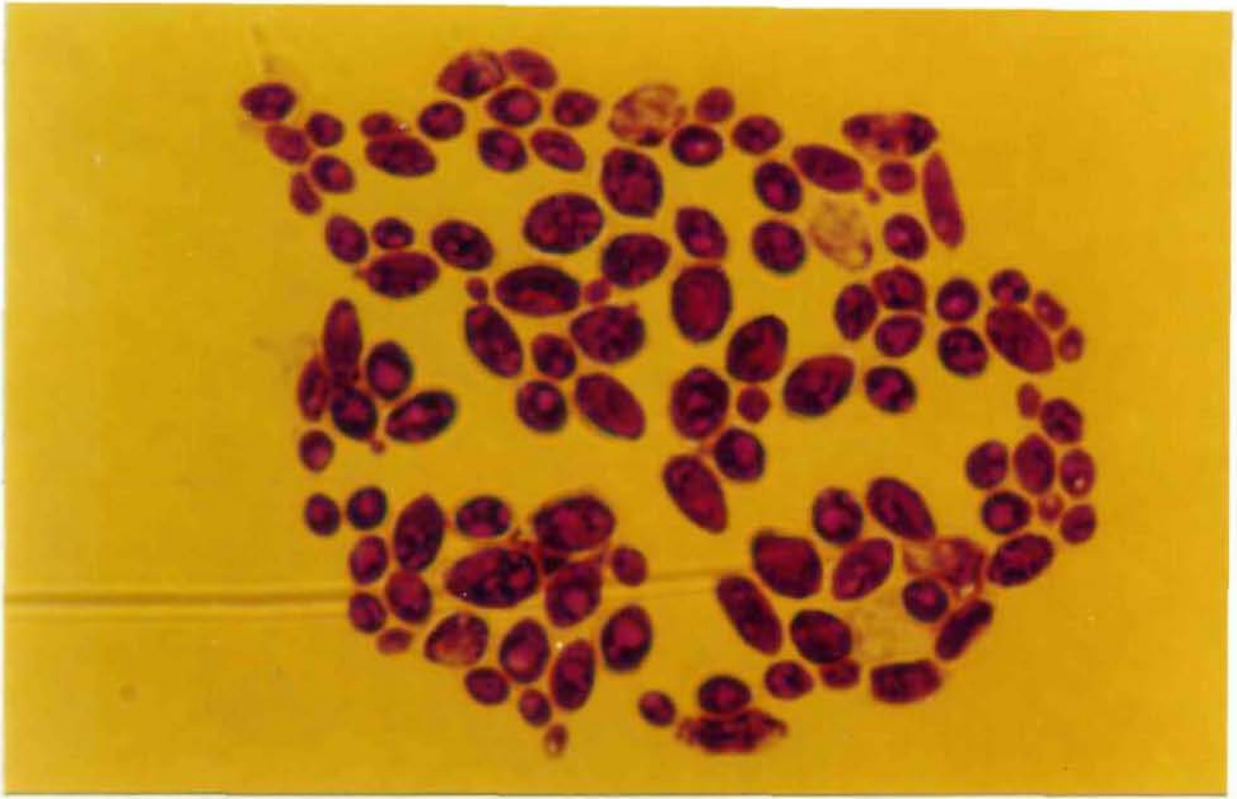


Plate-1 : *Saccharomyces cerevisiae* - Strain-B(SC-B)

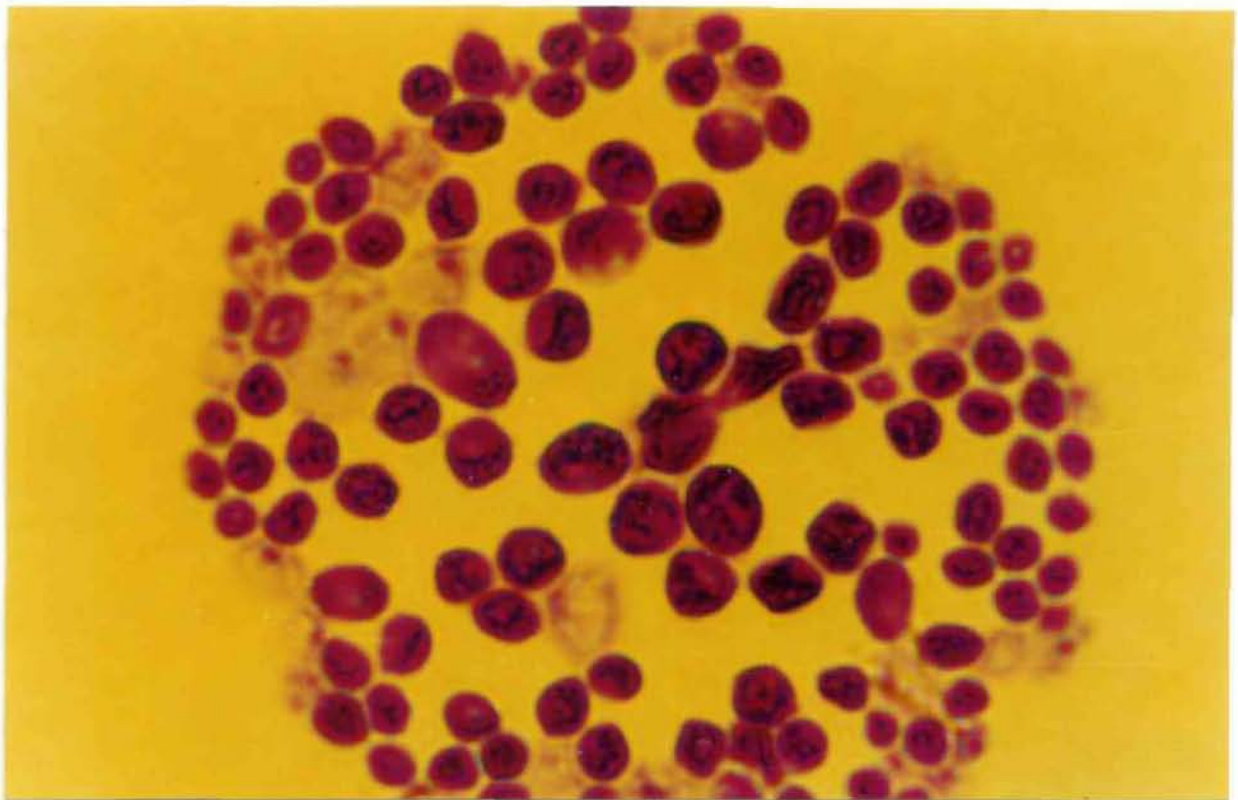


Plate-2 : *S.cerevisiae*-Strain-522 (SC-522)

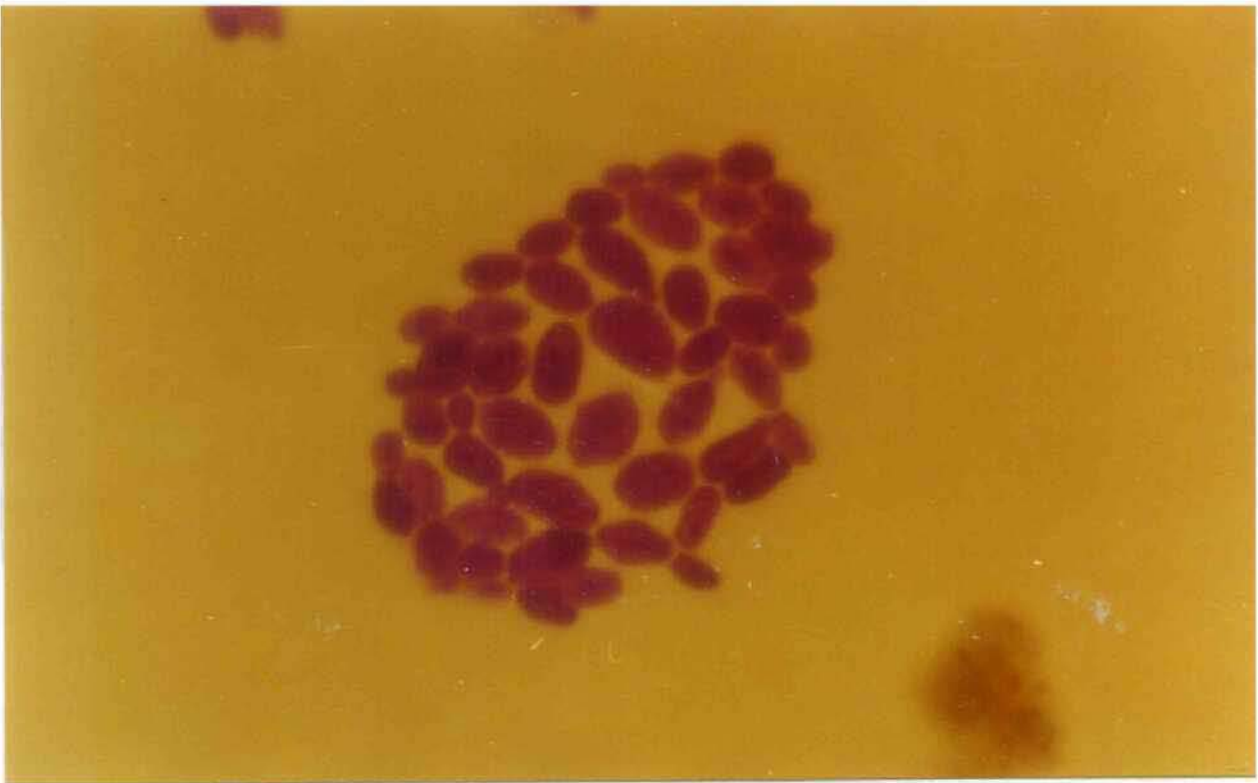


Plate-3 : Faecal yeast from SC-B treated calves



Plate-4 : Faecal yeast from SC-522 treated calves

by two orders of magnitude than that of control. Viable yeast persisted in the duodenum and ileum of experimental animals, at values 6.5 and 6.8 times higher than control (Newbold *et al.*, 1990). In present study, faecal yeast count remained 50% and 49% higher during treatment period over control in SC-B and SC-522 treatment groups (Table 4.3). The similar type of observations have been reported by Dawson (1988) and McLeod *et al.* (1990) who have shown increased number of viable yeast *in vitro* or *in vivo*. In Rusitech fermentors also viable cells were present when received the yeast supplement upto 14 hours after feeding (Dawson and Newman, 1987). Recent investigations also supported the same concept, wherein Kunj *et al.* (1997) observed the viability of yeast and the number of colonies remained constant after anaerobic incubation in ruminal fluid for 24h, thereafter the number of colonies declined. In contrast, Arambel and Rung-Syin Tung (1987) concluded that *S.cerevisiae* was unable to maintain a productive population in the rumen ecosystem, and similar type of observation was reported by Harrison *et al.* (1987) who found that supplementation of yeast did not affect yeast count in rumen fistulated Holstein cows.

4.1.2 Total Gastrointestinal Tract Survivability of Lactobacilli:-

4.1.2.1 Composition of Feed During Lactobacilli Feeding:-

The OM, CP, CF, EE, NFE and ash contents of concentrate mixture were 89.95, 21.12, 8.32, 4.38, 56.13 and 10.05 per cent, respectively. For oat fodder the respective values were 89.03, 12.57, 27.05, 3.02, 46.39 and 10.97 per cent (Table 4.1B).

4.1.2.2 Feed intake during Lactobacilli feeding:-

The intake data during survivability study of lactobacilli is presented in the Table 4.6. The total DM intake was 3.18 and 3.15 kg in *L.acidophilus* (LA) and *L.plantarum* (LP) supplemented groups. The DMI (kg)/100 kg b.wt and DMI(g) /W^{0.75} kg were 2.48 and 2.52; 83.46 and 84.10 in respective groups. The total OM intake (kg/day), OMI(kg)/100 kg b.wt and OMI(g)/W^{0.75} kg were

2.82 and 2.72; 2.20 and 2.17; 74.10 and 72.64 in LA and LP groups respectively. The total CP intake was 591.88 and 586.59 g/day in LA and LP groups. The CP intake (g)/100 kg b.wt and CPI(g)/W^{0.75} kg were 461.51 and 469.90; 15.54 and 15.69 in LA and LP fed groups of crossbred calves. The intake parameters between the two groups were statistically similar. The DMI and CPI in two groups of animals were comparable with NRC (1989) standards.

4.1.2.3 Effect of Lactobacilli Feeding on Their Total GI Tract Survivability:-

Faecal samples of experimental animals were cultured in MRS agar plate and the colonies in the agar plate were transferred to the litmus milk. After incubation upto 18-30h, it was observed that blue colour changed to red and there was curd formation. Therefore, the microorganisms isolated from faeces were acid producing and rod shaped (bacilli) in nature (Plate 7 and 8).

It was observed that faecal *L.acidophilus* counts (LSM of Log₁₀ cfu/g faeces) were statistically higher (P<0.01) during treatment period (6.365) as compared to pretreatment control (6.092) (Table 4.2). The counts during post treatment period were 6.214, and marginally higher over control, though the difference between control and post treatment counts was non-significant. In *L.plantarum* fed crossbred calves the faecal bacilli counts were similar during control (6.176), treatment (6.147) and post treatment period (6.258). The overall mean values were 6.224 and 6.193 in *L.acidophilus* and *L.plantarum* fed groups (Table 4.2). Comparison of faecal counts during treatment period of the two groups revealed that counts in *L.acidophilus* group (6.365) were higher (P<0.01) than *L.plantarum* group (6.147) (Table 4.4).

During treatment period of *L.acidophilus* there was 73% improvement of acid producing bacilli counts over control (Table 4.3) and remained 24% higher during post treatment period. Therefore, it appeared that some portion of lactobacilli passed through the rumen and intestine and were found viable in the faeces. Results of the present study are in agreement with earlier findings.



Plate-5 : *Lactobacillus acidophilus* (LA)



Plate-6 : *L. plantarum* (LP)

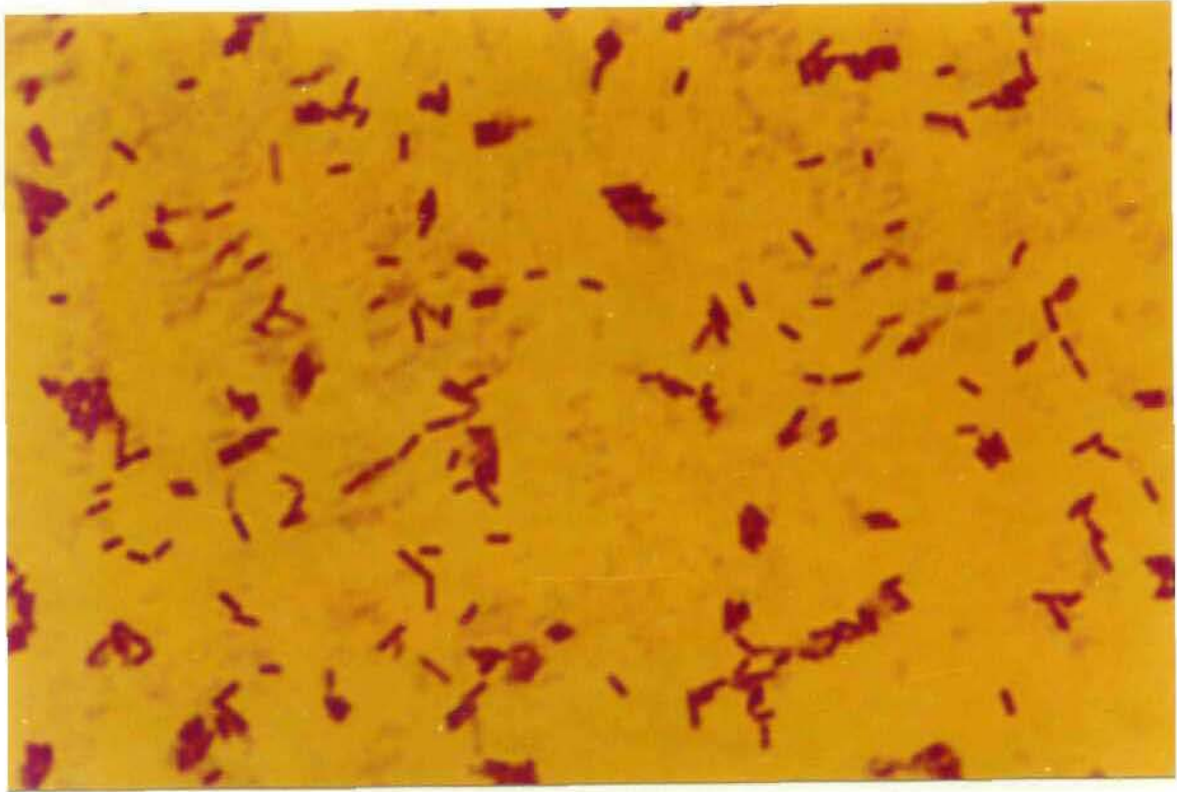


Plate-7 : Faecal acid producing Bacilli from LA treated calves



Plate-8 : Faecal acid producing Bacilli from LP treated calves

In calves faecal lactobacilli increased when fed lactobacilli in diet (Ellinger *et al.*, 1978; Gilliland *et al.*, 1980; Jenny *et al.*, 1991; Abu-Tarboush *et al.*, 1996). In different species of animals lactic acid bacteria survive gastroenteric passage and were found in the faeces (Garvie *et al.*, 1984; Ratcliffe *et al.*, 1986; Bianchi-Salvadori, 1986). Jenny *et al.* (1991) noted that counts of lactobacilli tended to remain higher than those for coliforms in calves given mixed concentrates containing lactobacilli.

Since in *L.plantarum* fed group no significant difference was observed among control, treatment and post treatment periods, it seems that *L.plantarum* cell could not pass live to the lower tract, and hence no change on faecal counts was observed. The present results are corroborated by earlier reports of Abu-Tarboush *et al.* (1996), who have shown that calves fed *L.acidophilus* 27SC had higher faecal lactobacilli counts than calves fed mixed Lactobacilli (*L.acidophilus* and *L.plantarum*). Sometimes faecal measurements may not be a satisfactory indicator of the behaviour of these organisms elsewhere in the GI tract (Pollmann *et al.*, 1980; Ward and Nelson, 1982). During comparison between two groups of lactobacilli feeding (*L.acidophilus* and *L.plantarum*) significantly higher faecal counts of acid producing bacilli were observed in *L.acidophilus* fed group (Table 4.4) than that of *L.plantarum* group. This observation is indicating that *L.acidophilus* is more resistant to the digestive process in calves than that of *L.plantarum*. Earlier reports by Abu-Tarboush *et al.* (1996) is also supportive in this context.

4.1.3 Total Gastrointestinal Tract Survivability of Streptococci:-

4.1.3.1 Composition of Feed:-

The chemical composition data of concentrate and oat fodder are presented in Table 4.1C. The OM, CP, CF, EE, NFE and ash per cent in concentrate mixture were 89.88, 20.66, 8.18, 4.45, 56.59 and 10.12, respectively. For oat fodder respective values were 91.23, 7.56, 29.97, 2.57, 51.13 and 8.57.

4.1.3.2 Feed Intake During Streptococci (*L.lactis* and *E.faecium*) Feeding:-

The feed intake during *L.lactis* and *E.faecium* feeding has been presented in the Table 4.7. The total DM intake (kg/day), DMI (kg)/100 kg b.wt and DMI(g)/W^{0.75} kg were 4.79 and 4.76; 3.63 and 3.68; 122.92 and 120.23 in *L.lactis* (LL) and *E.faecium* (EF) treated groups respectively. The OM intake data was in similar order. The CP intake (g) was 553.10 and 551.45 in LL and EF treated groups. The CP intake (g)/100 kg b.wt and CPI(g)/W^{0.75} kg were 418.24 and 425.91; 14.18 and 13.93g in LL and EF treated groups, respectively. Similar type of intake pattern of DM, OM and CP were recorded between these two probiotic cultures treated groups. The animals in both the groups fulfilled their nutrient requirements as per NRC (1989).

4.1.3.3 Effect of *Lactococcus lactis* and *Enterococcus faecium* Feeding on Their Total GI Tract Survivability:-

Faecal samples of experimental animals were cultured in LYPA agar plate and the colonies (the short chains of cocci, Plate 11 and 12) were transferred to the sterilised litmus milk. The tubes were incubated upto 18 to 24h and observed that blue colour of the litmus milk was changed to reddish and curdling of milk occurred, thereby indicating acid producing nature of the cultures.

It was observed that LSM values (Log₁₀ cfu/g faeces) of faecal colony counts of acid producing cocci were similar in three periods, i.e., control (5.950), treatment period (6.055) as well as post treatment period (5.807) in *L.lactis* (LL) supplemented calves (Table 4.2). During treatment period the counts (cfu/g faeces) appeared 2.6 times higher over control (Table 4.3). But the difference was not significant. Whereas, in EF supplemented group LSM value was marginally higher in treatment period (6.257) as compared to control (6.070) period. But during post treatment period the counts were significantly less ($P<0.01$) than control and treatment period (Table 4.2).

In *E.faecium* supplemented animals, faecal acid producing cocci were

non-significantly higher during treatment period over control, and number decreased significantly during post treatment period. During treatment period, faecal counts (cfu/g faeces) remained 1.75 times higher over control (Table 4.3). Therefore, it can be suggested that *E.faecium* remained partially viable in gastro-intestinal tract.

In a different kind of study, Muralidhara *et al.* (1977) observed that piglets given *L.lactis* in concentrate for upto 8 weeks after birth showed a progressive decline in coliform counts in faecal samples. Only mild diarrhoea was observed lasting 2-4 days in gnotobiotic pigs when inoculated with *Streptococcus faecium* prior to artificial *E.coli* infection (Underdahl *et al.*, 1982). Havrevoll *et al.* (1988) also found that diarrhoea was less pronounced in calves supplemented with *S.faecium* and less diarrhoea was mainly due to competitive attachment of receptor sites in the intestines between lactic acid bacteria and coliform (Muralidhara *et al.*, 1977). This finding indirectly indicated that a portion of *L.lactis* or *E.faecium*, which was fed to either pig or calves, were passing to the GI tract for competitive attachment. These findings supported our observations, indirectly, although the *E.coli* counts were not estimated in the present experiment. During comparison of two groups of crossbred calves treated with *L.lactis* and *E.faecium* no difference in faecal colony counts was observed during treatment period (6.055 Vs 6.257) (Table 4.4).

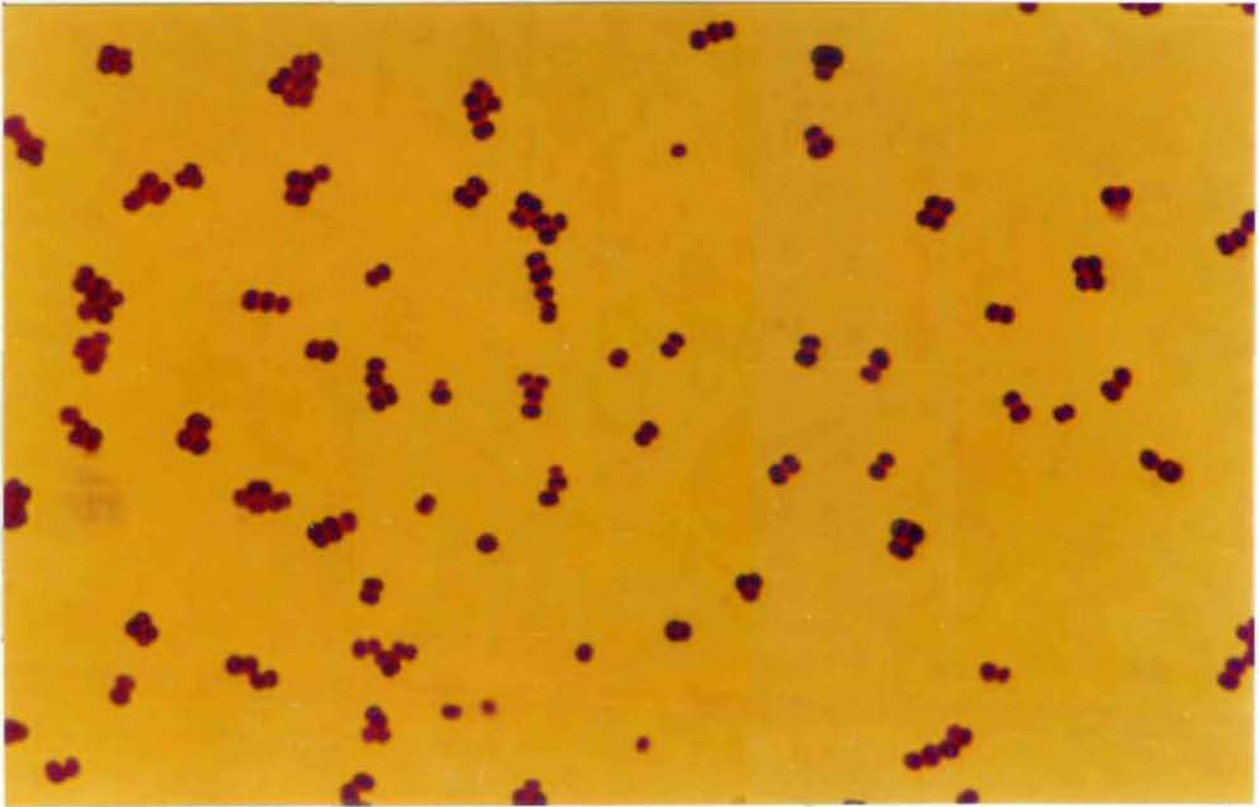


Plate-9 : *Lactococcus lactis* (LL)



Plate-10 : *Enterococcus faecium* (EF)

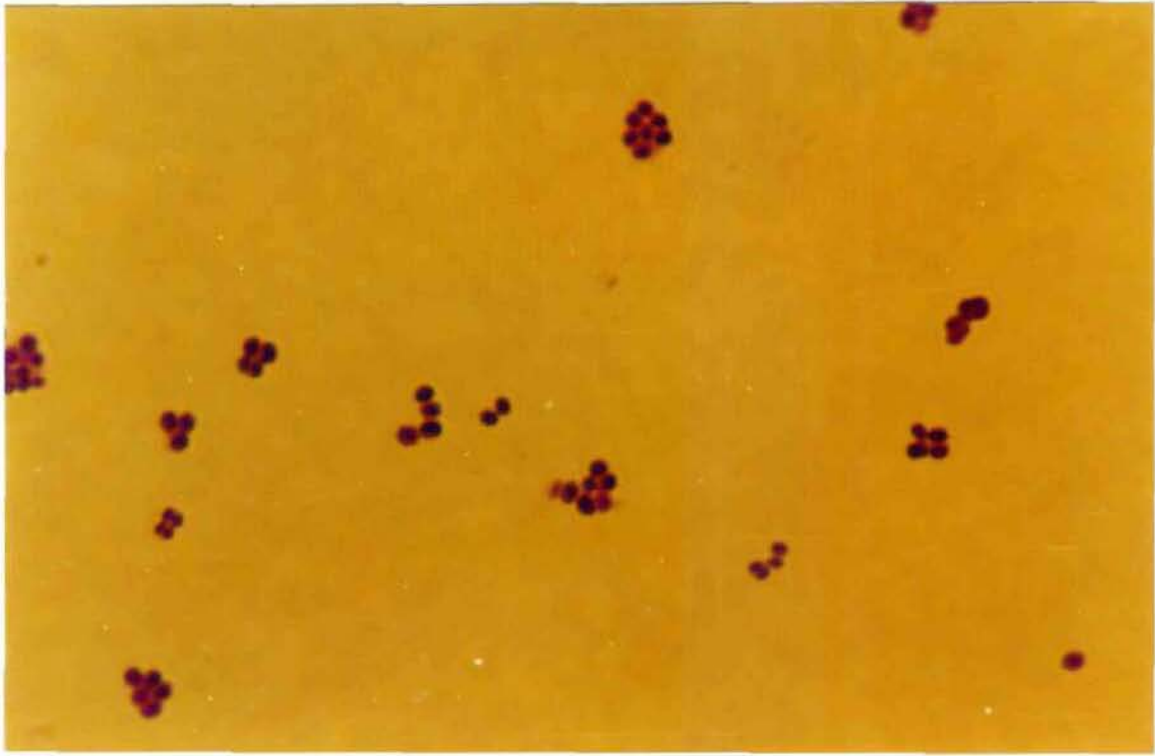


Plate-11 : Faecal acid producing cocci from LL treated calves



Plate-12 : Faecal acid producing cocci from EF treated calves

Table 4.1 Chemical composition of feedstuff fed during survivability study
(% DM basis)

A. During yeast feeding

Parameter	Concentrate	Maize fodder
OM	89.89	84.90
CP	20.65	11.69
CF	8.45	25.16
EE	4.41	1.88
NFE	56.38	46.17
Ash	10.11	15.10

B. During Lactobacilli feeding

Parameter	Concentrate	Oat fodder
OM	89.95	89.03
CP	21.12	12.57
CF	8.32	27.05
EE	4.38	3.02
NFE	56.13	46.39
Ash	10.05	10.97

C. During Streptococci Feeding

Parameter	Concentrate	Oat fodder
OM	89.88	91.23
CP	20.66	7.56
CF	8.18	29.97
EE	4.45	2.57
NFE	56.59	51.13
Ash	10.12	8.57

Table 4.2 Treatment and post-treatment effect of probiotic cultures on their presence in faeces (least square mean of Log₁₀ cfu/g faeces)

Parameter	Yeast feeding		Lactobacilli feeding		Streptococci feeding	
	SC-B	SC-522	LA	LP	LL	EF
Control	2.791±0.072	2.752±0.115	6.092±0.057 ^a	6.176±0.047	5.953±0.105	6.070±0.133 ^b
Treatment effect	2.919±0.056	2.944±0.089	6.365±0.044 ^b	6.147±0.036	6.055±0.081	6.257±0.103 ^b
Post-treatment effect	2.803±0.072	2.892±0.115	6.214±0.057 ^{ab}	6.258±0.047	5.807±0.105	5.666±0.133 ^a
Overall effect	2.838±0.039	2.863±0.062	6.224±0.031	6.193±0.025	5.938±0.056	5.997±0.071
Significance	NS	NS	**	NS	NS	**

NS = Non-significant; **P<0.01; a,b means figures within a column with different superscripts differ significantly

SC-B = *Sacharomyces cerevisiae*-strain B; SC -522 = *S.cerevisiae* - 522

LA = *Lactobacillus acidophilus*; LP = *L.plantarum*

LL = *Lactococcus lactis*; EF = *Enterococcus faecium*

Table 4.3 Average number of colony forming units per g of faeces of calves fed rations supplemented with probiotic cultures

Parameter	Yeast feeding		Lactobacilli feeding		Streptococci feeding	
	SC-B	SC-522	LA	LP	LL	EF
Control period	672.22	800.00	1.39×10^6	1.58×10^6	0.96×10^6	1.22×10^6
Treatment period	1020.00	1193.33	2.41×10^6	1.50×10^6	2.51×10^6	2.14×10^6
Post-treatment period	644.44	788.89	1.73×10^6	1.85×10^6	0.73×10^6	0.54×10^6

SC -B = *S.cerevisiae*-B; SC-522 = *S.cerevisiae* - 522

LA = *L.acidophilus*; LP = *L.plantarum*

LL = *Lactococcus lactis*; EF = *E.faecium*

Table 4.4 Comparison of probiotics LSM of Log₁₀ cfu/g of faeces during treatment period

Parameter	Treatment-I	Treatment-II	Significance
<u>Yeast feeding</u> @	2.919±0.089	2.944±0.089	NS
<u>Lactobacilli feeding</u> @@	6.365±0.037	6.147±0.037	**
<u>Streptococci feeding</u> @@@	6.055±0.124	6.257±0.124	NS

**P<0.01

NS = Non-significant

@ Treatment-I for SC-B and Treatment-II for SC-522

@@Treatment-I for *L.acidophilus* and Treatment-II for *L.plantarum*

@@@Treatment-I for *L.lactis* and Treatment-II for *E.faecium*

Table-4.5 Intake of calves during survivability study of *S.cerevisiae*-B(SC-B) and *S.cerevisiae*-522 (SC-522)

Parameter	SC-B	SC-522	Significance
<u>DM Intake</u>			
Total(kg)	4.06±0.02	3.88±0.11	NS
Kg/100kg b.wt	3.67±0.25	3.44±0.04	NS
g/W ^{0.75} Kg	119.09±6.31	112.21±1.71	NS
<u>OM Intake</u>			
Total(kg)	3.52±0.02	3.36±0.09	NS
Kg/100kg b.wt	3.18±0.22	2.98±0.04	NS
g/W ^{0.75} Kg	103.02±5.45	97.11±1.46	NS
<u>CP Intake</u>			
Total(g)	598.42±2.32	578.20±11.67	NS
g/100kg b.wt	540.48±36.70	513.20±3.65	NS
g/W ^{0.75} Kg	17.52±0.90	16.59±0.26	NS

NS= Non-significant

Table-4.6 Intake of crossbred calves during survivability study of *L.acidophilus*(LA) and *L.plantarum* (LP)

Parameter	LA	LP	Significance
<u>DM Intake</u>			
Total(kg)	3.18±0.04	3.15±0.06	NS
Kg/100kg b.wt	2.48±0.08	2.52±0.15	NS
g/W ^{0.75} Kg	83.46±1.81	84.10±3.67	NS
<u>OM Intake</u>			
Total(kg)	2.82±0.04	2.72±0.05	NS
Kg/100kg b.wt	2.20±0.07	2.17±0.13	NS
g/W ^{0.75} Kg	74.10±1.61	72.64±3.14	NS
<u>CP Intake</u>			
Total(g)	591.88±1.92	586.59±2.64	NS
g/100kg b.wt	461.51±16.64	469.90±33.09	NS
g/W ^{0.75} Kg	15.54±0.41	15.69±0.81	NS

Table-4.7 Intake of calves during survivability study of *L.lactis* (LL) and *E.faecium* (EF)

Parameter	LL	EF	Significance
<u>DM Intake</u>			
Total(kg)	4.79±0.02	4.76±0.07	NS
Kg/100kg b.wt	3.63±0.15	3.68±0.29	NS
g/W ^{0.75} Kg	122.92±3.79	120.23±3.93	NS
<u>OM Intake</u>			
Total(kg)	4.34±0.02	4.31±0.07	NS
Kg/100kg b.wt	3.28±0.14	3.33±0.26	NS
g/W ^{0.75} Kg	111.35±3.43	108.89±3.59	NS
<u>CP Intake</u>			
Total(g)	553.10±0.79	551.45±3.27	NS
g/100kg b.wt	418.24±18.45	425.91±32.02	NS
g/W ^{0.75} Kg	14.18±0.46	13.93±0.39	NS

PHASE-II

4.2 EFFECT OF PROBIOTIC CULTURES ON *IN VITRO* DM DIGESTIBILITY, GAS PRODUCTION AND TVFA PRODUCTION

In vitro trials have been conducted to screen the most promising probiotic cultures from following single, double and triple combinations of cultures. The cultures used in this study were as following:

Treatment	Probiotic culture	Abbreviation	Total dose per <i>in vitro</i> bottle (cfu)
<u>Control</u>	No culture	-	-
T ₁	<i>Saccharomyces cerevisiae</i> -B	SC-B	10 ⁹
T ₂	<i>S.cerevisiae</i> -522	SC-522	10 ⁹
T ₃	<i>Lactobacillus acidophilus</i>	LA	10 ⁹
T ₄	<i>L.plantarum</i>	LP	10 ⁹
T ₅	<i>Lactococcus lactis</i>	LL	10 ⁹
T ₆	<i>Enterococcus faecium</i>	EF	10 ⁹
<u>Double culture combination</u>			
	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i>	SC-B:LP	10 ⁹ (6:4)
T ₇	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i>	SC-B:LP	10 ⁹ (6:4)
T ₈	<i>S.cerevisiae</i> -B+ <i>E.faecium</i>	SC-B:EF	10 ⁹ (6:4)
T ₉	<i>S.cerevisiae</i> -B+ <i>L.lactis</i>	SC-B:LL	10 ⁹ (6:4)
T ₁₀	<i>S.cerevisiae</i> -	SC-522:LA	10 ⁹ (6:4)
T ₁₁	522+ <i>L.acidophilus</i>	SC-522:LP	10 ⁹ (6:4)
T ₁₂	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i>	SC-522:EF	10 ⁹ (6:4)
T ₁₃	<i>S.cerevisiae</i> -522+ <i>E.faecium</i>	SC-522:LL	10 ⁹ (6:4)
T ₁₄	<i>S.cerevisiae</i> -522+ <i>L.lactis</i>		

<u>Triple culture combination</u>			
T ₁₅	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i> + <i>E.faecium</i>	SC-B:LA:EF	10 ⁹ (6:2:2)
T ₁₆	<i>S.cerevisiae</i> -B+ <i>L.acidophilus</i> + <i>L.lactis</i>	SC-B:LA:LL	10 ⁹ (6:2:2)
T ₁₇	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i> + <i>E.faecium</i>	SC-B:LP:EF	10 ⁹ (6:2:2)
T ₁₈	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i> + <i>L.lactis</i>	SC-B:LP:LL	10 ⁹ (6:2:2)
T ₁₉	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i> + <i>E.faecium</i>	SC-522:LA:EF	10 ⁹ (6:2:2)
T ₂₀	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i> + <i>L.lactis</i>	SC-522:LA:LL	10 ⁹ (6:2:2)
T ₂₁	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i> + <i>E.faecium</i>	SC-522:LP:EF	10 ⁹ (6:2:2)
T ₂₂	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i> + <i>L.lactis</i>	SC-522:LP:LL	10 ⁹ (6:2:2)

A mixed feed consisting of oat fodder, wheat straw and concentrate (42:28:30, DM basis) was used as substrate.

4.2.1 Chemical Composition of Substrate:-

The substrate constituents were analysed for proximate principles (Table 4.9). The OM, CP, CF, EE, NFE contents were 89.55, 20.78, 8.26, 4.52 and 55.99 per cent, respectively for concentrate mixture. The wheat straw and oat fodder composition was comparable to reported values (NRC, 1989), and presented in table 4.9.

4.2.2 Effect of Probiotics Supplementation on DM Degradability:-

The substrate was incubated for 48 h in *in vitro* rumen fermentation system with individual and combinations of various probiotic cultures and analysed for DM disappearance (Table 4.13). The *in vitro* DM degradability (IVDMD%) at 48h incubation ranged from 68.24 (control) to 77.65 (T₉). The IVDMD was significantly ($P<0.01$) affected due to treatments. Maximum DM degradability was observed in T₇, T₉, T₁₂, T₁₃, T₁₄, T₁₇, T₂₂ in double and triple combinations, respectively. This contention also received support from total gas production which was also increased in *in vitro* system (Table 4.13). These results are in agreement with the earlier reports (Chademana and Offer, 1990), that addition of Yea-Sacc to concentrate/forage diets consisting 20 to 60 per cent concentrate consistently increased DM degradability of hay upto 24 h (37.3 to 42.2 per cent). Other workers (Williams *et al.*, 1991; Kim *et al.*, 1992c; Carro *et al.*, 1992a,b; Mpofu and Ndlovu, 1994; Malik, 1993; Newbold *et al.*, 1995) have also reported improvement in degradability pattern of nutrients either *in vitro* or *in sacco* due to supplementation of yeast culture. Similar trend was observed in *in sacco* DM disappearance in fistulated Holstein cows (Williams *et al.*, 1991). Carro *et al.* (1992a) observed higher filter paper (cellulose) degradability (24h) for the *in vitro* vessels supplemented with yeast culture than for the control (33% Vs 27%). These results suggested that yeast culture increased the population of fibre-degrading bacteria and/or their activity. In the mixed culture of *S.cerevisiae*-B and *L.acidophilus*, *in sacco* DM degradability was higher by 16.55, 11.66 and 4.97 per cent at 6, 12 and 24h of incubation (Malik, 1993). In the present study, the maximum DM degradability was observed in double (T₇, T₉, T₁₂, T₁₃ and T₁₄) and triple culture combinations (T₁₇, T₂₂) in which yeast was having major proportion (60%).

4.2.3 Response of Probiotics on Total Volatile Fatty Acid (TVFA) Concentration:-

Data on TVFA concentration on various treatment groups are presented in the table 4.13. The value of TVFA (mmol/100 ml SRL) was 7.23 in control whereas in various treatments the concentration ranged between 4.57(T₅) to 9.70 (T₂₀). Higher (P<0.05) TVFA production was recorded in triple combination of yeast, lactobacilli and streptococci groups (T₁₇, T₁₈, T₂₀, T₂₁ and T₂₂) than control. The trend of TVFA production was also encouraging in other triple combination treatment groups (T₁₅, T₁₆, T₁₉) as well as in some double combination groups (T₉, T₁₀, T₁₂, T₁₃, T₁₄). The concentration was similar in single culture treatments, except, T₄ and T₅ which showed depression of VFA production as compared to control.

The TVFA concentration was statistically similar among all single and double combination of probiotic culture treatment groups (P<0.05) except *Lactobacillus plantarum* (T₄) and *Lactococcus lactis* (T₅) treatment groups where significant (P<0.05) reduction of TVFA concentration was observed (Table 4.13). Maximum TVFA was measured in T₁₇ and T₂₀. In this study, the substrate contained high roughage (70%), which may be one of the factors of non-significant change in TVFA production in yeast treatment groups (T₁ and T₂) over control. Harrison *et al.* (1988), Carro *et al.* (1992a), Flachowsky *et al.* (1993), Newbold *et al.* (1995) and Kung *et al.* (1997) have observed no positive response of yeast culture supplementation on TVFA production in rumen either *in vitro* or *in vivo*. Dawson *et al.* (1990) reported that neither the yeast culture supplement nor the mixed microbial (yeast, lactobacilli or enterococci) supplement consistently alter the pattern of VFA production in the continuous cultures (Rusitech) or in the rumen of steers. Whereas other workers have reported improvement in VFA production due to addition of yeast culture in ruminants (Harrison *et al.*, 1987; Weidmeier *et al.*, 1987; Martin *et al.*, 1989).

and Williams, 1990a,b). Our results are in agreement with Chademana and Offer (1990) who reported either small or no change in TVFA when yeast culture supplemented to a high roughage diet.

4.2.4 Concentration of Individual Fatty Acids As Affected By Probiotics in the *in vitro* System:-

The concentration of acetate, propionate and butyrate on different probiotic treatments are presented in Table 4.14. It was observed that there was significant ($P<0.05$) reduction of acetate in triple combination groups T_{15} , T_{16} , T_{17} , T_{18} , T_{19} , T_{20} , T_{21} and T_{22} by 11.17, 11.75, 12.80, 13.24, 14.50, 12.69, 14.05 and 10.14 per cent respectively over control after 48h of incubation. Whereas, acetate proportion reduced by 6.99 per cent in *Lactobacillus plantarum* (T_4) treated group and 6.19 per cent in double combination of *S.cerevisiae*-522 and *L.acidophilus* treated group (T_{11}).

Propionate concentration improved ($P<0.05$) in all the triple combination treated groups, i.e., T_{15} , T_{16} , T_{17} , T_{18} , T_{19} , T_{20} , T_{21} and T_{22} by 8.6, 9.66, 10.87, 12.22, 12.56, 11.54, 12.17 and 8.78 per cent and in T_{11} (SC-522+LA) also, there was ($P<0.05$) an improvement. However, there was reduction ($P<0.05$) in propionate concentration in *Lactococcus lactis* treated (T_5) group over control. In the treatment groups of T_2 , T_3 , T_4 , T_6 , T_7 and T_8 the propionate concentration was comparable with that of control.

Butyrate production was similar in all the treatment groups except T_4 and T_6 which have shown higher ($P<0.05$) butyrate production over control by 7.07 and 5.62 per cent, respectively. Other treatment groups showed higher trend of butyrate production over control, but the difference was non-significant.

The data on acetate to propionate ratio have been presented in the Table 4.14. Maximum reduction ($P<0.05$) of acetate to propionate ratio was recorded in all the triple combination groups as well as in some of the double combination groups such as T_{10} (1.81) and T_{11} (1.67) as compared to control

(2.21). Depressing trend of acetate to propionate ratio was also observed in T_4 , T_9 , T_{12} , T_{13} , T_{14} , whereas, higher ($P<0.05$) ratio of acetate to propionate was observed in *Lactococcus lactis* treated group (T_5) by 21.72% over control. However, acetate to propionate ratio observed in T_1 , T_2 , T_3 , T_6 , T_7 and T_8 groups remained similar to control. The results of present study indicated that though individual culture of yeast (*S.cerevisiae*-B and 522) had little effect on acetate proportion but in combination with lactobacilli and enterococci or lactococci resulted significant reduction in acetate level.

The present results are not in agreement with the earlier reports, in lactating cows, wherein Harrison *et al.* (1988) observed higher propionate production and reduction of A/P ratio due to supplementation of yeast culture to lactating cows. Similarly, enhanced production of propionate and reduction of acetate to propionate was reported by Martin *et al.* (1989), Dawson *et al.* (1990), Plata *et al.* (1994), Moloney and Drennan (1994). Supplementation of commercial yeast to the high concentrate diet of steer reduced acetate, acetate to propionate ratio, and increased molar proportion of butyrate and isoacids (Moloney and Drennan, 1994).

In the present study, substrate consisted high roughage (70%) proportion in a closed *in vitro* study, which could probably be the reason for not having positive impact of individual yeast culture on acetate to propionate ratio, but triple combination of cultures having yeast as major component have positive impact on acetate to propionate ratio. Although in another study addition of *S.cerevisiae* improved acetate *in vitro* (Martin *et al.*, 1989) and *in vivo* (Rouzbehan *et al.*, 1994).

4.2.5 Influence of Probiotics on Total Gas Production (ml) at Different Hours of Incubation:-

Total *in vitro* gas production data at 4h interval in single, double and triple combination of viable culture treatment are presented in Tables 4.10, 4.11 and 4.12. The total gas production (ml) increased in all the treatment groups

of individual, double and triple combinations of probiotic cultures over control at different incubation intervals. The triple culture combination (*S. cerevisiae*-B, *L. plantarum* and *E. faecium*) showed maximum cumulative gas production at 24 and 48 h (84.80 ml and 125.70 ml) as compared to respective incubation periods of control (52.65 ml and 79.58 ml) (Table 4.13).

In the treatment groups, total gas production (ml) ranged between 60.93 (T₁₁) to 84.80 (T₁₇) and 99.73 (T₁₁) to 125.70 ml (T₁₇) at 24 and 48h, respectively. The average (117.05 ml) gas production was higher in triple culture treatments. Treatment groups of T₁, T₂, T₁₅, T₁₇ and T₁₈ (consisting yeast) showed higher (P<0.05) gas production. These observations indicated that the treatment groups having yeast culture significantly (P<0.05) increased the rate of fermentation. The improvement in total gas production could mainly be due to increase in DM degradability. Increase in anaerobic bacterial counts (Dawson and Newman, 1987; Dawson, 1990; Newbold *et al.*, 1995) might have increased the rate of fermentation due to probiotics supplementation and hence total gas production was increased.

Very less information is available regarding the effect of yeast culture on total gas production. The present observation was in contrast with earlier observations, wherein Mutsvangwa *et al.* (1992) reported *in vitro* gas production in bulls receiving yeast culture was lower compared to control (P<0.05) and methane production was significantly reduced after 12h incubation. Whereas no added effect of *in vitro* yeast supplementation was observed on total gas production (Rouzbehan *et al.*, 1994).

Due to supplementation of yeast culture methane production was reduced (Williams, 1989a; Carro *et al.*, 1992a). Whereas rate of methane production reported on high concentrate diet was higher in yeast supplemented vessels (Rusitech) (Carro *et al.*, 1992a). The response of probiotics culture has been reported to depend on level of culture as well as diet. In present study, the level of probiotics culture was 10⁹ cfu /*in vitro* bottle which was very high

(300 to 400 times) under *in vitro* system and may be another reason for higher total gas production.

Based on these *in vitro* studies of the probiotic cultures in single, double and triple combination, following six cultures or culture combinations were selected for detailed study under *in vitro* system of phase III.

1. *Saccharomyces cerevisiae*-B (T₂)
2. *Enterococcus faecium* (T₆)
3. *S.cerevisiae*-522+*L. plantarum* (6:4) (T₁₂)
4. *S.cerevisiae*-522+*E.faecium* (6:4)(T₁₃)
5. *S.cerevisiae*-522+*L.acidophilus*+*Lactococcus lactis*(6:2:2)(T₁₇)
6. *S.cerevisiae*-522+*L.plantarum*+*E.faecium*(6:2:2)(T₂₀)
7. *S.cerevisiae*-B+*L.plantarum*+*E.faecium*(6 :2 :2)(T₂₁)

Following observations were taken into consideration above six treatments:-

- i) DM degradability was significantly ($P<0.01$) increased due to supplementation of above cultures.
- ii) Total gas production upto 48h was also increased due to above treatments.
- iii) Maximum TVFA concentration was recorded in triple combinations of T₁₇, T₂₀ and T₂₁; and in double combinations of T₁₂ and T₁₃. *S.cerevisiae*-B and *E.faecium* were the component cultures in T₁₇ which has given maximum response on total gas production, IVDMD and total VFA concentration.

E.faecium was also component culture alongwith yeast in T₁₂, T₁₃, T₂₀ and T₂₁ treatments which has also given higher values in DM degradability and TVFA concentration. Hence, among individual cultures *S.cerevisiae*-B and *E.faecium* were selected for detailed study under *in vitro* system.

iv) Significant ($P<0.05$) reduction of acetate level and improvement in propionate level were observed in T₁₇, T₂₀ and T₂₁ groups.

Based on the above points, two treatments (T₁ and T₆) from individual cultures, two from (T₁₂ and T₁₃) double combinations and three from triple culture combinations (T₁₇, T₂₀ and T₂₁) were screened for detailed fermentation study under *in vitro* system (Phase-III).

Table 4.8 Composition of concentrate mixture used in *in vitro* studies (Phase-II)

Ingredient	Part(kg)	CP(kg)	TDN(kg)
Maize	40	4.00	36.00
Groundnut cake	25	10.00	19.75
Mustard cake	10	3.70	7.50
Wheat bran	22	3.52	14.30
Min.mix.	2	-	-
Common salt	1	-	-
	100	21.22	77.55

Table 4.9 Chemical composition of feedstuffs used during *in vitro* trials
(% DM basis)

Ingredient	OM	CP	CF	EE	NFE	Ash
Conc. mix.	89.55	20.78	8.26	4.52	55.99	10.45
Oat fodder	90.87	7.70	30.21	2.69	50.27	9.13
Wheat straw	88.50	3.30	37.50	1.50	46.20	11.50

Table 4.10: Effect of individual probiotic culture on 4-hourly *in vitro* gas production (ml) (Phase-II)

Incuba- tion Period(h)	Treatments						
	Control	T ₁ (SC-B)	T ₂ (SC-522)	T ₃ (LA)	T ₄ (LP)	T ₅ (LL)	T ₆ (EF)
4	2.67	0.33	0.00	0.00	0.00	0.00	0.00
8	8.50	9.00	8.50	8.75	9.00	3.83	3.17
12	13.87	15.53	13.60	14.27	14.07	14.80	14.50
16	13.13	16.80	16.07	15.73	16.43	16.33	17.00
20	9.83	14.00	14.00	15.20	16.00	17.00	16.33
24	8.00	15.83	15.67	14.17	15.17	14.33	15.33
28	7.50	14.67	14.67	12.50	13.50	13.33	14.33
32	6.33	10.83	10.83	9.67	10.33	9.50	12.33
36	4.17	7.17	8.50	7.00	8.00	6.83	8.00
40	3.33	5.33	5.83	5.83	5.67	5.33	5.67
44	3.23	4.67	5.33	5.00	5.00	5.00	4.83
48	2.33	3.50	3.90	3.50	3.50	4.00	4.07

Each value is based on average of three observations

T₁ = *Saccharomyces cerevisiae*-B, T₂ = *S.cerevisiae*-522, T₃ = *Lactobacillus acidophilus*,

T₄ = *L.plantarum*; T₅ = *Lactococcus lactis*; T₆ = *Enterococcus faecium*

Table 4.11: Combined effect of two probiotic cultures on 4-hourly *in vitro* gas production (ml)(Phase-II)

Incuba- tion Period(h)	Treatments								
	Control	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄
		(B-LA)	(B-LP)	(B-EF)	(B-LL)	(522-LA)	(522-LP)	(522-EF)	(522-LL)
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	6.50	5.00	6.90	6.83	6.67	3.83	4.23	4.33	4.97
12	8.80	11.07	12.87	12.47	13.10	12.36	13.83	13.93	14.93
16	11.20	19.27	20.03	21.43	18.73	17.40	18.26	18.40	19.60
20	9.00	16.17	19.50	20.67	17.17	16.33	17.83	19.00	18.83
24	8.75	12.33	12.83	12.50	11.73	11.00	11.66	12.00	12.67
28	5.50	8.67	9.17	9.40	8.27	9.50	8.90	8.83	9.00
32	4.75	7.40	7.50	8.07	7.83	6.90	7.03	8.00	7.67
36	4.00	6.93	6.83	6.93	7.07	6.57	6.93	7.50	7.00
40	4.00	5.93	5.73	5.97	5.90	5.67	6.00	6.00	5.90
44	2.90	5.87	5.73	5.87	5.50	5.17	5.43	5.83	5.67
48	3.25	5.07	4.83	4.83	5.07	5.00	5.40	5.27	5.16

Each value is based on average of three observations.

T₇ = *S.cerevisiae*-B+*L.acidophilus*; T₈ = *S.cerevisiae*-B+*L.plantarum*; T₉ = *S.cerevisiae*-B+*E.faecium*; T₁₀ = *S.cerevisiae*-B+*L.lactis*;
 T₁₁ = *S.cerevisiae*-522+*L.acidophilus*; T₁₂ = *S.cerevisiae*-522+*L.plantarum*; T₁₃ = *S.cerevisiae*-522+*E.faecium*; T₁₄ = *S.cerevisiae*-
 522+*L.lactis*

Table 4.12: Combined effect of three probiotic cultures on 4-hourly *in vitro* total gas production (ml)(Phase-II)

Incuba- tion Period(h)	Treatments								
	Control	T ₁₅ (B-LA-EF)	T ₁₆ (B-LA-LL)	T ₁₇ (B-LP-EF)	T ₁₈ (B-LP-LL)	T ₁₉ (522-LA-EF)	T ₂₀ (522-LA-LL)	T ₂₁ (522-LP-EF)	T ₂₂ (522-LP-LL)
4	1.00	0.00	0.67	1.73	0.00	0.00	0.00	1.33	0.73
8	9.85	9.23	7.83	13.07	10.10	8.90	10.57	9.07	9.83
12	12.25	13.67	14.50	19.00	15.67	15.33	15.50	15.33	15.17
16	12.00	16.00	15.83	18.67	16.67	16.67	17.80	17.00	17.27
20	10.00	15.85	15.00	17.07	15.40	15.83	16.67	15.67	16.27
24	10.00	16.60	16.60	15.27	15.10	14.67	14.33	15.17	16.13
28	8.00	12.70	11.73	11.67	11.50	10.43	10.67	11.13	10.93
32	6.30	9.30	9.07	8.80	9.40	9.00	8.57	9.10	8.60
36	5.25	7.77	7.73	7.27	7.37	7.13	7.10	7.23	7.17
40	4.50	6.47	6.06	5.70	6.10	5.83	5.50	5.83	6.07
44	3.30	4.93	5.03	4.87	5.07	5.00	4.73	4.77	4.73
48	2.70	4.40	4.40	4.27	5.07	4.40	4.50	4.43	4.80

Each value is based on average of three observations:

- T₁₅ = *S.cerevisiae*-B+*L.acidophilus*+*E.faecium*; T₁₆ = *S.cerevisiae*-B+*L.acidophilus*+*L.lactis*
 T₁₇ = *S.cerevisiae*-B+*L.plantarum*+*E.faecium*; T₁₈ = *S.cerevisiae*-B+*L.plantarum*+*L.lactis*
 T₁₉ = *S.cerevisiae*-522+*L.acidophilus*+*E.faecium*; T₂₀ = *S.cerevisiae*-522+*L.acidophilus*+*L.lactis*
 T₂₁ = *S.cerevisiae*-522+*L.plantarum*+*E.faecium*; T₂₂ = *S.cerevisiae*-522+*L.plantarum*+*L.lactis*

Table 4.13: Effect of different probiotics on *in vitro* DM degradability (48h); total gas and total VFA production (48h) using high roughage substrate (Roughage: Concentrate, 70:30)

Treat- ment	DM degradability (%)	Total gas(24h) (ml)	Total gas(48h) (ml)	Total VFA mmol/ 100ml
Control	68.24±1.36 ^a	52.65±2.13 ^a	79.58±3.73 ^a	7.23±0.03 ^{cdef}
T ₁	73.52±0.99 ^b	71.50±1.04 ^{cdef}	117.67±1.17 ^{fg}	7.02±1.43 ^{cde}
T ₂	73.45±0.79 ^b	67.83±0.60 ^{bcd}	116.90±0.56 ^{fg}	6.07±0.59 ^{bc}
T ₃	74.56±0.62 ^{bcd}	64.50±7.05 ^{bcd}	109.67±6.57 ^{cdef}	6.23±0.71 ^{bcd}
T ₄	73.42±0.89 ^b	68.33±6.19 ^{bcd}	114.33±5.76 ^{def}	5.53±0.10 ^{ab}
T ₅	74.85±0.38 ^{bcd}	66.30±0.35 ^{bcd}	110.30±2.45 ^{cdef}	4.57±0.36 ^a
T ₆	74.29±0.58 ^{bc}	66.33±0.60 ^{bcd}	115.57±1.55 ^{ef}	7.43±0.73 ^{def}
T ₇	77.26±1.15 ^{cd}	63.83±1.83 ^{bc}	103.70±1.76 ^{bc}	7.22±0.03 ^{cdef}
T ₈	75.87±0.27 ^{bcd}	72.13±1.55 ^{cdef}	111.93±1.32 ^{cdef}	7.17±0.40 ^{cdef}
T ₉	77.65±0.87 ^d	73.90±0.80 ^{ef}	111.97±1.28 ^{ef}	7.80±0.18 ^{efg}
T ₁₀	74.95±1.22 ^{bcd}	67.40±2.75 ^{cdef}	107.03±2.81 ^{bcd}	7.72±0.18 ^{efg}
T ₁₁	75.65±0.97 ^{bcd}	60.93±0.92 ^{ab}	99.73±0.63 ^b	7.32±0.32 ^{cdef}
T ₁₂	76.49±0.48 ^{bcd}	65.83±2.43 ^{bcd}	105.53±1.74 ^{bcd}	8.40±0.03 ^{fgh}
T ₁₃	76.27±0.49 ^{bcd}	67.67±0.82 ^{bcd}	109.07±1.65 ^{bcd}	8.05±0.26 ^{efgh}
T ₁₄	76.39±0.46 ^{bcd}	71.00±0.87 ^{cdef}	111.40±0.46 ^{cdef}	7.82±0.15 ^{efg}
T ₁₅	75.30±2.29 ^{bcd}	71.33±1.64 ^{cdef}	116.87±1.23 ^{fg}	8.32±0.14 ^{fgh}
T ₁₆	73.53±1.08 ^b	70.43±2.27 ^{cdef}	114.47±2.44 ^{def}	8.23±0.13 ^{efgh}
T ₁₇	77.60±2.00 ^d	84.80±9.46 ^g	125.70±11.13 ^g	9.68±0.83 ⁱ
T ₁₈	75.33±0.52 ^{bcd}	72.77±0.39 ^{def}	117.43±0.05 ^{fg}	8.70±0.26 ^{ghi}
T ₁₉	74.77±0.62 ^{bcd}	71.90±3.07 ^{cdef}	113.70±2.86 ^{def}	8.15±0.29 ^{efgh}
T ₂₀	75.28±0.30 ^{bcd}	74.87±1.42 ^f	115.93±1.92 ^{ef}	9.70±0.87 ⁱ
T ₂₁	75.07±0.37 ^{bcd}	73.57±1.33 ^{ef}	116.07±1.44 ^{ef}	9.23±0.22 ⁱ
T ₂₂	77.03±1.90 ^{cd}	73.40±1.91 ^{ef}	116.20±1.85 ^{ef}	9.13±0.93 ^{hi}
CD	3.12**	8.67 *	9.38 *	1.25 *

Figures with different superscripts (a,b,c,d,e,f,g,h,i) in the same column differ significantly; *P<0.05; **P<0.01

Table 4.14: Effect of different probiotic cultures on *in vitro* fractions VFA and acetate to propionate ratio on high roughage substrate (Roughage: concentrate,70:30)

Treat- ment	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate/ Propionate
Control	62.55±0.61 ^{fg}	28.59±0.50 ^{bcd}	8.86±0.11 ^a	2.21±0.04 ^{ef}
T ₁	60.83±0.70 ^{efg}	27.77±1.24 ^{abc}	11.39±0.54 ^{ab}	2.19±0.12 ^{ef}
T ₂	62.17±0.43 ^{fg}	26.62±0.26 ^{ab}	11.22±0.17 ^{ab}	2.34±0.04 ^{fg}
T ₃	60.37±1.46 ^{defg}	27.51±1.18 ^{abc}	12.11±0.27 ^{ab}	2.20±0.15 ^{ef}
T ₄	55.56±2.41 ^{bcd}	28.50±0.86 ^{bcd}	15.93±1.55 ^c	1.95±0.14 ^{def}
T ₅	64.61±2.94 ^g	24.20±1.73 ^a	11.20±1.21 ^{ab}	2.69±0.31 ^g
T ₆	58.32±2.19 ^{def}	26.87±1.72 ^{abc}	14.48±4.20 ^{bc}	2.18±0.22 ^{ef}
T ₇	64.42±1.33 ^g	26.16±1.18 ^{ab}	9.42±0.15 ^a	2.47±0.16 ^{fg}
T ₈	62.48±3.06 ^{fg}	27.50±1.79 ^{abc}	10.02±1.26 ^a	2.29±0.26 ^{fg}
T ₉	59.81±2.52 ^{defg}	29.40±0.77 ^{bcd}	10.78±1.76 ^a	2.04±0.14 ^{def}
T ₁₀	57.99±0.92 ^{def}	32.02±0.29 ^{de}	9.99±0.62 ^a	1.81±0.04 ^{cde}
T ₁₁	56.36±0.02 ^{cde}	33.60±0.14 ^{ef}	10.03±0.17 ^a	1.67±0.05 ^{bcd}
T ₁₂	57.76±1.70 ^{def}	30.80±2.74 ^{cdc}	11.43±1.03 ^{bc}	1.89±0.22 ^{de}
T ₁₃	59.23±3.13 ^{def}	29.92±2.95 ^{bcd}	10.85±0.18 ^a	2.01±0.30 ^{de}
T ₁₄	57.54±2.96 ^{def}	31.84±2.16 ^{de}	10.62±0.80 ^a	1.82±0.22 ^{cde}
T ₁₅	51.38±0.44 ^{abc}	36.37±0.87 ^{fg}	12.24±0.43 ^{ab}	1.41±0.04 ^{abc}
T ₁₆	50.80±0.60 ^{ab}	37.43±0.22 ^{fg}	11.76±0.38 ^{ab}	1.35±0.02 ^{ab}
T ₁₇	49.75±0.80 ^a	38.64±0.14 ^g	11.60±0.65 ^{ab}	1.28±0.02 ^{ab}
T ₁₈	49.31±1.81 ^a	39.99±2.27 ^g	10.70±0.45 ^a	1.24±0.12 ^a
T ₁₉	48.05±0.97 ^a	40.33±1.42 ^g	11.61±0.45 ^{ab}	1.19±0.06 ^a
T ₂₀	49.86±0.27 ^a	39.31±0.53 ^g	10.82±0.26 ^a	1.26±0.02 ^{ab}
T ₂₁	48.50±1.10 ^a	39.94±1.27 ^g	11.55±0.17 ^{ab}	1.21±0.06 ^a
T ₂₂	52.41±2.75 ^{abc}	36.55±2.32 ^{fg}	11.03±0.43 ^a	1.44±0.16 ^{ab}
CD	5.03*	4.03*	3.41*	0.43*

Figures with different superscripts(a,b,c,d,e,f,g)n the same column differ significantly

*P<0.05

PHASE-III

4.3 EFFECT OF PROMISING CULTURES ON *IN VITRO* RUMEN FERMENTATION PATTERN

Mixed feeds consisting of maize fodder, wheat straw, concentrate were used as substrate in three ratios (DM basis) 24:16:60, 36:24:40, 42:28:30 for the three *in vitro* fermentation studies.

4.3.1 Composition:-

The feed components were analysed for proximate principles (Table 4.16). The values for OM, CP, CF, EE and NFE were 89.55, 20.78, 8.26, 4.52 and 55.99 per cent, respectively in concentrate mixture, corresponding values for maize fodder were 87.54, 11.69, 25.16, 1.88 and 48.81 and for wheat straw 88.50, 3.30, 37.50, 1.50 and 46.20 per cent.

4.3.2 Details of Treatments:-

Based on the results of 2nd phase *in vitro* trials, two promising individual cultures, two double combination and three triple combination cultures were selected for detailed fermentation study under *in vitro* system. The selected cultures were as following:

Treatment	Probiotic culture	Abbrevia- tion	Total dose per <i>in vitro</i> bottle(cfu)
<u>Control</u>	no culture	-	-
P ₁	<i>S.cerevisiae</i> -B	SC-B	10 ⁹
P ₂	<i>E.faecium</i>	EE	10 ⁹
<u>Double culture combination</u>			
P ₃	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i>	SC-522+LP	10 ⁹ (6:4)
P ₄	<i>S.cerevisiae</i> -522+ <i>E.faecium</i>	SC-522+EF	10 ⁹ (6:4)

<u>Triple culture combination</u>			
P ₅	<i>S.cerevisiae</i> -522+ <i>L.acidophilus</i> + <i>L.lactis</i>	SC-522+LA+ LL	10 ⁹ (6:2:2)
P ₆	<i>S.cerevisiae</i> -522+ <i>L.plantarum</i> + <i>E.faecium</i>	SC-522+LP+ EF	10 ⁹ (6:2:2)
P ₇	<i>S.cerevisiae</i> -B+ <i>L.plantarum</i> + <i>E.faecium</i>	SC-B+LP+EF	10 ⁹ (6:2:2)

4.3.3 DM Degradability as Affected by Probiotics:-

In vitro DM degradability (IVDMD) in high concentrate (Roughage:concentrate = 40:60), medium concentrate (Roughage:concentrate = 60:40) and low concentrate substrate (Roughage:concentrate = 70:30) has been presented in Table 4.17, 4.20 and 4.23. In high concentrate study, DM degradability was lowest ($P<0.01$) in control (62.05%) and it was highest in P₁(67.65), P₄ (68.51), P₆ (68.07) and P₇ (67.25). In medium concentrate also (Table 4.20) increased ($P<0.05$) DM degradability was observed due to treatments, and it ranged between 55.17(P₂) to 57.11(P₅) in various treatments against 49.03 per cent for control. In low concentrate substrate (Table 4.23) also IVDMD was higher ($P<0.01$) in all treatments, highest being in P₁ (52.16), P₂ (51.41), P₃ (52.09), P₄ (51.05) and P₅ (52.43) as compared to control (46.70). Substrate showed significant ($P<0.01$) influence on DM degradability; and in high concentrate diet, DM degradability was highest followed by medium and low concentrate substrate (Table 4.26).

The present findings are in agreement with earlier observations, wherein workers have reported improvement in degradability patterns of nutrients either *in vitro* or *in sacco* due to supplementation of yeast culture (Chadema and Offer, 1990; Williams *et al.*, 1991; Kim *et al.*, 1992a; Carro *et al.*, 1992a,b; Mporfu and Ndlovu, 1994; Malik, 1993; Newbold *et al.*, 1995). Chadema and Offer (1990) reported that addition of Yea-Sacc to concentrate/forage diets, in

which the proportion of concentrate ranged from 20 to 60 per cent of the diet consistently increased degradability of hay upto 24h (37.3 to 42.2 per cent), but had no effect on the 48h degradability. Supplementing the high concentrate diet with yeast culture resulted in significantly higher DM and NDF degradabilities (Carro *et al.*, 1992a,b). Moreover, filter paper (cellulose) degradability (24h) tended to be higher for the vessels supplemented with yeast culture than for control (33% Vs 27%). However, yeast culture had no significant effect on DM and NDF degradabilities with medium or low concentrate diets (Carro *et al.*, 1992a). Degradability of straw in the rumen tended to increase by yeast addition from 16h onward and this increase was significant for *S.cerevisiae* (NCYC1026) at 72 and 96h. But in present study, higher DM degradability was observed in all the treatments over control with high, medium or low concentrate substrate. This could be assigned to higher dose of probiotic culture (10^9 cfu/0.5 of substrate) in *in vitro* supplementation.

4.3.4 Total Gas Production as Influenced by Probiotic Cultures:-

In high concentrate substrate total gas (ml) production at 18h incubation was maximum ($P<0.01$) in P_1 (70.20), P_2 (71.50) and P_4 (68.90 ml) and other treatment groups also showed higher ($P<0.01$) gas production than control. Total gas production (ml) from 18 to 24h of incubation was also higher in all treatment groups than control (Table 4.17). Similar trend was observed in cumulative gas production (ml) upto 24h wherein maximum ($P<0.01$) gas production (ml) was recorded in P_1 (94.53), P_2 (96.83), P_3 (92.33), P_4 (93.90) and P_7 (92.67) as compared to control (66.00).

In medium concentrate substrate (Table 4.20) *in vitro* total gas production (ml) at 18h was maximum in P_1 (45.17), P_2 (45.83), P_3 (44.10), P_4 (44.17) and P_5 (46.67) and all the treatments showed ($P<0.05$) increase in gas production than control., whereas maximum gas production was recorded in P_2 , P_3 , P_4 , P_5 and P_7 groups when the *in vitro* bottles were incubated for 18-24 h. Cumulative gas production upto 24h was higher in P_2 (71.17), P_3 (69.77), P_4

(69.83) and P₅ (73.17) followed by P₁ (68.83), P₆ (64.50), P₇ (64.03) and control (50.90). In high roughage substrate, the *in vitro* gas production at 18h was recorded highest in treatment group P₁ (39.13) and lowest in the control group (30.00) (Table 4.23). From 18 to 24h of incubation, gas production ranged from 15.17 ml (control) to 24.67 ml (P₆), whereas, cumulative gas production (ml) upto 24h was highest in P₁ (62.52) followed by P₂ (60.30) , P₅ (60.93), P₆ (59.33), P₄ (58.56), P₃ (58.33), P₇ (57.17) and control (45.17). The difference was found significant (P<0.01). The increase in total gas production appeared to be associated with higher DM degradability in probiotic culture supplemented groups as well as high dose of probiotics per bottle (10⁹ cfu/bottle/0.5g substrate).

Anaerobic bacterial counts in the rumen were increased due to supplementation of yeast culture (Dawson and Newman, 1987; Harrison *et al.*, 1987; Weidmeier *et al.*, 1987; Dawson, 1990; Newbold *et al.*, 1995). Due to increase in anaerobic bacterial counts rate of fermentation might have increased in probiotic supplemented groups and hence total gas production was increased in present studies. As such results on total gas production were in variance with earlier reports of Harrison *et al.*, (1988) who showed that *in vitro* gas production was not affected due to yeast supplementation.

In the present study substrate had significant impact on total gas production, i.e., rate of fermentation as indicated by total gas production was higher in high concentrate followed by medium and low concentrate substrate (Table 4.26). Treatment effect was also significant (P<0.01) at 18h, 18-24h and 24h cumulative gas production. All effect interaction was significant at 18h (P<0.05) and 24h cumulative gas production (P<0.01) but not at 18-24h gas production.

4.3.5 Effect of Probiotic Culture on Methane Production:-

In high concentrate diet (Table 4.17) methane production per centage upto 18h, 18-24h and cumulative methane production upto 24h was similar

among all the treatments and control. Cumulative methane production upto 24h of incubation ranged from 17.62 per cent in P₁ and P₂ to 19.58 per cent in P₇ group. Whereas quantitative methane production (ml /0.5g substrate) was recorded higher in all probiotic treatment groups by 18h of incubation (P<0.01) as well as 18 to 24h of incubation (P<0.05) over control (Table 4.17).

In medium concentrate substrate the methane production (per cent of total gas) in 18h and upto 24h of incubation was recorded higher (P<0.01) in all the probiotic treatment groups except P₃ group over control (Table 4.20). When methane production was calculated quantitatively higher (P<0.01) values were recorded in treatment groups in 18h as well as in 18-24h of incubation.

In high roughage substrate, methane production per cent in 18h and upto 24h was estimated to be similar among all treatment groups and control (Table 4.23). Same trend was observed in quantitative methane(ml) during 18h of incubation., but significantly higher (P<0.01) in P₅, P₆ and P₇ treatments during 18-24h of incubation.

The quantitative increase in methane production in high, medium and low concentrate substrate might be due to higher DM degradability. The present observations are in partial agreement with earlier report by Carro *et al.*(1992a). They showed lower methane production for medium concentrate diet, but it was higher for high concentrate diet when supplemented with yeast culture. Whereas, Williams (1989a) showed reduction of methane production in steers by 28% when supplemented with yeast culture.

4.3.6 Effect of Probiotic Cultures on pH:-

The data on pH as influenced by treatments at different types of substrates are presented in Table 4.18, 4.21 and 4.24. The pH of *in vitro* rumen liquor was significantly (P<0.05) higher in P₁ (6.88) as compared to control (6.73) in high concentrate substrate, in other treatments also there was increasing tendency of pH (Table 4.18). In medium concentrate diet, pH

ranged from 6.72 in P₂ group to 6.82 in P₅ and P₆ groups. There was an increase ($P<0.01$) in pH in the treatment groups of P₅, P₆ and P₇ as compared to control (Table 4.21). In high roughage substrate (Roughage : concentrate = 70:30) lowest pH (6.75) was observed in P₁ and P₅ and highest (6.86) in P₄ group. The difference was found non-significant.

These results can be substantiated by earlier reports that supplementation of *S.cerevisiae* decreased lactic acid concentration which ultimately helped in elevation of ruminal pH (Williams, 1989a,1990a) and was sufficient to raise the pH by 0.2 to 0.5 units (Dawson, 1987; Williams *et al.*, 1991). These pH changes in order of 0.2 units may have considerable effect on cellulolysis when the mean pH level are low or in the region of 6.0 (Williams, 1989a,1990a). In the present study yeast culture improved pH in high and medium concentrate substrate, it was also increased in triple combination cultures where 60 per cent yeast was present. The basic reason for raised pH had been assigned to higher uptake of lactate by *Selenomonas ruminantium* and *Megasphaera elsdenii* in high concentrate diets when supplemented with yeast culture (Nisbet and Martin, 1990,1991). Thus in concentrate diets, there would be more cellulolytic activity due to addition of yeast culture (Carro *et al.*, 1992a).

4.3.7 Total VFA Concentration as Affected by Probiotics Culture:-

In high concentrate substrate (Table 4.18), concentration of total VFA ranged from 7.27 mmol/100 ml (control) to 9.40 mmol/100 in P₁. The addition of cultures resulted in improved ($P<0.01$) TVFA production in all treatments. In medium concentrate diet, higher ($P<0.01$) VFA production was observed in all treatments except P₇ than control (Table 4.21). Whereas, in high roughage diet, maximum TVFA concentration was observed in yeast treated group (P₁ - 7.50) and in double combination of *S.cerevisiae*-522 and *L.plantarum* (P₃ - 7.47)(Table 4.24). In other treatments also higher ($P<0.01$) VFA production was observed as compared to control. These data indicated that yeast culture was also effective in high roughage substrate.

Diet had significant ($P<0.01$) impact on TVFA concentration, i.e., higher TVFA concentration was observed in high concentrate diet followed by medium and low concentrate diet. All effect interaction was found significant ($P<0.01$). The present results could be substantiated by previous reports. The TVFA concentration was increased from 172.2 to 184.5 mmol/d when supplemented with yeast culture (Harrison *et al.*, 1987). Similar results were observed by Martin *et al.* (1989). Recently enhanced production of TVFA was revealed by Kim *et al.* (1992), Moloney and Drennan (1994) and Rouzbehan *et al.* (1994), who showed higher TVFA in rumen due to addition of yeast culture. Addition of yeast culture to barley grain based diet given to growing bulls increased concentrations of VFA (Mutsvangwa *et al.*, 1992). Dawson *et al.* (1990) have reported that neither the yeast culture supplement nor the mixed microbial (yeast, lactobacilli and enterococci) supplement consistently alter the patterns of VFA production in the continuous cultures or in the rumen of steers.

4.3.8 Effect of Probiotics on Proportion of Individual VFA:-

In high concentrate diet, proportion of acetate, propionate and butyrate have been presented in the Table 4.18. It was observed that acetate proportion was lowest in P_1 (51.28) and P_7 (51.80) and other treatments also showed lower ($P<0.01$) acetate proportion as compared to control (64.29). Hence, inclusion of SC-B (P_1) alone or in combination with LP and EF (P_7) reduced acetate level in *in vitro* system. Due to lowering of acetate level, propionate ratio was increased significantly ($P<0.01$) in P_1 , P_2 , P_3 , P_5 and P_7 . Propionate proportion varied from 25.94 per cent (control) to 34.33 per cent in *S.cerevisiae*-B treated group. Butyrate proportion was also statistically ($P<0.01$) higher in all the treatment groups over control in high concentrate substrate. Maximum butyrate per cent was observed in double and triple combination of probiotic cultures.

In medium concentrate diet (Table 4.21) acetate proportion was reduced significantly ($P<0.01$) in P_3 (49.44) and was highest in control (70.16). Similar

concentration of acetate was observed in other treatment groups. Propionate proportion ranged from 24.63 (control) to 37.81 per cent (P_3). Significantly higher ($P<0.05$) propionate and butyrate ($P<0.01$) concentration was observed in all the groups over control.

In high roughage substrate (Table 4.24), acetate proportion was lowest in P_7 (49.68) and highest in P_2 (72.79). Treatment groups of P_1 , P_4 , P_5 and P_6 showed similar type of acetate proportion but significantly ($P<0.01$) lower than control (66.84). Acetate proportion in P_3 (62.62) was similar to that of control. Propionate proportion was observed highest in P_3 (36.48) and lowest in control (24.94). Numerically higher propionate per cent was observed but the difference was statistically non-significant. Butyrate per cent was highest in ($P<0.05$) P_7 (13.84) followed by P_6 (13.05), P_5 (13.05), P_4 (9.72), P_3 (9.16), control (8.22), P_1 (8.08) and P_2 (6.89).

Reduction of acetate and increase in propionate level was reported by earlier workers also (Harrison *et al.*, 1988; Martin *et al.*, 1989; Dawson *et al.*, 1990; Plata *et al.*, 1994 and Moloney and Drennan, 1994). In lactating cows, Harrison *et al.* (1988) observed that there was higher production of propionate and reduction of acetate to propionate ratio due to supplementation of yeast culture. Addition of commercial yeast to the high concentrate diet of steer reduced acetate, and molar proportion of butyrate and isoacids were higher (Moloney and Drennan, 1994). In the present experiment also, similar type of observation were recorded in high and medium concentrate diets.

In the present experiment maximum effect was obtained in *S.cerevisiae*-B treatment as well as triple combination groups. The combined culture of *S.cerevisiae*-B+ *L.plantarum* +*E.faecium* showed maximum response in high roughage substrate in terms of propionate production. The effect of substrate (high, medium and low concentrate) was significant ($P<0.01$) on acetate and butyrate proportion, but not on propionate level. Whereas, treatment and all effect interaction (substrate x treatment) were found significant ($P<0.01$) on

acetate, propionate and butyrate proportion (Table 4.27).

4.3.9 Acetate to Propionate Ratio As Influenced by Probiotic Culture Supplementation:-

In high concentrate substrate (Table 4.18), acetate to propionate ratio was significantly ($P < 0.01$) depressed in all the probiotic culture treatments as compared to control. In the medium concentrate (60:40), there was also decrease ($P < 0.01$) in acetate to propionate ratio due to supplementation of probiotic cultures (Table 4.21). In high roughage substrate, highest acetate to propionate ratio was observed in control (3.01) and lowest in P_7 (1.37). Treatment groups had significantly ($P < 0.05$) lower acetate to propionate ($P_1, P_3, P_4, P_5, P_6, P_7$) as compared to control (Table 4.24).

In lactating cows, higher level of propionate in rumen might increase milk yield, whereas higher acetate proportion increases butter fat per centage. The results of present experiment could be substantiated by earlier observations. Due to supplementation of yeast cultures enhanced production of propionate and reduction of A:P ratio was reported by Harrison *et al.* (1988), Martin *et al.* (1989), Dawson *et al.* (1990), Plata *et al.* (1994), Moloney and Drennan (1994). In lactating cow, Harrison *et al.* (1988) observed that there was higher production of propionate and reduction of acetate to propionate ratio due to supplementation of yeast culture. In steer, there was reduction of A:P ratio when fed high concentrate diet supplemented with commercial yeast. In sheep also, yeast culture addition reduced A:P ratio from 5.01 to 3.81 mainly due to increase in propionate (Newbold *et al.*, 1990).

4.3.10 Effect of Probiotic Culture on Ammonia-N under *in vitro* System:-

Influence of supplementation of different probiotics to various substrates on ammonia-N was presented in Table 4.18, 4.21 and 4.24. It was observed that maximum ammonia-N (mg/100 ml SRL) was in the control (16.43) and P_2 (15.37) groups in the *in vitro* system with high concentrate substrate (60%)(Table 4.18). Whereas treatments P_1, P_3, P_4, P_5, P_6 and P_7 showed significantly ($P < 0.05$) lower ammonia-N concentration. In medium concentrate

also, reduction ($P < 0.01$) of ammonia-N was observed in all the treatments over control (Table 4.21). Lowest value was observed in the treatment groups of P_6 (7.73) and P_7 (7.97) and highest in control (12.63). Similar trend was observed with high roughage substrate where reduction ($P < 0.05$) of ammonia-N was also observed in all the treatment groups over control (Table 4.24). The values varied from 6.03 to 10.73 mg in P_7 and control. Maximum reduction of ammonia-N (mg/100 ml SRL) was observed in the combined culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* (P_7) in high and low concentrate.

In the present experiment reduction of ammonia levels have not been associated with decreased protein degradation or deamination (Williams and Newbold, 1990a). Incorporation of ammonia into microbial protein was enhanced due to supplementation of yeast (Dawson, 1987; Weidmeier *et al.*, 1987; Chademana and Offer, 1990; Carro *et al.*, 1992b; Olson *et al.*, 1994b), which was confirmed by greater microbial yield and microbial true protein reaching the duodenum (Gomez-Alarcon *et al.*, 1987; Erasmus, 1991). The concentration of ammonia was decreased by 10 to 35 per cent *in vitro* (Dawson and Newman, 1987, 1988; Carro *et al.*, 1992a) due to addition of yeast culture. Similar results have been reported by Harrison *et al.* (1988), Newbold *et al.* (1990), Chademana and Offer (1990), Williams and Newbold (1990a,b); Edwards (1991) and Sahn and Song (1996). Thus, it could be inferred that probiotics supplementation resulted decrease in NH_3 level because of its utilization for microbial protein synthesis.

4.3.11 Effect of Probiotic Cultures on Total Bacterial Counts:-

Probiotic treatments increased ($P < 0.01$) bacterial population in *in vitro* studies using high concentrate substrate (Table 4.19). $\log_{10}$ values of counts varied between 8.51 and 8.81 in control and P_7 . In medium concentrate diet also same trend was observed (Table 4.22). The values ranged between 8.46 to 8.76 in control and P_7 . In all treatments, higher total bacterial counts ($\log_{10}$ value) were recorded over control, maximum counts were in P_6 and P_7 .

groups. Whereas in high roughage substrate (Table 4.25) total bacterial counts were similar among control and treatment groups. The values ranged from 8.44 (control) to 8.67 (T_7).

The data implied that both combined cultures having three species either resulted increased ($P < 0.01$) bacterial population or tended to increase on high roughage substrate. The same corroborates with earlier reports. In yeast culture supplementation studies, there are reports indicating stimulation of growth of certain types of anaerobic bacteria in the rumen (Dawson and Newman, 1987; Harrison *et al.*, 1987; Weidmeier *et al.*, 1987; Dawson, 1990; Newbold *et al.*, 1995). The stimulation of fibre degrading bacteria has been observed in both rumen simulating fermentor (Dawson and Newman, 1987) and in rumen of dairy cattle (Harrison *et al.*, 1987; Weidmeier *et al.*, 1987). It was particularly pronounced when forage containing diets were fed (Dawson *et al.*, 1990). Non-significant improvement of total anaerobic and cellulolytic bacteria was observed in Holstein cows when fed yeast or mixed culture of yeast and *Aspergillus oryzae* (Kim *et al.*, 1992a).

4.3.12 Effect of Probiotics on Protozoal Population:-

The total protozoal population as influenced by probiotics culture in high, medium and low concentrate diets are presented in Table 4.19, 4.22 and 4.25. It was observed that total protozoal population was statistically similar in all the treatments and control in present studies. In high concentrate the values ($\log_{10}$ no/ml SRL) ranged from 2.96 (P_2) to 3.75 (P_5); in medium concentrate from 2.80 (P_2) to 3.23 in control. On high roughage, the value varied between 3.06 (P_6 and P_7) to 3.38 (control).

Individual protozoal counts have been tabulated in Table 4.19. Total holotrichs per cent in high concentrate substrate ranged from 28.58(P_3) to 49.90 (P_6) and spirotrichs from 50.10 in P_6 to 71.42 in P_3 , respectively. In medium concentrate (Table 4.22) per cent of holotrichs was minimum in control (3.47) and maximum in P_3 (10.37). Spirotrichs population ranged from 89.63 (P_3) to

96.53 per cent (control). In high roughage substrate the response of probiotics on holotrichs and spirotrichs was quite variable (Table 4.25) and Holotrichs and spirotrichs per centage varied from 7.04 (P_4) to 19.83 in P_5 and 80.17 (P_5) to 92.96(P_4), respectively.

Total protozoal population remained unchanged due to supplementation of probiotics culture under these *in vitro* studies. But the diet was having significant impact ($P<0.01$) on total protozoal population, i.e., in high concentrate substrate significantly more protozoal population was observed as compared to medium and low concentrate diet. In high concentrate diet more holotrichs were found followed by low and medium concentrate (Plate 13 and 14). The reverse trend was observed for spirotrichs (entodiniomorphs) population (Plate 14,15,16,17 and 18). Viable yeast culture (*S.cerevisiae*-B) supplementation increased holotrichs per cent in all three ration systems, whereas combined cultures had variable response on holotrichs as well as spirotrichs population.

In the present study response of probiotic cultures on total protozoal population was in consonance with earlier reports. Kim *et al.* (1992a) observed no response of yeast with or without *A.oryzae* on rumen protozoa in non-lactating Holstein cows. Whereas, increased rumen protozoal population was demonstrated by Maurya (1993), Panda (1994), and Plata *et al.* (1994) due to supplementation of yeast culture. Reduction of entodiniomorphids or spirotrichs due to addition of yeast in the present study was in agreement with the observation of Miranda *et al* (1994) who have shown decreased entodiniomorphid counts in Holstein heifers due to *S.cerevisiae* addition. Variable response of different probiotic cultures on holotrichs and spirotrichs was in partial agreement with Newbold *et al.* (1995) who found strains variability of *S.cerevisiae*; NCYC-1026 and NCYC-1088 to improve protozoal population, whereas NCYC-240, NCYC-694 and Yea Sacc depressed protozoal population. However, the difference was found non-significant.

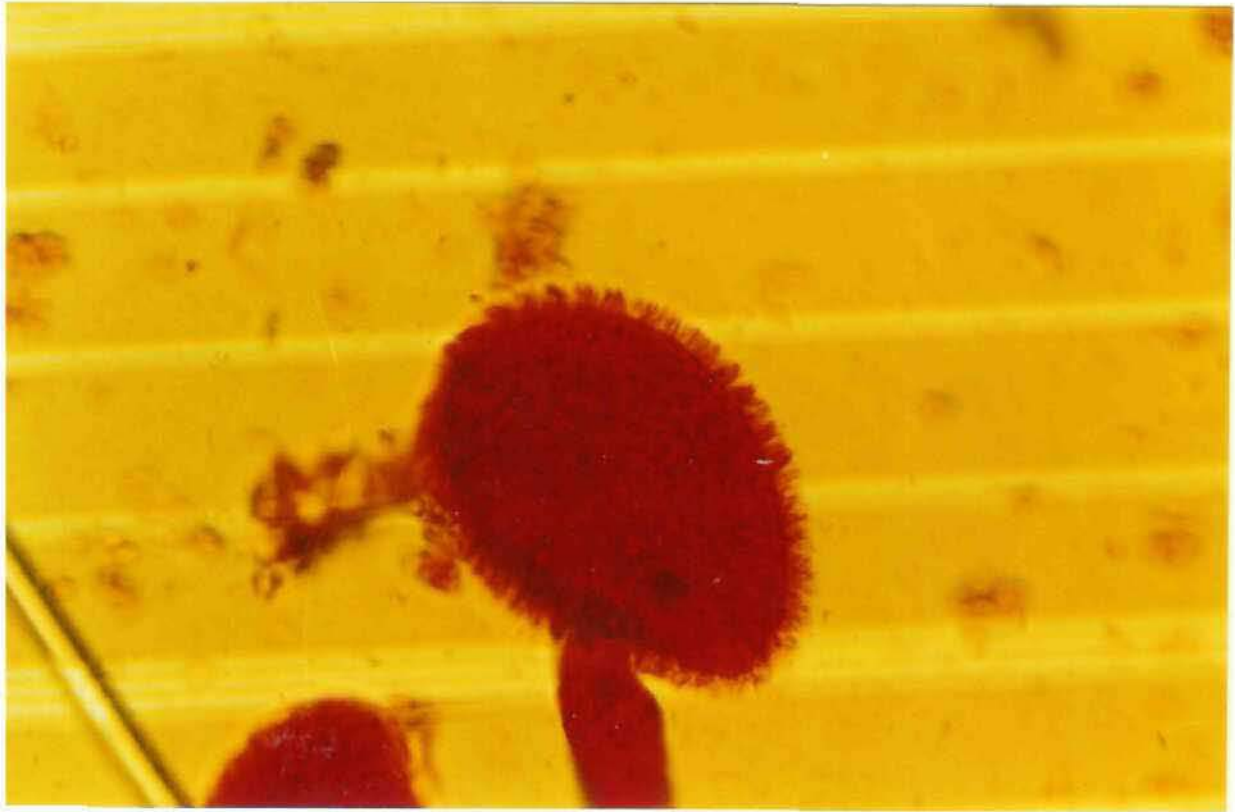


Plate-13 : Isotricha (Large Holotrichs)



Plate-14 : A. Dasytricha (Small Holotrichs) and B. La

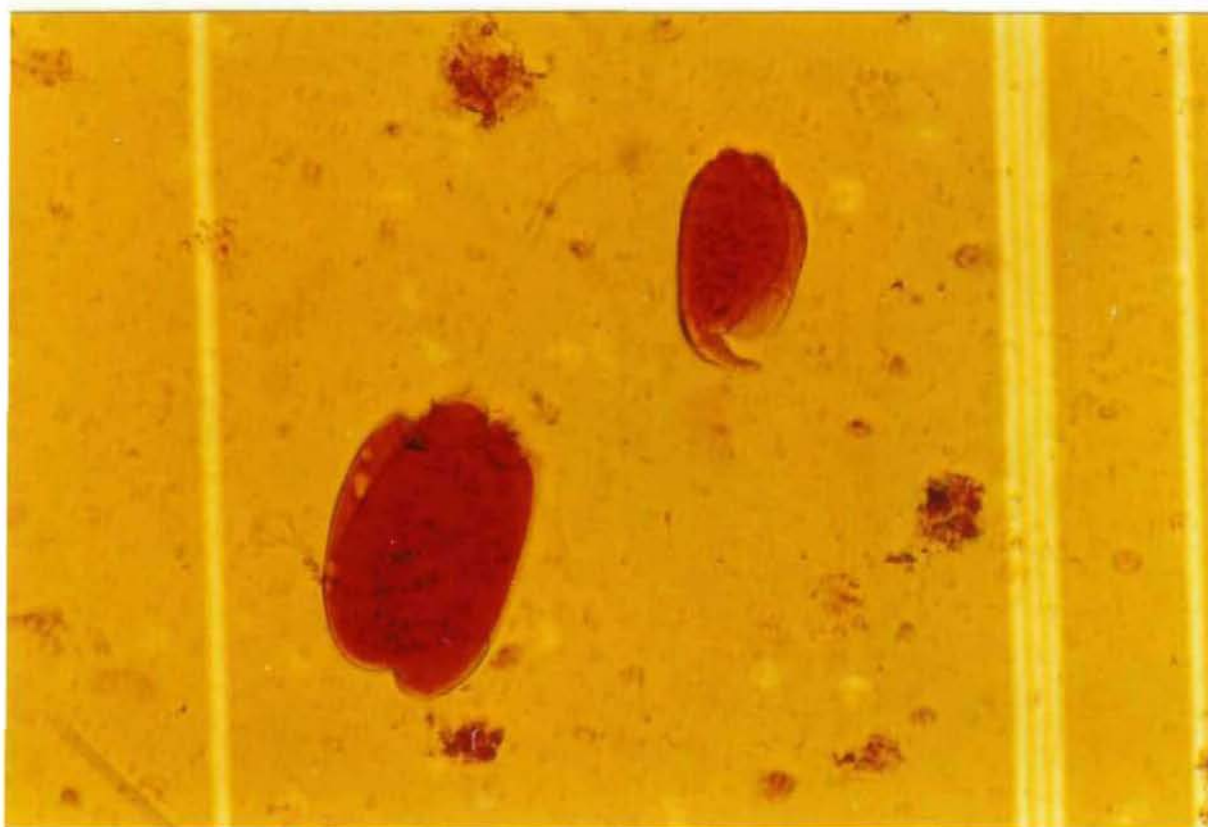


Plate-15 : A Small Spirotrichs and B. Large Spirotrichs



Plate-16 : Different species of Smaller Spirotrichs

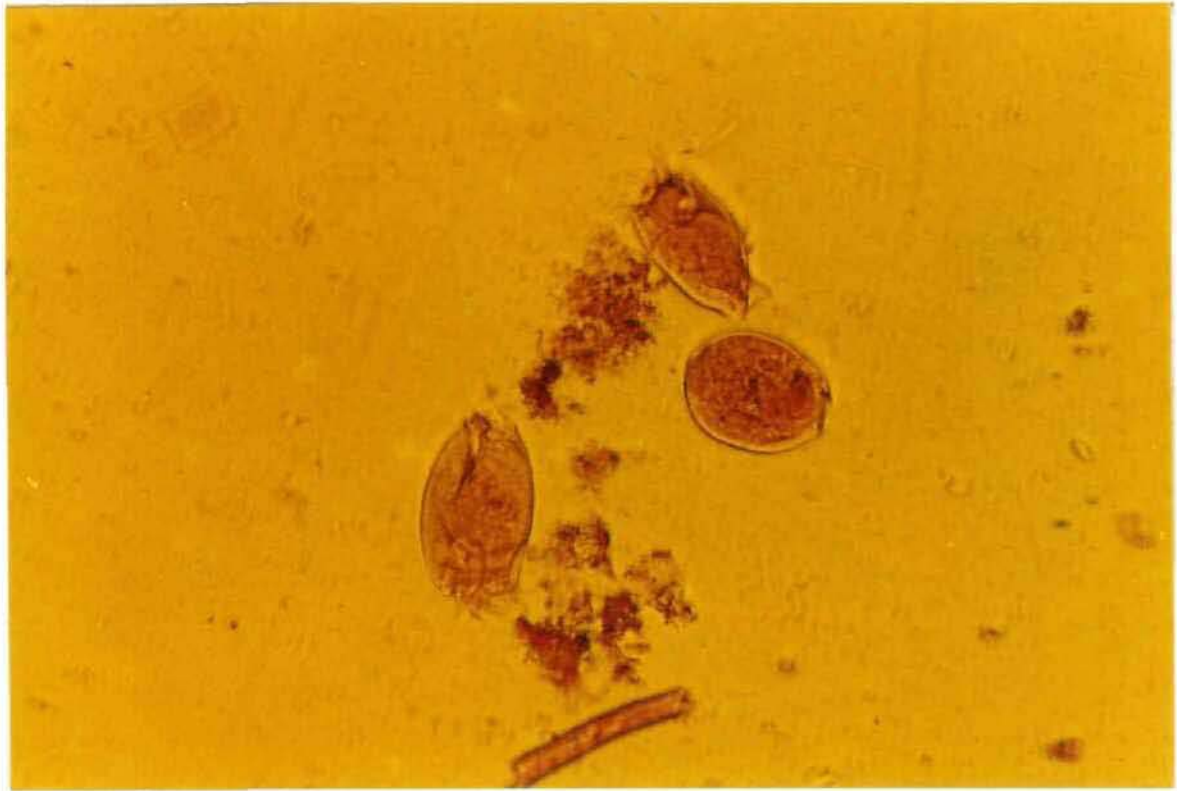


Plate-18 : Different species of Smaller Spirotrichs



Plate-17 : A. Large Spirotrichs; B and C Small Spirotrichs of different species

4.3.13 Effect of Probiotics on Total-N, NPN, TCA-ppt-N and ³⁵S Incorporation Into Microbial Protein:-

Two most promising culture treatments were taken for *in vitro* ³⁵S incorporation into microbial protein, total-N, NPN and TCA-ppt-N. The cultures were as following:

S₁ = *S.cerevisiae*-B

S₂ = *S.cerevisiae*-B+*L.plantarum*+*E.faecium*

4.3.13.1 ³⁵S incorporation into Microbial Protein:-

The rate of ³⁵S incorporation into microbial protein was significantly (P< 0.05) improved in *S.cerevisiae*-B treated (S₁) bottles (51.92) and in combined cultures of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* treated (P₂) group (56.69) over control (46.52) in high concentrate substrate (Table 4.28). In medium concentrate substrate also there was increasing tendency of ³⁵S incorporation in S₁ (47.83) and S₂ (47.91) over control (41.30). In low concentrate diet the values were 38.60, 41.71 and 41.97 per cent in control, S₁ and S₂, respectively. However, in medium and low concentrate the difference was non-significant.

4.3.13.2 Effect of Probiotic Cultures on Total and Non Protein-N:-

Total-N in *in vitro* rumen liquor has been presented in the Table 4.28. In high concentrate substrate, total-N concentration (mg/100 ml SRL) was 50.17, 51.22 and 51.92 in control, *S.cerevisiae*-B (S₁) and *S.cerevisiae*-B, *L.plantarum* and *E.faecium* combined culture treated group (S₂) and the variation was non-significant. In medium and low concentrate also the variations among control and treatments were non-significant.

Non-protein-N (NPN) (mg/100ml SRL) was depressed significantly due to treatment of probiotic cultures in high(P<0.01), medium and low concentrate(P<0.05) substrate (Table 4.28). The values were 25.72 (control), 23.45 (S₁) and 22.05 (S₂) in high concentrate; 22.87 (control), 21.35 (S₁) and 20.48 (S₂) in medium concentrate and 21.53 (control), 19.25 (S₁) and 18.03 (S₂) in low concentrate substrate.

4.3.13.3 TCA-Precipitable-N as Influenced by Probiotics:-

The TCA precipitable-N (mg/100ml SRL) was increased ($P<0.05$) due to supplementation of probiotics in high, medium and low concentrate substrate (Table 4.28). The respective values were 24.45 (control), 27.76 (S_1) and 29.86 (S_2) in high concentrate; 22.28 (control), S_1 (24.03) and 26.42 (S_2) in medium concentrate; 21.17 (control), 24.03 (S_1) and 25.61 (S_2) in low concentrate substrate.

Results of the present study could be substantiated with the earlier reports. Ammonia incorporation into microbial protein was enhanced due to supplementation of yeast (Dawson, 1987; Weidmeier *et al.*, 1987; Chademana and Offer, 1990; Carro *et al.*, 1992a; Olson *et al.*, 1994b) and this was confirmed by greater microbial yield and microbial true protein reaching the duodenum (Gomez-Alarcon *et al.*, 1987; Erasmus, 1991). The concentration of ammonia was decreased by 10 to 35 per cent *in vitro* when supplemented with yeast (Dawson and Newman, 1987, 1988; Carro *et al.*, 1992a). In the present study also there was reduction in ammonia-N concentration which in turn reduced NPN concentration. TCA precipitable N was increased due to supplementation of probiotics which was mainly due to higher microbial yield. This was confirmed by higher rate of ^{35}S incorporation into microbial protein. The ^{35}S incorporation was more in high concentrate diet.

Type of substrate and treatment have significant impact on total-N, NPN, TCA-precipitable-N and ^{35}S incorporation into microbial protein (Table 4.29), i.e., the values were higher in high concentrate substrate followed by medium and low concentrate substrate. In the present study higher microbial yield was supported by higher TCA-precipitable-N, ^{35}S incorporation, and total bacterial counts in the *in vitro* system when supplemented with probiotics.

Based on the data of *in vitro* (Phase III) rumen fermentation the culture combination, namely, *S.cerevisiae*-B (SC-B), *L.plantarum* and *E.faecium* (SC-B+LP+EF) appeared to be most promising. The resume for the same can be as

following:

- i) In high concentrate diet highest DM degradability and TVFA concentration was recorded in SC-B and SC-B+LP+EF treatments. Lowest acetate to propionate ratio was observed in these treatments which was due to higher propionate level. Higher bacterial counts, TCA-precipitable-N and microbial protein synthesis as indicated by ^{35}S incorporation into microbial protein were seen in the combined cultures of SC-B+LP+EF treatment.
- ii) In medium concentrate diet also combined culture of SC-B+LP+EF showed better performance in terms of higher $\text{NH}_3\text{-N}$ incorporation into microbial protein as indicated by the higher bacterial counts, TCA-precipitable-N and ^{35}S incorporation into microbial protein.
- iii) It was observed that *S.cerevisiae*-B and combined culture of SC-B+LP+EF showed better performance in high fodder (70%) substrates also (70%). Highest TVFA was recorded in SC-B group; whereas, A/P ratio was lowest in SC-B+LP+EF group due to higher propionate and lower acetate value. TCA precipitable-N was highest in the combined culture group. ^{35}S incorporation into microbial protein was also comparatively higher in the combined culture group.

Based on the above observations, combined culture of *S.cerevisiae*-B, *L.plantarum*+ *E.faecium* showed better performance to improve rumen fermentation over other treatment groups. The benefits of *S.cerevisiae*-B was incorporated in this combination as SC-B contributed 60% of this combination. The *L.plantarum* and *E.faecium* may improve digestive activity (Szabo, 1979; Speck, 1983; Kolars *et al.*, 1984), control diarrhoea (Kopeeny *et al.*, 1989; Abu-Tarbousch *et al.*, 1996), and enhance health and performance (Collington *et al.*, 1988; Porter, 1977) of the animals. Therefore, combined culture of *S.cerevisiae*-B+*L.plantarum*+*E.faecium* were finally selected to supplement the diet of dairy cows to evaluate the effect on lactation performance.

Table 4.15: Concentrate mixture constituents and their calculated CP and TDN values (*in vitro* studies) Phase-III

Ingredient	Part (kg)	CP(kg)	TDN (kg)
Maize	40	4.00	36.00
Groundnut cake	25	10.00	19.75
Mustard cake	10	3.70	7.50
Wheat bran	22	3.52	14.30
Min.mix.	2	-	-
Common salt	1	-	-
	100	21.22	77.55

Table 4.16: Chemical composition of feedstuffs (% DM basis) used for *in vitro* studies (Phase III)

Parameter	Conc.mix.	Maize fodder	Wheat straw
OM	89.55	87.54	88.50
CP	20.78	11.69	3.30
CF	8.26	25.16	37.50
EE	4.52	1.88	1.50
NFE	55.99	48.81	46.20
Ash	10.45	12.46	11.50

Table 4.17: Response of promising probiotic cultures on DM degradability(%), total gas and methane production under *in vitro* system having high concentrate substrate(concentrate: roughage = 60:40)

Treat ment	DM Degra- dability	Total gas (ml) in 18h	Total gas(ml) in 18-24h	Cumulative gas (ml)in 24h	Methane (%) in 18h	Methane (ml) in18h	Methane (%) in 18-24 h	Methane (ml) in 8-24h	Methane(%) upto 24h
Control	62.05±0.41 ^a	49.33±2.68 ^a	16.67±0.93 ^a	66.00±3.61 ^a	18.40±0.50	9.10±0.72 ^a	17.71±0.37	2.95±0.19 ^a	18.23±0.45
P ₁	67.65±1.29 ^{cd}	70.20±0.20 ^{de}	24.33±0.88 ^b	94.53±1.07 ^{bc}	17.85±0.14	12.53±0.09 ^b	16.95±0.37	4.13±0.23 ^b	17.62±0.15
P ₂	66.52±0.04 ^{bc}	71.50±0.50 ^c	25.33±0.67 ^b	96.83±0.60 ^c	17.71±0.24	12.67±0.25 ^b	17.37±0.27	4.40±0.16 ^{bc}	17.62±0.12
P ₃	65.39±0.48 ^b	67.67±0.33 ^{bcd}	24.67±0.33 ^b	92.33±0.33 ^{bc}	17.85±0.36	12.08±0.27 ^b	17.90±0.84	4.40±0.25 ^{bc}	17.87±0.11
P ₄	68.37±0.24 ^d	68.90±0.56 ^{cde}	25.00±0.50 ^b	93.90±0.87 ^{bc}	18.54±0.24	12.77±0.80 ^b	18.20±0.28	4.45±0.14 ^{bc}	18.45±0.24
P ₅	66.56±0.84 ^{bc}	65.83±0.44 ^b	25.33±1.30 ^b	91.17±0.88 ^b	18.27±0.14	12.06±0.01 ^b	18.33±0.24	4.65±0.27 ^{bc}	18.32±0.12
P ₆	68.07±0.29 ^{cd}	66.23±0.43 ^{bc}	24.50±1.04 ^b	90.73±1.37 ^b	18.96±0.86	12.47±0.60 ^b	18.20±0.14	4.46±0.18 ^{bc}	18.42±0.80
P ₇	67.25±0.33 ^{cd}	65.67±0.73 ^b	27.00±3.05 ^b	92.67±3.68 ^{bc}	19.78±0.84	13.00±0.55 ^b	19.03±0.50	5.17±0.72 ^c	19.58±0.57
Level of Sign.	**	**	**	**	NS	**	NS	*	NS
CD	1.61	2.70	3.55	5.15		1.04		0.86	

Figures with different superscripts (a,b,c,d,e) in a column differ significantly

*P<0.05

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.18 : Effect of promising probiotic cultures on *in vitro* rumen fermentation pattern (substrate, concentrate:roughage = 60:40)

Treatment	pH	Total VFA mmol/100ml	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate/ Propionate	NH ₃ -N (mg/100ml)
Control	6.73±0.11 ^a	7.27±0.22 ^a	64.29±1.68 ^b	25.94±1.04 ^a	9.76±0.94 ^a	2.51±0.14 ^a	16.43±0.63 ^e
P ₁	6.88±0.02 ^b	9.40±0.26 ^d	51.28±0.51 ^a	34.33±0.20 ^c	14.39±0.31 ^b	1.49±0.03 ^a	13.63±0.43 ^{bc}
P ₂	6.82±0.04 ^{ab}	8.97±0.03 ^{bcd}	52.51±0.54 ^a	32.45±0.64 ^{bc}	15.04±0.14 ^{bc}	1.62±0.05 ^{ab}	15.37±0.03 ^{bc}
P ₃	6.83±0.04 ^{ab}	8.93±0.03 ^{bcd}	53.32±1.72 ^a	30.46±0.33 ^{bc}	15.23±0.65 ^{bc}	1.70±0.12 ^{ab}	12.97±0.23 ^{abc}
P ₄	6.78±0.01 ^{ab}	8.73±0.67 ^{bc}	54.10±0.91 ^a	29.64±0.68 ^{ab}	16.26±0.43 ^c	1.82±0.68 ^b	14.37±0.34 ^{cd}
P ₅	6.82±0.01 ^{ab}	8.50±0.20 ^b	54.28±0.45 ^a	30.98±0.64 ^{bc}	15.74±0.45 ^c	1.72±0.47 ^{ab}	13.17±0.67 ^{abc}
P ₆	6.77±0.02 ^a	8.90±0.11 ^{bc}	54.23±2.28 ^a	29.75±2.21 ^{ab}	16.03±0.25 ^c	1.85±0.22 ^b	12.67±0.53 ^{ab}
P ₇	6.75±0.00 ^a	9.17±0.33 ^{cd}	51.80±0.82 ^a	32.27±0.87 ^{bc}	15.93±0.11 ^c	1.61±0.67 ^{ab}	12.13±0.74 ^a
Level of Sign. CD	** 0.11	** 0.49	** 3.42	** 4.07	** 1.22	** 0.29	** 1.40

Figures with different superscripts (a,b,c,d,e) in a column differ significantly *P<0.05 **P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.19: Effect of different promising probiotic cultures on microbial counts under *in vitro* system having high concentrate substrate (concentrate to roughage = 60:40)

Treatment	Total Bacteria (no/ml SRL) Log ₁₀ value	Total protozoa (no/ml SRL) Log ₁₀ value	Holotrichs %	Spirotrichs %
Control	8.51±0.01 ^a	3.48±0.10	41.68	58.32
P ₁	8.66±0.02 ^a	3.31±0.16	46.84	53.16
P ₂	8.59±0.01 ^b	2.96±0.14	45.13	54.87
P ₃	8.68±0.01 ^{cd}	3.33±0.25	28.58	71.42
P ₄	8.61±0.02 ^b	3.60±0.03	30.19	69.81
P ₅	8.71±0.02 ^d	3.75±0.09	45.97	54.03
P ₆	8.79±0.01 ^a	3.63±0.17	49.90	50.10
P ₇	8.81±0.02 ^a	3.30±0.12	33.96	66.04
Level of Sign.	**	NS		
CD	0.04			

Figures with different superscripts (a,b,c,d,e) in a column differ significantly

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF
P₇ = SC-B+LP+EF

Table 4.20: Response of supplementation of promising probiotic cultures to medium concentrate (concentrate:roughage = 40:60) substrate on *in vitro* DM degradability(%), total gas and methane production

Treat ment	DM Degrada- bility	Total gas (ml) in 18h	Total gas (ml) in 18-24h	Cumulative gas (ml)in 24h	Methane (%) in 18h	Methane (ml) in 18h	Methane (%) in 18-24 h	Methane (ml) in 8-24h	Methane(%) upto 24h
Control	49.03±0.91 ^a	35.73±0.82 ^a	15.17±0.17 ^a	50.90±0.67 ^a	16.16±0.14 ^a	5.76±0.17 ^a	15.59±0.24 ^a	2.36±0.02 ^a	15.99±0.17 ^a
P ₁	56.03±0.37 ^b	45.17±1.45 ^d	23.67±0.73 ^b	68.83±2.12 ^{cd}	16.86±0.50 ^b	7.63±0.46 ^{cd}	16.55±0.14 ^{bc}	3.92±0.94 ^b	16.76±0.28 ^{bc}
P ₂	55.17±2.08 ^b	45.83±0.93 ^d	25.33±0.33 ^c	71.17±0.60 ^d	17.56±0.00 ^{cd}	8.05±0.16 ^d	16.69±0.14 ^{bcd}	4.23±0.81 ^{bcd}	17.25±0.05 ^{cd}
P ₃	55.77±1.67 ^b	44.10±2.01 ^{cd}	25.66±0.67 ^c	69.77±2.66 ^d	16.58±0.14 ^{ab}	7.32±0.36 ^{bcd}	16.13±0.14 ^{ab}	4.14±0.15 ^{bcd}	16.41±0.12 ^{ab}
P ₄	56.88±0.33 ^b	44.17±1.17 ^{cd}	25.67±0.88 ^c	69.83±1.59 ^d	17.70±0.14 ^d	7.82±0.22 ^{cd}	16.69±0.28 ^{bcd}	4.29±0.21 ^{cd}	17.34±0.11 ^{cd}
P ₅	57.11±0.53 ^b	46.67±0.67 ^d	26.50±0.29 ^c	73.17±0.44 ^d	17.00±0.61 ^{bc}	7.93±0.32 ^{cd}	16.69±0.28 ^{bcd}	4.42±0.87 ^d	16.89±0.47 ^{bcd}
P ₆	56.94±1.39 ^b	40.83±1.96 ^{bc}	23.67±1.01 ^b	64.50±2.75 ^{bc}	17.70±0.28 ^d	7.22±0.29 ^{bc}	16.97±0.14 ^{cd}	4.02±0.20 ^{bc}	17.43±0.21 ^d
P ₇	54.59±1.10 ^b	38.60±1.53 ^{ab}	25.43±0.30 ^c	64.03±1.75 ^b	17.56±0.42 ^{cd}	6.79±0.41 ^b	17.25±0.24 ^d	4.39±0.11 ^d	17.03±0.44 ^{cd}
Level of Sign.	*	**	**	**	**	**	**	**	**
CD	2.99	3.69	1.60	4.75	0.66	0.76	0.56	0.35	0.59

Figures with different superscripts (a,b,c,d) in a column differ significantly

*P<0.05

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.21: Effect of promising probiotic cultures supplementation to substrate containing concentrate:roughage = 40:60 on *in vitro* rumen fermentation

Treatment	pH	Total VFA (mmol/100ml)	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate/Propionate	NH ₃ -N (mg/100ml)
Control	6.75±0.07 ^{abc}	6.66±0.08 ^a	70.16±1.16 ^c	24.63±1.08 ^a	5.21±0.36 ^a	2.86±0.16 ^c	12.63±0.57 ^c
P ₁	6.73±0.06 ^{ab}	7.23±0.09 ^{bcd}	53.98±0.31 ^{ab}	32.87±0.34 ^b	13.15±0.59 ^{bc}	1.64±0.01 ^{ab}	8.23±0.03 ^{ab}
P ₂	6.72±0.01 ^a	7.60±0.20 ^{de}	54.77±1.74 ^{ab}	32.90±1.55 ^b	12.53±0.59 ^{bc}	1.67±0.13 ^{ab}	8.70±0.45 ^{ab}
P ₃	6.77±0.01 ^{cd}	7.70±0.32 ^e	49.44±4.69 ^a	37.81±3.73 ^c	12.77±0.96 ^{bc}	1.35±0.24 ^a	9.13±0.47 ^b
P ₄	6.76±0.02 ^{bc}	7.47±0.20 ^{cde}	54.75±1.55 ^{ab}	32.73±1.47 ^b	12.51±0.68 ^{bc}	1.68±0.12 ^{ab}	8.43±0.61 ^{ab}
P ₅	6.82±0.01 ^e	7.10±0.06 ^{bc}	53.47±3.52 ^{ab}	34.80±2.37 ^{bc}	11.73±1.32 ^b	1.54±0.20 ^{ab}	8.67±0.47 ^{ab}
P ₆	6.82±0.01 ^e	7.60±0.10 ^{de}	54.11±1.46 ^{ab}	31.69±1.08 ^b	14.15±1.68 ^c	1.71±0.79 ^{ab}	7.73±0.23 ^a
P ₇	6.80±0.00 ^{de}	7.03±0.13 ^{ab}	56.06±0.52 ^c	30.92±0.66 ^b	13.02±0.47 ^{bc}	1.82±0.05 ^b	7.97±0.23 ^a
Level of Sign.	**	**	**	**	**	**	**
CD	0.03	0.40	6.14	4.88	2.25	0.38	1.12

Figures with different superscripts (a,b,c,d,e) in a column differ significantly *P<0.05 **P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.22 : Effect of different promising probiotic cultures on microbial counts in *in vitro* study having medium concentrate substrate (concentrate to roughage = 40:60)

Treatment	Total Bacteria (no/ml SRL) Log ₁₀ value	Total protozoa (no/ml SRL) Log ₁₀ value	Holotrichs %	Spirotrichs %
Control	8.46±0.01 ^a	3.23±0.12	3.47	96.53
P ₁	8.59±0.01 ^{bc}	3.13±0.13	7.78	92.22
P ₂	8.56±0.03 ^b	2.80±0.10	9.72	90.28
P ₃	8.57±0.01 ^b	3.13±0.13	10.37	89.63
P ₄	8.57±0.03 ^b	3.20±0.10	9.30	90.70
P ₅	8.65±0.04 ^c	3.22±0.14	7.52	92.48
P ₆	8.72±0.03 ^d	3.22±0.04	9.79	90.21
P ₇	8.76±0.02 ^d	3.16±0.09	9.21	90.79
Level of Sign. CD	** 0.06	NS		

Figures with different superscripts (a,b,c,d) in a column differ significantly

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.23: Response of promising probiotics culture on *in vitro* DM degradability(%), total gas and methane production using low concentrate substrate (concentrate :roughage = 30:70)

Treat- ment	DM Degra- dability	Total gas (ml) in 18h	Total gas (ml) in 18-24h	Cumulative gas (ml)in 24h	Methane (%) in 18h	Methane (ml) in18h	Methane (%) in 18-24 h	Methane (ml) in 8-24h	Methane(%) upto 24h
Control	46.70±0.29 ^a	30.00±1.04 ^a	15.17±0.17 ^a	45.17±1.01 ^a	11.10±0.81	3.34±0.33	10.86±0.21 ^a	1.65±0.05 ^a	11.02±0.61
P ₁	52.16±1.05 ^c	39.13±1.10 ^c	23.67±0.45 ^b	62.52±1.06 ^d	11.38±0.28	4.46±0.23	11.56±0.50 ^{abc}	2.97±0.10 ^d	11.44±0.36
P ₂	51.41±0.77 ^c	36.43±1.29 ^{cd}	23.87±0.59 ^{bc}	60.30±1.85 ^{cd}	10.96±0.51	3.99±0.26	11.42±0.01 ^{ab}	2.72±0.06 ^{bc}	11.14±0.31
P ₃	52.09±0.34 ^c	34.60±0.70 ^{bc}	23.73±0.37 ^{ab}	58.33±0.52 ^{bc}	11.10±0.14	3.84±0.13	11.00±0.64 ^a	2.61±0.14 ^a	11.06±0.35
P ₄	51.05±0.53 ^{bc}	35.40±0.31 ^{cd}	23.10±0.49 ^b	58.56±0.54 ^{bc}	10.54±0.28	3.73±0.11	11.14±0.14 ^a	2.58±0.06 ^a	
P ₅	52.43±1.01 ^c	37.40±0.31 ^{dc}	23.53±0.38 ^{bc}	60.93±0.27 ^{cd}	10.96±0.28	4.10±0.13	12.12±0.51 ^{bc}	2.86±0.16 ^{cd}	10.77±0.21
P ₆	49.26±0.31 ^b	34.67±0.73 ^{bc}	24.67±0.67 ^c	59.33±1.36 ^{bc}	11.38±0.28	3.95±0.17	13.11±0.01 ^d	3.24 ±0.08 ^c	11.42±0.05
P ₇	49.38±0.88 ^b	33.17±1.09 ^b	24.00±0.58 ^{bc}	57.17±1.59 ^b	11.80±0.37	3.92±0.21	12.27±0.01 ^c	2.94±0.07 ^{cd}	12.10±0.16
Level of Sign	**	**	**	**	NS	NS	**	**	12.00±0.22
CD	1.84	2.21	1.28	2.88			0.80	0.24	NS

Figures with different superscripts (a,b,c,d,e) in a column differ significantly

*P<0.05

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.24: Effect of probiotic cultures on *in vitro* rumen fermentation having a substrate containing concentrate to roughage (30:70)

Treatment	pH	Total VFA (mmol/100ml)	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate: Propionate	NH ₃ -N (mg/100ml)
Control	6.84±0.02	6.30±0.10 ^a	66.84±4.98 ^{de}	24.94±5.74	8.22±2.39 ^{ab}	3.01±0.72 ^d	10.73±0.38 ^f
P ₁	6.75±0.03	7.50±0.09 ^c	58.25±3.29 ^{bc}	33.67±3.28	8.08±0.44 ^a	1.72±0.25 ^{abc}	6.57±0.17 ^{bc}
P ₂	6.81±0.03	7.07±0.09 ^b	72.79±5.50 ^c	26.99±2.10	6.89±1.69 ^a	2.51±0.36 ^{cd}	7.03±0.09 ^{de}
P ₃	6.81±0.04	7.47±0.14 ^c	62.64±0.31 ^{cd}	28.20±0.74	9.16±0.52 ^{ab}	2.22±0.07 ^{bc}	7.30±0.12 ^c
P ₄	6.86±0.02	6.93±0.06 ^b	59.24±0.82 ^{bc}	31.04±0.63	9.72±0.22 ^{ab}	1.91±0.06 ^{abc}	6.37±0.17 ^{ab}
P ₅	6.75±0.03	7.10±0.12 ^b	57.95±2.93 ^{bc}	30.91±2.19	11.14±0.87 ^{bc}	1.90±0.22 ^{abc}	6.73±0.12 ^{bcd}
P ₆	6.84±0.04	7.03±0.03 ^b	52.23±1.14 ^b	34.72±0.78	13.05±0.38 ^c	1.51±0.06 ^{ab}	7.00±0.11 ^{cde}
P ₇	6.84±0.03	6.93±0.23 ^b	49.68±1.79 ^a	36.48±1.22	13.84±0.74 ^c	1.37±0.09 ^a	6.03±0.09 ^a
Level of Sign.	NS	**	**	NS	*	**	**
CD		0.32	7.17		3.01	0.77	0.45

Figures with different superscripts (a,b,c,d,e,f) in a column differ significantly

*P<0.05

**P<0.01

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EFP₇ = SC-B+LP+EF

Table 4.25 : Effect of different promising probiotics on *in vitro* microbial counts having low concentrate substrate (concentrate to roughage = 30:70)

Treatment	Total Bacteria(no/ml SRL) Log ₁₀ value	Total protozoa (no/ml SRL) Log ₁₀ value	Holotrichs %	Spirotrichs %
Control	8.44±0.03	3.38±0.19	11.03	88.97
P ₁	8.55±0.06	3.08±0.25	18.21	81.79
P ₂	8.46±0.04	3.18±0.01	9.12	90.88
P ₃	8.51±0.02	3.25±0.07	10.69	89.31
P ₄	8.53±0.05	3.16±0.09	7.04	92.96
P ₅	8.55±0.04	3.29±0.06	19.83	80.17
P ₆	8.55±0.07	3.06±0.06	17.14	82.86
P ₇	8.67±0.04	3.06±0.06	6.73	91.19
Level of Sign.	NS	NS		

NS = Non-significant

P₁ = SC-B; P₂ = EF; P₃ = SC-522+LP; P₄ = SC-522+EF; P₅ = SC-522+LA+LL; P₆ = SC-522+LP+EF

P₇ = SC-B+LP+EF

Table 4.26: Analysis of variance for IVDMD, total gas, and methane production

Source of variation	D F	Mean sum of squares								
		IVDMD	Total gas (ml) in 18h	Total gas (ml) in 18-24h	Cumulative gas (ml) in 24h	Methane (%) in 18h	Methane (ml) in 18h	Methane (%) in 18-24 h	Methane (ml) in 18-24h	Methane (%) upto 24h
Diet (Substrate)	2	1597.302**	6085.911**	14.151**	6558.585**	361.236**	403.970**	260.733**	18.682**	319.621**
Treatment	7	29.463**	164.080**	93.823**	468.554**	1.581**	4.502**	2.590**	2.900**	1.463**
All effect interaction	14	3.289	23.885*	1.918	27.315**	0.642	1.078**	0.580	0.151	0.566
Error	46	2.401	3.854	2.514	8.511	0.447	0.291	0.358	0.136	0.310

*P<0.05; **P<0.01

Table 4.27: Analysis of variance for pH, VFA production, NH₃-N and microbial population

Source of variation	DF	Mean Sum of Squares								
		pH	Total VFA mmol/100ml	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate/ Propionate	NH ₃ -N mg/100 ml	Total Bacteria (no/ml) Log ₁₀ value	Total protozoa (no/ml) Log ₁₀ value
Diet (Substrate)	2	19.932**	0.023**	201.876**	18.008	139.684**	0.449**	283.333**	0.117**	0.548**
Treatment	7	1.559**	0.008*	202.876**	63.166**	39.328**	1.362**	17.789**	0.066**	0.172**
All effect interaction	14	0.252**	0.013**	64.229**	22.757*	7.844**	0.215	1.195*	0.003	0.057
Error	46	0.803	0.003	17.168	11.504	2.412	0.131	0.516	0.003	0.050

*P<0.05; **P<0.01

Table 4.28: *In vitro* N-fractions and ^{35}S incorporation into microbial protein as influenced by probiotics

Parameter	High concentrate ¹	Medium concentrate ²	Low concentrate ³
Total-N(mg)/100 ml SRL)			
Control	50.17±0.31	45.15±0.85	42.70±1.01
S ₁	51.22±0.91	45.38±0.47	43.63±0.51
S ₂	51.92±0.51	46.90±0.20	43.63±0.51
CD	NS	NS	NS
Non-Protein-N(mg)/100 ml SRL)			
Control	25.72±0.10 ^c	22.87±0.26 ^b	21.53±0.56 ^b
S ₁	23.45±0.18 ^b	21.35±0.18 ^a	19.25±0.71 ^a
S ₂	22.05±0.10 ^a	20.48±0.44 ^a	18.03±0.17 ^a
CD	0.44**	1.08*	1.91*
TCA-precipitable-N (mg)/100ml SRL			
Control	24.45±0.38 ^a	22.28±0.59 ^a	21.17±0.79 ^a
S ₁	27.76±1.07 ^b	24.03±0.58 ^b	24.03±0.35 ^b
S ₂	29.86±0.61 ^c	26.42±0.30 ^c	25.61±0.36 ^b
CD	1.96*	1.62*	1.68*
^{35}S incorporation into microbial protein (%)			
Control	46.52±2.01 ^a	41.30±0.78	38.60±0.88
S ₁	51.92±0.91 ^b	47.83±3.34	41.71±1.87
S ₂	56.69±1.52 ^c	47.91±1.66	41.97±1.06
CD	3.99*	NS	NS

*P<0.05; **P<0.01 a,b,c means with different superscripts differ significantly

S₁ = *S.cerevisiae*-B

S₂ = *S.cerevisiae*-B+*L.plantarum*+*E.faecium*(6:2:2)

1. Roughage:Concentrate (40:60)

2. Roughage:Concentrate (60:40)

3. Roughage:Concentrate (70:30)

Table 4.29: Analysis of variance for total-N, NPN, TCA-ppt-N and ³⁵S incorporation into microbial protein

Source of variation	DF	Mean sum of squares			
		Total-N(mg)/100 ml SRL	Non-Protein-N(mg)/ 100 ml SRL	TCA-precipitable protein (mg)/100ml SRL	³⁵ S incorporation into microbial protein
Substrate	2	145.625**	38.577**	36.322**	270.481**
Treatment	2	4.956	23.374**	49.215**	109.679**
All effect interaction	4	0.374	0.368	0.630	10.830
Error	16	0.913	0.442	0.949	9.398

**P<0.01

PHASE-IV

4.4 EFFECT OF PROBIOTICS ON LACTATION PERFORMANCE AND DIGESTIBILITY OF NUTRIENTS IN CROSSBRED COWS

Ten multiparous crossbred (Tharparker x Holstein Friesian) cows of similar stage of lactation and milk yield were distributed into two groups of 5 each. Both the groups were fed same concentrate mixture and green fodder according to requirements (NRC, 1989), however, the animals in experimental group were supplemented with a triple combination of probiotics (*S.cerevisiae*-B + *L.plantarum* + *E.faecium* in the ratio of 6:2:2) @ 10×10^9 cfu/d/animal for a period of 90d. During the course of trial, daily milk yield, DM intake, and fortnightly milk composition were recorded. During the last phase of experiment, a digestibility trial was conducted. The results obtained during the experiment are presented as follows:

4.4.1 Meteorological Observations During Lactation Trial:-

The lactation trial was conducted during winter season (23rd Oct'97 to 24th Jan'98). Lowest minimum temperature was recorded during the last 4 weeks of the experiment. Highest relative humidity was recorded during 7, 8 and 9th week of the observation period due to cloudy weather and rainfall. Sunshine hours were minimum during this period. Due to this inclement weather, slightly lower milk yield was recorded in both the groups during this period (Table 4.30).

4.4.2 Fortnightly Nutrient Intake As Influenced by Probiotics:-

4.4.2.1 DM Intake:-

Average DM intake was about 10.2 kg in both the groups during first fortnight and thereafter increased about 10% in both the groups upto 5th fortnight (Table 4.34). During sixth fortnight, the DM intake increased by 20 per cent in both the groups, and was statistically similar in both the groups. Fodder intake was significantly higher ($P < 0.10$) during 2nd, 4th and 6th

fortnights in experimental group than control, without affecting concentrate intake. The supplementation of probiotics tended to improve the DM intake/100 kg b.wt. significantly during 2nd ($P<0.10$), 4th and 6th fortnight ($P<0.05$), however, the improvement was not consistent during whole of the experimental period.

4.4.2.2 OM Intake:-

The OM intake ranged from 9.72 to 11.27 kg in control and 9.66 to 11.25 kg in treatment group, however, the difference between control and treatment groups was non-significant in all six fortnights. But the OM intake/100 kg b.wt. followed the same trend with that of DM intake/100kg b.wt., i.e., OM intake/100 kg b.wt. was significantly improved in 2nd ($P<0.10$), 4th and 6th ($P<0.05$) fortnight.

4.4.2.3 CP Intake:-

The total CP intake varied from 1.46 to 2.15 kg and 1.48 to 2.18 kg from 1st to 6th fortnight in control and probiotics groups. Variations between two groups was non-significant in all the fortnight. CP intake per 100 kg body weight was 0.404 and 0.428 kg/day in control and treatment groups during the first fortnight. Total CP intake was increased because of inclusion of berseem fodder from 2nd to 6th fortnight in both the groups.

The fortnightly DM intake and CP intake fulfilled the requirements of the animals in both the groups as per NRC, 1989. The observations in the present study were in partial agreement with the earlier reports. In lactating cows, yeast culture supplementation significantly increased the DM intake by 1.2 to 1.6 kg/day with higher milk production (Williams, 1990a, Williams *et al.*, 1991). Greater DMI during first 6 weeks of lactation was observed due to yeast supplementation in dairy cattle when fed with silage:grain (1:1, DM basis) and hay (0.9 kg/day) (Wohlt *et al.*, 1991). Whereas, Gomez-Alarcon (1987), Harrison *et al.* (1988), Piva *et al.* (1993) and Scott *et al.* (1994) observed non-significant improvement of DMI by lactating cows fed with yeast

adjunct. In the present study, total DMI was not influenced by the addition of combined culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium*, but DMI per 100 kg body weight and fodder intake *per se* was significantly higher in some fortnights. However, some studies indicated no added advantage of yeast culture supplementation to lactating cows on DMI (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz *et al.*, 1994; Kung *et al.*, 1997). Differences in response to added probiotics might have been due to interactions among yeast, diet or stage of lactation.

4.4.3 Change in Body Weight as Affected by Probiotics:-

Body weight gain in treatment group was higher ($P<0.05$) than in control group (Table 4.35). Animals in both the groups gained the body weight and maximum gain in both groups was recorded during the fifth fortnight, however, during the sixth fortnight the animals in both the groups lost the body weight probably due to inclement weather, but the loss was higher in control than in treatment group. The animals would have diverted the body reserves for maintaining body temperature and milk production. These results indicated that dietary supplementation of probiotics improved feed utilization for weight gain.

4.4.4 Milk Yield and Composition as Influenced by Probiotics Supplementation to Crossbred Cows:-

4.4.4.1 Milk Yield:-

Mean daily milk yield was 9.29 and 9.21 kg in control and treatment groups respectively during the first fortnight (Table 4.31). Milk yield gradually increased in both the groups during 2nd fortnight onwards and the magnitude of increase was higher in treatment groups than in control. However, variation between the groups during each fortnight was non-significant. The overall average milk yield/day was 10.16 and 10.60 kg in control and treatment groups, respectively. The application of t-test revealed the significantly higher ($P<0.01$) milk yield in probiotic supplemented group

than in control group.

Several workers have reported that supplementation of yeast culture to the lactating cows improved milk yield or FCM yield (Hoyos *et al.*, 1987; Williams, 1989b; Piva *et al.*, 1993; Swartz *et al.*, 1994; Adams *et al.*, 1995; Kung *et al.*, 1997). In the high producing cows, there was a 6% increase in milk production due to supplementation of viable mixed microbial feed additives (*S.cerevisiae*, *Streptococcus faecium* and *L.acidophilus*), whereas, there was no treatment effect in the low productive cows (Hoyos *et al.*, 1987). Milk production by primiparous Holstein cows fed corn silage:grain (1:1, DM basis) and hay (0.9 kg/d) improved by addition of yeast culture (Wohlt *et al.*, 1991). European commercial farm trials demonstrated increase in milk yield in maximum cases as a consequence of including Yea-Sacc in the diet (Williams, 1989b). In early (Scott *et al.*, 1994) and mid lactation (Piva *et al.*, 1993; Adams *et al.*, 1995) cows there was increase in milk yield due to addition of yeast culture. Whereas, some workers reported that milk yield was unaffected by the addition of yeast culture to the diet of cows (Malcolm and Kiesling, 1986; Arambel and Kent, 1990; Kim *et al.*, 1992b, Higginbotham *et al.*, 1994). Dawson (1993) stated that yeast culture supplementation did not have equal beneficial effects with all types of diets and it is currently not possible to define the dietary conditions that provide for the optimum response to yeast culture supplementation.

4.4.4.2 FCM Yield:-

The average FCM yield was 8.64 and 8.37 kg/day during the first fortnight and thereafter it followed the trend of milk yield during each fortnight (Table 4.31). However, the FCM yield was significantly higher ($P<0.10$) during 3rd, 4th and 5th fortnights. Overall average FCM yield was 9.94 and 10.31 kg/day in control and experimental groups, respectively and the variation between the groups was significant ($P<0.10$). Earlier, Huber *et al.* (1989) reported higher milk and FCM yield of dairy cows when fed commercial

preparation of yeast (Yea-Sacc). Gunther (1989) has also shown the same trend in dairy cows indicating that the inclusion of Yea-Sacc increased FCM from 30.10 kg/day to 35.35 kg/day. In early (Harris and Lobo, 1988; Swartz *et al.*, 1994; Putnam *et al.*, 1997; Kung *et al.*, 1997) and mid lactation cows (Piva *et al.*, 1993) more FCM yield was recorded due to addition of yeast culture.

4.4.4.3 Effect of Mixed Culture Addition to Crossbred Cows on Milk Production Efficiency:-

The milk production efficiency (kg/kg DMI) was 0.91 and 0.89 in control and treatment groups, respectively, during the first fortnight. During the 2nd fortnight the efficiency improved in both the groups (Table 4.31). Thereafter the efficiency declined upto 6th fortnight in both groups but the decline in treatment group was lower than control group. However, statistically there was no significant variation between the treatments in the different fortnights. The overall milk production efficiency was 0.88 in control and 0.90 in experimental group and it was significantly higher ($P<0.10$) in experimental group than in control.

FCM production efficiency (Table 4.31) was 0.84 and 0.81 in control and treatment groups, during first fortnight. There was increasing tendency in FCM efficiency during 2nd, 3rd, 4th and 5th fortnight in control as well as treatment groups, but the increasing tendency was more in probiotic supplemented group. The overall FCM production efficiency was 0.86 and 0.88 in control and treatment groups which was statistically similar.

4.4.4.4 Milk composition:-

The parameters, viz., total solids, fat, protein, SNF, lactose and ash were estimated. The results are presented as follows:

4.4.4.4.1 Total Solids:-Total solids (TS) content in milk (Table 4.32) of control and experimental group was 13.26 and 13.08% during the first fortnight. Thereafter, the TS content varied in a narrow range in both the groups. The values were significantly higher ($P<0.10$) in probiotic

supplemented group during 3rd fortnight, whereas, in 4th ($P<0.05$) and 6th ($P<0.01$) fortnight there was significant reduction in TS in treatment group as compared to control. Overall TS per centage was found similar in both the groups (13.12 and 12.99 per cent). Quantitative TS was significantly ($P<0.10$) higher in treatment group over control during 3rd fortnight (Table 4.33), whereas, in other fortnights, quantitative TS was found similar, but there was increasing trend in TS in milk due to probiotic culture supplementation.

4.4.4.4.2 Fat:- In the first fortnight the fat content (Table 4.32) in milk was 3.52 and 3.42 per cent in control and treatment groups respectively, and the variation between the groups was not significant. Rising trend in fat content was observed during 2nd fortnight onwards in both the groups. The fat content was significantly higher in treatment group than control during 3rd ($P<0.05$), 4th ($P<0.01$) and 5th ($P<0.01$) fortnights. During 1st, 6th fortnight, and overall fat per cent was statistically similar in both groups. Total fat yield in milk followed the similar trend with that of fat per cent (Table 4.33).

These results revealed that the dietary supplementation of probiotics occasionally improve the fat content in milk of crossbred cows. The improved milk fat per centage was also reported by earlier workers. Addition of mixed microbial culture to the lactating cows improved milk fat per cent significantly in the top producers (Averaging 19.4% higher) and in lower producing cows (averaging 14% higher) (Hoyos *et al.*, 1987). Addition of commercial culture (Yea Sacc) to lactating cows improved butter fat per centage (Williams, 1989b; Gunther, 1989). Some others also observed significant improvement in milk fat per centage of lactating cows due to supplementation of yeast culture (Harris and Lobo, 1988; Dobos *et al.*, 1989; Piva *et al.*, 1993; Putnam *et al.*, 1997). Whereas others did not record any difference in milk fat per cent due to yeast culture supplementation (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz *et al.*, 1994).

4.4.4.4.3 Protein:- During the first fortnight the average protein content (Table

4.32) in milk of control and experimental animals was 2.94 and 2.96 per cent. The variation between the groups was not significant. However, there was an increasing trend in protein content in milk during the subsequent experimental period in both the groups. Milk protein varied from 2nd fortnight onwards in the range of 3.17 to 3.43 per cent in control group and 3.12 to 3.23 per cent in experimental group. However, the variation between groups was non-significant. Similar trend was observed in milk protein yield (kg) irrespective of treatment and fortnight (Table 4.33), thereby indicating that the protein content in milk was not influenced by the addition of probiotics culture to lactating crossbred cows.

Literature also indicated that no added advantage of yeast culture treatment to lactating cows was observed on milk proteins (Quinonez *et al.*, 1988; Huber *et al.*, 1989; Scott *et al.*, 1994; Higginbotham *et al.*, 1994; Kung *et al.*, 1997). However, some workers reported improvement in milk protein per cent of lactating cows due to yeast culture addition (Williams, 1989b; Gunther, 1989; Williams *et al.*, 1991; Putnam *et al.*, 1997).

4.4.4.4 Solid Not Fat (SNF):- During the first fortnight the highest value (9.74%) of SNF content (Table 4.32) in control group as well as in treatment (9.66%) group was registered. Thereafter, the milk SNF content declined in both the groups. Second fortnight onwards the milk SNF content varied in the range of 8.86 to 9.32 per cent in control group and 8.80 to 9.40 per cent in treatment group. Overall SNF per cent was significantly ($P < 0.01$) higher in control group than probiotic supplemented group.

4.4.4.5 Lactose:- In the first fortnight, the lactose content (Table 4.32) was 6.08 and 5.96 per cent in control and treatment groups, respectively, and the variation between the groups was significant ($P < 0.05$). Declining trend was registered in lactose content from 2nd fortnight onwards, and the values varied in the range of 4.89 to 5.26 per cent in control group and 4.88 to 5.55 per cent in treatment group. Average variation in lactose per cent of six fortnights

was non-significant between two groups. When calculated the total yield of lactose (kg) it was found that the variation between two groups was non-significant in all six fortnights (Table 4.33).

Kim *et al.* (1992b) have reported significant improvement of milk lactose per cent in early to mid lactation dairy cows when supplemented with yeast culture. Whereas, similar to our observations, Arambel and Kent (1990), Scott *et al.* (1994) and Kim *et al.* (1994) have shown no added effect of yeast culture on milk lactose per cent in dairy cows. However, per centage of lactose was lower for cows fed yeast culture plus *Aspergillus oryzae* (Higginbotham *et al.*, 1994).

4.4.4.4.6 Milk Ash:-During the first fortnight (Table 4.32) the milk ash content was 0.71 and 0.73 per cent in control and treatment groups. The milk ash content varied in the range of 0.71 to 0.74 per cent in control group and 0.70 to 0.74 per cent in treatment group during the six fortnights. Statistical analysis of data revealed that overall variation between the groups was non-significant.

4.4.5 Effect of Supplementation of Probiotics to Lactating Cows on Intake and Digestibility Coefficients of Nutrients During Digestibility Trial:-

A digestibility trial was conducted in order to study the effects of probiotic cultures on intake and digestibility co-efficients of nutrients in crossbred lactating cows.

4.4.5.1 Chemical Composition of Feedstuffs:-

CP, NDF, Cellulose and Lignin contents of concentrate mixture were 20.56, 40.00, 10.50 and 2.10 per cent (Table 4.36). The respective values were 9.55, 54.31, 31.41 and 7.98 per cent in oat fodder; and 18.16, 42.31, 21.69 and 7.94 per cent in berseem fodder.

4.4.5.2 Dry Matter and Crude Protein Intake:-

The concentrate mixture intake in control and treatment group was 4.13 and 4.29 kg/d, and the variation between the groups was not significant. DMI

through oat and berseem was similar in two groups. Total DM intake was 11.36 and 11.53 kg in control and treatment groups respectively. DMI per 100 kg body weight and DMI per kg metabolic body size ($W^{0.75}$ kg) were 3.07 and 3.25 kg; 134.77 and 141.10 g in control and treatment groups respectively. The variations between the groups were non-significant. Roughage to concentrate mixture ratio in the entire ration in control and treatment groups was 63.64:36.36 and 62.79:37.21. The oat fodder to berseem ratio (on DM basis) was 57.32:42.68 and 59.72:40.27 in control and treatment groups respectively.

CP intake was 1.82 and 1.87 kg in control and treatment groups respectively. CPI(g)/100 kg body weight and CPI(g)/ $W^{0.75}$ kg were slightly but non-significantly higher in treatment group (527.41 g and 22.86g) than control group (493.95g and 21.66g). These results revealed that the DM and CP intake from various feedstuffs were not influenced by the difference in dietary probiotics treatments.

Studies in past indicated no added advantage of yeast culture supplementation to lactating cows on DMI (Erdman and Sharma, 1989; Arambel and Kent, 1990; Wohlt *et al.*, 1991; Swartz *et al.*, 1994; Kung *et al.*, 1997). Whereas, non-significant improvement of DMI by lactating cows was observed when fed with yeast adjunct (Gomez-Alarcon, 1987; Harrison *et al.*, 1988; Piva *et al.*, 1993; Scott *et al.*, 1994). Increased DMI has also been reported due to yeast feeding in several other studies (Malcolm and Kiesling, 1986; Quinonez *et al.*, 1988, 1989; Williams *et al.*, 1991; Piva *et al.*, 1993).

4.4.5.3 Digestible Nutrient Intake:-

Digestible OM intake (Table 4.38) was 7.54 and 7.78 kg in control and treatment groups, respectively. The difference was found non-significant. Whereas, DOMI (kg)/100 kg body weight ($P<0.05$) and DOMI (g)/ $W^{0.75}$ kg ($P<0.01$) were significantly higher in treatment group (2.19 kg and 95.02 g) than control (2.04 kg and 89.41 g). TDN intake was 7.90 and 8.15 kg in control

and treatment groups, respectively, which was statistically similar. But TDN intake/100 kg b.wt and TDN intake/ $W^{0.75}$ kg was significantly higher ($P<0.01$) in probiotic treated group (2.29 kg and 99.55 g) than control (2.14 kg and 93.68 g). DCP intake followed the trend of TDN intake, i.e., DCP intake per unit body weight was significantly ($P<0.10$) higher in treatment group than control. TDN per cent of the ration was statistically higher ($P<0.05$) in treatment group than control, but DCP per cent was similar in both the groups. The intake of metabolizable energy (ME) and net energy for lactation (NEL) was 125.77 and 75.31 in control, 130.02 and 77.73 MJ in probiotic supplemented group. The ME and NEL (MJ) per kg DM of feed was 11.05 and 11.32; 6.61 and 6.76 MJ in control and treatment groups, respectively; and the treatment group showed significantly higher values ($P<0.05$). The data revealed that 4.25 MJ extra ME was consumed by probiotic supplemented group at similar level of DM intake in both the groups. The treatment group also consumed 2.42 MJ more NEL which could be the reason for higher level of milk yield due to probiotic culture addition to crossbred lactating cows.

TDN, ME and NEL intake were almost comparable with that of NRC (1989) requirements in both the groups. But CP intake was higher than NRC (1989) in both the groups. This could be assigned to berseem fodder addition in the feeding schedule which was having about 18% CP.

The results on digestible nutrient intake revealed that supplementation of triple combination of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* (Ratio 6:2:2) to crossbred milch cows increased TDN, DCP and DOM intake per unit body size at similar level of DM intake with that of control.

4.4.5.4 Digestibility Co-efficients of Nutrients as Influenced by Viable Probiotics Culture:-

Digestibility coefficients (Table 4.37) of nutrients for DM, OM, CF, CP, EE, NEF, NDF, ADF, cellulose and hemicellulose were 68.97, 75.68, 63.22, 75.12, 69.93, 81.43, 54.61, 44.91, 79.02 and 69.67 in control group, and

corresponding values in treatment group were 68.50, 76.03, 64.40, 75.70, 73.77, 80.61, 57.47, 47.07, 79.59 and 72.01. These results showed that there was no significant difference in digestibility of DM, OM, CF and CP. But NDF ($P<0.10$), ADF ($P<0.10$), hemicellulose ($P<0.01$) and EF ($P<0.01$) digestibilities were significantly increased due to probiotic culture addition to lactating cows. These results indicated that fibre digestibility was increased due to probiotic culture supplementation in the ration of milch cows having medium milk yield. In the present study higher cell wall component digestibility could be due to stimulation of fibre degrading bacteria in the rumen of dairy cattle by the addition yeast culture (Harrison *et al.*, 1987; Weidmeier *et al.*, 1987).

Earlier reports also suggested the similar trend. Kim *et al.* (1992b) have shown significantly higher CF, NDF and ADF digestibility due to supplementation of yeast to lactating cows. Yeast culture with high concentrate diet resulted in significantly higher DM and NDF digestibility in rusitech studies (Carro *et al.*, 1992a). In another study, yeast culture had similar effect on DM, NDF, ADF and hemicellulose digestibilities but CP and cellulose digestibilities tended to be improved (Wohlt *et al.*, 1991). Some reports suggested that addition of yeast culture to ruminants had no added advantage on digestibility of DM (Harrison *et al.*, 1988; Chademana and Offer, 1990) and OM (Chademana and Offer, 1990; Carro *et al.*, 1992b).

4.4.6 Addition of Probiotics on Economics of Milk Production:-

Fortnightly cost of milk production as influenced by probiotic culture supplementation is presented in the Table 4.39. Lowest feed cost (Rs)/kg of milk was recorded during 1st fortnight in both the groups. Whereas from 2nd to 6th fortnight feed cost/kg milk was higher due to berseem fodder addition in the feeding schedule. From 2nd fortnight onward feed cost/kg milk production ranged from Rs.5.24 to Rs.6.93 in control group and Rs.5.20 to Rs.6.74 in probiotics supplemented group. Cost of probiotics/animal/day was

calculated as Rs.1.44. Slightly lower feed cost/kg of milk was recorded due to probiotic treatment from 3rd to 6th fortnight. Fortnight average feed cost/kg of milk production was recorded, Rs.5.85 and Rs.5.81 in control and treatment groups, respectively.

For the 3 months observation period variable cost and fixed cost has been calculated to see the net income benefit due to probiotics culture supplementation (Table 4.40). Fixed cost values were taken from the study of Kumar (1997). It was observed that net income/animal for 3 months period was Rs.2918 in control group and Rs.3125 in probiotic supplemented group i.e. total benefit due to probiotic culture supplementation was Rs.207 per animal for a period of 3 months. Observation on economics study revealed that probiotics culture addition to the crossbred cows during mid lactation improved milk yield and profit margin.

Table 4.30: Meteorological Observations during Lactation Trial(Oct 97 to Jan 98)

Week	Max. Temp. °C	Min. Temp. °C	Relative humidity		Sunshine hours
			8.00 A.M.	2.30 P.M.	
1st week	26.6	14.6	90.0	55.0	6.6
2nd week	27.8	13.4	90.9	48.0	7.4
3rd week	25.0	12.2	85.6	48.7	6.9
4th week	25.0	9.7	83.3	54.6	4.4
5th week	20.6	10.8	93.7	68.1	4.4
6th week	21.6	8.3	96.4	55.4	6.4
7th week	18.7	11.2	94.9	81.9	2.0
8th week	14.4	9.1	96.1	88.1	1.0
9th week	12.2	7.0	92.6	86.4	1.3
10th week	15.9	4.1	93.3	75.5	6.8
11th week	17.8	5.9	92.7	74.1	5.1
12th week	19.5	6.4	91.9	72.8	6.1
13th week	18.1	2.6	92.7	76.5	7.9

Table 4.31:Fortnightly milk yield and milk production efficiency as influenced by probiotics culture (SC-B+LP+EF) supplementation to crossbred cows

Parameter	Group	Fortnight						Fortnight average
		1	2	3	4	5	6	
<u>Yield</u>								
Milk yield (kg/day)	I	9.29±0.59	10.45±0.89	10.61±1.03	10.08±1.20	10.19±0.88	10.33±1.03	10.16±0.19 ^a
	II	9.21±0.87	10.78±0.96	11.14±1.15	10.74±1.25	10.71±1.28	11.02±1.39	10.60±0.29 ^b
FCM yield (kg/day)	I	8.64±0.65	10.16±0.99	10.54±0.93 ^a	10.04±1.15 ^a	10.17±0.81 ^a	10.13±1.12	9.94±0.27 ^a
	II	8.37±0.73	9.78±0.75	11.24±1.04 ^b	10.95±1.15 ^b	10.90±1.22 ^b	10.61±1.09	10.31±0.44 ^b
<u>Milk production efficiency</u>								
Milk(kg/kg DMI)	I	0.91±0.06	0.93±0.07	0.89±0.05	0.89±0.08	0.85±0.06	0.81±0.06	0.88±0.02 ^a
	II	0.89±0.05	0.92±0.06	0.95±0.07	0.92±0.08	0.89±0.07	0.85±0.09	0.90±0.01 ^b
FCM (kg/ kg DMI)	I	0.84±0.05	0.90±0.07 ^b	0.89±0.04	0.89±0.08	0.85±0.05 ^a	0.80±0.07	0.86±0.02
	II	0.81±0.04	0.84±0.04 ^a	0.97±0.06	0.94±0.07	0.91±0.07 ^b	0.82±0.07	0.88±0.03

Figures with different superscripts (a,b) in group I and group II differ significantly

Table 4.32 :Effect of addition of probiotics culture (SC-B+LP+EF) on milk composition (%) of crossbred milch cows

Fortnight								
Parameter	Group	1	2	3	4	5	6	Fortnight average
Total solids	I	13.26±0.22	12.90±0.28	13.10±0.14 ^a	13.32±0.39 ^b	12.88±0.25	13.27±0.44 ^a	13.12±0.24
	II	13.08±0.24	12.83±0.28	13.36±0.26 ^b	12.98±0.34 ^a	12.97±0.31	12.72±0.44 ^b	12.99±0.23
Fat	I	3.52±0.16	3.80±0.19 ^b	3.99±0.08 ^a	4.00±0.06 ^a	4.01±0.07 ^b	3.86±0.20	3.86±0.08
	II	3.42±0.11	3.42 ±0.16 ^a	4.10±0.09 ^b	4.18±0.09 ^b	4.15±0.07 ^a	3.84±0.16	3.85±0.10
Protein	I	2.94±0.08	3.17±0.14	3.43±0.31	3.36±0.26	3.24±0.17	3.20±0.12	3.22±0.17
	II	2.96±0.11	3.12±0.15	3.15 ±0.07	3.19±0.05	3.23±0.07	3.23 ±0.08	3.14±0.07
SNF	I	9.74±0.16 ^b	9.10±0.17 ^a	9.11±0.07	9.32±0.37 ^b	8.86±0.22	9.19±0.36	9.22±0.17 ^b
	II	9.66±0.18 ^a	9.40±0.19 ^b	9.26 ±0.20	8.80±0.27 ^a	8.82±0.32	8.92±0.43	9.14±0.17 ^a
Lactose	I	6.08±0.19 ^b	5.20±0.15 ^a	4.93 ±0.25 ^a	5.21±0.31 ^b	4.89±0.17	5.26±0.25	5.28±0.13
	II	5.96±0.18 ^a	5.55±0.24 ^b	5.37 ±0.19 ^b	4.91±0.32 ^a	4.88±0.32	4.98±0.43	5.26 ±0.14
Ash	I	0.71±0.01 ^a	0.74±0.01	0.74±0.01	0.74±0.02 ^b	0.73±0.02	0.73±0.02	0.73±0.01
	II	0.73±0.01 ^b	0.73±0.02	0.74±0.01	0.70±0.01 ^a	0.72±0.03	0.72 ±0.03	0.72±0.02

Figures with different superscripts (a,b) in group I and group II differ significantly

Table 4.33 Milk constituents yield/d(Kg) due to probiotics culture (SC-B+LP+EF) supplementation to crossbred cows

Fortnight								
Parameter	Group	1	2	3	4	5	6	Fortnight average
Total solid	I	1.234±0.874	1.349±0.124	1.384±0.125 ^a	1.337±0.164	1.313±0.121	1.372±0.150	1.333±0.120
	II	1.205±0.120	1.370±0.097	1.475±0.130 ^b	1.373±0.126	1.380±0.150	1.405±0.178	1.379±0.133
Fat	I	0.328±0.029	0.399±0.044	0.420±0.035 ^a	0.401±0.045 ^a	0.406±0.031 ^a	0.400±0.048	0.392±0.035
	II	0.313±0.026	0.364±0.027	0.452±0.039 ^b	0.444±0.043 ^b	0.441±0.047 ^b	0.413±0.037	0.403±0.033
Protein	I	0.273±0.019	0.326±0.022	0.350±0.019	0.325±0.026	0.328±0.028	0.327±0.028	0.322±0.021
	II	0.291±0.029	0.334±0.027	0.350±0.035	0.340±0.036	0.344±0.040	0.428±0.094	0.331±0.032
Lactose	I	0.567±0.043	0.547±0.057	0.535±0.069	0.537±0.089	0.505±0.061	0.541±0.057	0.539±0.058
	II	0.554±0.066	0.592±0.048	0.590±0.050	0.514±0.039	0.518±0.058	0.561±0.091	0.556±0.055

Figures with different superscripts (a,b) in group I and group II differ significantly

Table 4.34:Fortnightly intake pattern of crossbred lactating cows as influenced by probiotics (SC-B+LP+EF) addition

Parameter Fortnightly intake	Control	Treatment	Sig.	t-value
<u>1st fortnight</u>				
Fodder, DM(kg)	6.71±0.44	6.62±0.28	NS	
Conc.mix., DM(Kg)	3.56±0.20	3.57±0.28	NS	
Total DMI(kg)	10.27±0.39	10.20±0.49	NS	
DMI(Kg)/100kg b.wt.	2.83±0.15	2.95±0.24	NS	
OMI (kg)	9.72±0.34	9.66±0.42	NS	
OMI (Kg)/100 kg b.wt	2.68±0.13	2.79±0.22	NS	
CPI (kg)	1.46±0.05	1.48±0.08	NS	
CPI(Kg)/100 kg.b.wt.	0.404±0.017	0.428±0.038	NS	
<u>2nd fortnight</u>				
Fodder, DM(kg)	7.68±0.49	8.04±0.30	P<0.10	1.68
Conc.mix.,DM(kg)	3.55±0.27	3.59±0.29	NS	
Total DMI(kg)	11.23±0.57	11.63±0.50	NS	
DMI(kg)/100kg b.wt.	3.06±0.15	3.33±0.26	P<0.10	1.79
OMI(kg)	10.17±0.53	10.48±0.45	NS	
OMI(kg)/100kg b.wt.	2.77±0.14	3.00±0.23	P<0.10	1.77
CPI(kg)	1.78±0.08	1.81±0.08	NS	
CPI(kg)/100kg b.wt.	0.485±0.020	0.519±0.043	NS	
<u>3rd fortnight</u>				
Fodder,DM (kg)	7.96±0.29	7.59±0.40	NS	
Conc.mix.,DM(kg)	3.87±0.31	4.02±0.41	NS	
Total DMI(kg)	11.84±0.55	11.60±0.69	NS	
DMI(kg)/100kg b.wt.	3.23±0.18	3.31±0.32	NS	
OMI(kg)	10.45±0.48	10.25±0.61	NS	
OMI(kg)/100kg b.wt.	2.85±0.16	2.93±0.28	NS	
CPI(kg)	1.96±0.09	1.94±0.13	NS	
CPI(kg)/100kg b.wt.	0.533±0.030	0.555±0.058	NS	

.....contd.

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Parameter <u>Fortnightly intake</u>	Control	Treatment	Significance	t-value
<u>4th fortnight</u>				
Fodder, DM(kg)	7.28±0.27	7.52±0.18	P<0.10	1.81
Conc.mix.,DM(kg)	3.86±0.31	4.00±0.41	NS	
Total DMI(kg)	11.14±0.44	11.52±0.40	NS	
DMI(kg)/100kg b.wt.	3.03±0.15	3.26±0.21	P<0.05	2.81
OMI(kg)	9.73±0.39	10.06±0.36	NS	
OMI(kg)/100kg b.wt.	2.65±0.13	2.85±0.18	P<0.05	2.72
CPI(kg)	1.85±0.08	1.91±0.08	P<0.05	3.25
CPI(kg)/100kg b.wt.	0.504±0.025	0.541±0.041	P<0.10	2.04
<u>5th fortnight</u>				
Fodder, DM(kg)	7.73±0.16	7.65±0.15	NS	
Conc.mix.,DM(kg)	4.13±0.27	4.19±0.40	NS	
Total DMI(kg)	11.86±0.28	11.84±0.42	NS	
DMI(kg)/100kg b.wt.	3.12±0.09	3.26±0.21	NS	
OMI(kg)	10.39±0.25	10.37±0.38	NS	
OMI(kg)/100kg b.wt.	2.73±0.08	1.96±0.09	NS	
CPI(kg)	1.96±0.05	2.85±0.19	NS	
CPI(kg)/100kg b.wt.	0.516±0.016	0.540±0.040	NS	
<u>6th fortnight</u>				
Fodder, DM(kg)	8.51±0.22	8.68±0.27	P<0.10	1.55
Conc.mix.,DM(kg)	4.10±0.27	4.14±0.40	NS	
Total DMI(kg)	12.61±0.39	12.82±0.24	NS	
DMI(kg)/100kg b.wt.	3.35±0.11	3.54±0.16	P<0.05	2.18
OMI(kg)	11.27±0.28	11.25±0.22	NS	
OMI(kg)/100kg b.wt.	2.95±0.10	3.11±0.14	P<0.05	3.11
CPI(kg)	2.15±0.07	2.18±0.06	P<0.10	1.57
CPI(kg)/100kg b.wt.	0.571±0.016	0.604±0.040	NS	

Table 4.35: Body weight change of lactating crossbred cows as-influenced by viable probiotics culture (SC-B+LP+EF) supplementation

Parameter	Control	Treatment	Significance	t-value
<u>Body wt change (kg)per day</u>				
1st fortnight	0.067±0.068	0.134±0.082		
2nd fortnight	0.093±0.177	0.067±0.086		
3rd fortnight	0.080±0.017	0.120±0.048		
4th fortnight	0.033±0.069	0.033±0.106		
5th fortnight	0.627±0.081	0.546±0.328		
6th fortnight	-0.093±0.129	-0.013±0.134		
<u>Overall body weight change (kg/day)</u>	0.131±0.047	0.223±0.023	P<0.05	3.24

Table 4.36: Chemical composition of feedstuffs during milch trial (per cent DM)

Parameter	Conc.mix.	Oat fodder	Berseem fodder
OM	90.04	86.49	84.98
CP	20.56	9.55	18.16
CF	8.83	33.37	17.66
EE	4.70	2.97	2.69
NFE	55.95	40.60	46.47
Ash	9.96	13.51	15.02
NDF	40.00	54.31	42.31
ADF	8.00	40.99	32.79
Hemicellulose	32.00	13.32	9.52
Cellulose	10.50	31.41	21.69
Lignin	2.10	7.98	7.94

Table 4.37: Effect of probiotics culture (SC-B+LP+EF) on intake and digestibility coefficients of nutrients in crossbred lactating cows

Parameter	Control	Treatment	Significance	t-value
<u>Intake, DM</u>				
Conc.mix (kg)	4.13±0.30	4.29±0.45	NS	
Oat fodder (kg)	4.11±0.20	4.36±0.33	NS	
Berseem (kg)	3.06±0.03	2.94±0.06	NS	
Total DMI(kg)	11.36±0.31	11.53±0.46	NS	
DMI(kg)/100kg b.wt.	3.07±0.12	3.25±0.24	NS	
DMI(g)/W ^{0.75} Kg	134.77±4.74	141.10±9.21	NS	
<u>Intake, CP</u>				
CPI(kg)	1.82±0.06	1.87±0.09	NS	
CPI(kg)/100kg b.wt.	493.95±22.45	527.41±43.58	NS	
CPI(g)/W ^{0.75} Kg	21.66±0.92	22.86±1.67	NS	
<u>Digestibility coefficients of nutrients (%)</u>				
DM	68.97±1.58	68.50±1.67	NS	
OM	75.68±1.22	76.03±1.49	NS	
CF	63.22±1.77	64.60±2.75	NS	
CP	75.12±0.75	75.70±1.99	NS	
EE	69.93±2.76	73.77±3.16	P<0.01	5.36
NFE	81.43±1.52	80.61±1.63	P<0.05	2.18
NDF	54.61±2.14	57.47±2.06	P<0.01	3.76
ADF	44.91±1.75	47.07±2.86	P<0.10	1.61
Cellulose	79.02±1.03	79.59±2.93	NS	
Hemicellulose	69.67±2.91	72.01±2.11	P<0.10	1.78

Table 4.38: Response of probiotics culture (SC-B+LP+EF) on digestible nutrient intake of crossbred milch cows

Parameter	Control	Treatment	Significance	t-value
Dig. OMI(kg)	7.54±0.33	7.78±0.16	NS	
DOMI(kg)/100kg b.wt.	2.04±0.10	2.19±0.13	P<0.05	3.57
DOMI(g)/W ^{0.75} Kg	89.41±4.35	95.02±4.56	P<0.01	5.73
TDN intake (kg)	7.90±0.35	8.15±0.19	NS	
TDNI(kg)/100kg bwt	2.14±0.11	2.29±0.14	P<0.01	3.79
TDNI(g)/W ^{0.75} Kg	93.68±4.59	99.55±4.91	P<0.01	6.56
TDN(%)	69.42±1.66	70.87±1.31	P<0.05	2.56
DCP Intake(kg)	1.37±0.05	1.41±0.05	NS	
DCPI(kg)/100kg b.wt	371.25±18.47	397.00±28.53	P<0.10	1.77
DCP(g)/W ^{0.75} Kg	16.28±0.76	17.23±1.08	P<0.10	1.70
DCP(%)	12.06±0.16	12.02±0.53	NS	
ME(MJ) intake	125.77±6.01	130.02±2.71	NS	
NEL(MJ) intake	75.31±3.47	77.73±1.68	NS	
<u>Nutrient requirement*</u>				
TDN (kg)	7.56±0.49	8.45±0.42	P<0.10	2.11
CP(g)	1403.93±126.01	1566.91±114.61	P<0.01	4.09
ME(MJ)	121.31±7.87	135.49±6.78	P<0.01	5.72
NEL(MJ)	72.15±4.47	75.92±6.34	NS	
<u>Energy value of feed</u>				
DE(MJ/Kg DM)	12.74±0.27	13.08±0.24	P<0.01	3.97
ME(MJ/Kg DM)	11.05±0.31	11.32±0.24	P<0.05	2.56
NEL(MJ/Kg DM)	6.61±0.17	6.76±0.13	P<0.05	2.33

*NRC (1989)

Table 4.39: Cost of milk production as influenced by probiotics (SC-B+LP+EF) culture supplementation to crossbred cows

Fortnight	Milk yield (kg)	Total cost of feed (Rs)	Cost of probiotics/ day	Feed cost/kg milk (Rs)
<u>1st Fortnight</u>				
I	9.29	35.41		3.81
II	9.21	35.26	1.44	3.98
<u>2nd Fortnight</u>				
I	10.45	54.79		5.24
II	10.78	56.66	1.44	5.38
<u>3rd Fortnight</u>				
I	10.61	57.28		5.40
II	11.14	56.58	1.44	5.20
<u>4th Fortnight</u>				
I	10.08	68.25		6.77
II	10.74	70.50	1.44	6.71
<u>5th Fortnight</u>				
I	10.19	69.44		6.81
II	10.71	68.95	1.44	6.57
<u>6th Fortnight</u>				
I	10.33	71.63		6.93
II	11.02	72.82	1.44	6.74
<u>Fortnight Average</u>				
I	10.16	59.47		5.85
II	10.60	60.13	1.44	5.81

Table 4.40 : Net income(Rs.) from lactating cow as influenced by probiotics(SC-B+LP+EF) supplementation

Items of cost and income	Rs./animal for 3 months	
	Group-I	Group-II
A. <u>Variable cost</u>		
Fodder	3405.00	3427.35
Concentrate	1947.00	1984.20
Probiotics	-	129.60
Veterinary and Misc.cost	18.87	18.87
Labour	675.00	675.00
Total variable cost	6045.87	6235.02
B. <u>Fixed cost*</u>		
Depreciation and interest on fixed capital	186.66	186.66
Insurance charges	66.45	66.45
Total fixed cost	253.11	253.11
C. <u>Gross cost</u>	6298.98	6488.13
D. <u>Income</u>		
Value of milk	9144.00	9540.00
Value of dung	73.00	73.00
Gross income	9217.00	9613.00
E. <u>Net income</u>	2918.02	3124.87

* Ashok Kumar(1997)

PHASE-V

4.5 PRESERVATION OF PROBIOTICS CULTURE IN POWDER FORM AND EVALUATION OF SURVIVABILITY OF THE CULTURES IN POWDER

Most promising cultures, namely, *Saccharomyces cerevisiae*-Strain B (SC-B), *Lactobacillus plantarum* (LP) and *Enterococcus faecium* (EF) were selected for preservation in form of powder. Skim milk powder and sucrose were taken as basal materials. Adonitol and mono-sodium glutamate were selected as cryoprotectants during the preparation of powder. Different combination of treatments were made in order to study the effectiveness of basal material alongwith protective agents which can keep the above mentioned cultures viable in powder form during preservation (Table 4.41).

4.5.1 Preparation of Culture Powder:-

As mentioned in the Table 4.42, different combination of the culture powders were prepared. The doses of cultures were 17.8×10^9 cfu/vial for *S.cerevisiae*-B; 17.3×10^9 cfu/vial for *L.plantarum* and 17.0×10^9 cfu/vial for *E.faecium*. The skim milk based culture suspension was freeze dried (12h) under vacuum. The dried culture powders were then kept in the sterilized glass vial and sealed under vacuum. The sealed vials were preserved in the refrigerator (4°C to 6°C) and tested for viability of cultures at fortnightly intervals.

4.5.2 Viability of Probiotic Culture Powders:-

Each culture powder was dissolved in 10 ml of autoclaved saline water. The culture suspension was diluted serially upto 10^8 times. For plating of *S.cerevisiae*-B, *L.plantarum* and *E.faecium*, Malt extract, MRS and LYPA agar media were taken, respectively. Viability of the cultures in powder at fortnight intervals are presented in the Table 4.41. It was observed that in general, addition of adonitol and mono-sodium glutamate in the skim milk and skim milk plus sucrose powder affected the viability per cent of SC-B, LP and EF cultures differently. Fortnight average of SC-B counts indicated that Y_2 , Y_3 ,

Y₅ and Y₆ preservatives gave most promising results as the counts of live cells were higher. Y₄, where only sucrose was added resulted least viability after preservation. After 3rd fortnights only 7.3% (Table 4.43) cells were in viable form due to this treatment. Adonitol and sucrose together (Y₅) resulted in highest survivability per cent (48.31%) after 3rd fortnight which was comparable to mono-sodium glutamate (Y₃) containing treatment (47.75%). Earlier report by Berny and Hennebert (1991) also indicated improvement in viability rate of *S.cerevisiae* cells by using appropriate protective medium such as sodium glutamate.

The preservation of LP appeared to be most effective ($P < 0.01$) in presence of mono sodium glutamate alone (L₃) and alongwith sucrose (L₆). This also could be substantiated by the survivability of LP cells to the extent to 67.05 (L₃) and 65.32 (L₆) after 3rd fortnight. Adonitol alone or with sucrose was not found beneficial to improve the viability per cent even after 1st fortnight. The same was true for sucrose alone which even appeared to have slightly negative effect on the cell viability per centage (Table 4.43). Present findings corroborate with the observation that sodium glutamate was found effective cryoprotectant for *L.plantarum* (Pereda-Alardin *et al.*, 1990; Dave *et al.*, 1992). Whereas, in present studies adonitol was not found to have any protective effect on LP cells contrary to the findings of Valdez *et al.* (1983).

Preservation of *E.faecium* (EF) in powder form consisting skim milk alone appeared to be quite effective, though addition of adonitol and mono sodium glutamate appeared to give slight protection as indicated by survivability per cent (Table 4.43). But the same was not statistically improved (Table 4.41). Sucrose alone or alongwith adonitol appeared to have negative effect. Whereas, sucrose with mono-sodium glutamate gave best protection upto one month (61.76%), but it could not be confirmed after 3rd fortnight. The study done by Valdez *et al.* (1993) demonstrated that adonitol has strong protective effect during freeze drying of *S.faecium*. In present study also

protective effect has been recorded which was indicated by improved survivability per centage by more than 10 units. However, with sucrose the survivability of cells decreased drastically.

The findings of the present experiment indicated that highest survivability of SC-B was recorded with the addition of sucrose + adonitol (Y_3) which was comparable with mono-sodium glutamate addition (Y_3). For LP culture mono-sodium glutamate was found most effective protectant alone or along with sucrose. Whereas, viability of EF was non-significantly improved due to adonitol addition. While sucrose + mono sodium glutamate, protection was maximum upto one month. Based on the findings of this study, probiotics could be successfully stored/preserved in powder form which will be an appropriate technology for field application of the cultures.

Table 4.41: Viability of preserved probiotics culture powders (Log₁₀ value of cfu)

Treatment	Initial counts	1st fortnight	2nd fortnight	3rd fortnight	Fortnight average
<u>Yeast (SC-B)</u>					
Y ₁ (SMP)	10.26±0.00	9.70±0.08	9.48±0.07	9.41±0.01 ^b	9.71±0.19 ^b
Y ₂ (SMP+AD)	10.26±0.00	9.89±0.01	9.84±0.00	9.81±0.07 ^c	9.95±0.10 ^{bc}
Y ₃ (SMP+GL)	10.26±0.00	9.98±0.07	9.97±0.02	9.93±0.03 ^c	10.03±0.08 ^c
Y ₄ (SMP+S)	10.26±0.00	9.21±0.10	9.15±0.15	9.09±0.09 ^a	9.43±0.28 ^a
Y ₅ (SMP+S+AD)	10.26±0.00	9.90±0.12	9.80±0.15	9.93±0.10 ^c	9.97±0.10 ^c
Y ₆ (SMP+S+GL)	10.26±0.00	10.03±0.09	9.93±0.03	9.88±0.10 ^c	10.02±0.08 ^c
<u><i>L. plantarum</i>(LP)</u>					
L ₁ (SMP)	10.24±0.00	9.68±0.08	9.62±0.02	9.56±0.03 ^c	9.77±0.16 ^a
L ₂ (SMP+AD)	10.24±0.00	9.53±0.23	9.70±0.01	9.65±0.00 ^c	9.78±0.31 ^a
L ₃ (SMP+GL)	10.24±0.00	10.12±0.04	10.11±0.03	10.07±0.00 ^d	10.13±0.04 ^b
L ₄ (SMP+S)	10.24±0.00	9.50±0.10	9.38±0.02	9.20±0.20 ^a	9.58±0.23 ^a
L ₅ (SMP+S+AD)	10.24±0.00	9.49±0.21	9.32±0.09	9.30±0.10 ^a	9.59±0.22 ^a
L ₆ (SMP+S+GL)	10.24±0.00	10.17±0.09	10.17±0.02	10.05±0.05 ^d	10.16±0.04 ^b
<u><i>E. faecium</i>(EF)</u>					
E ₁ (SMP)	10.24±0.00	9.85±0.05	9.76±0.06	9.77±0.03 ^d	9.90±0.11 ^b
E ₂ (SMP+AD)	10.24±0.00	9.97±0.03	9.39±0.52	9.88±0.04 ^d	9.87±0.18 ^b
E ₃ (SMP+GL)	10.24±0.00	9.88±0.10	9.88±0.02	9.85±0.00 ^d	9.96±0.09 ^b
E ₄ (SMP+S)	10.24±0.00	9.08±0.08	9.15±0.03	9.18±0.23 ^a	9.41±0.28 ^a
E ₅ (SMP+S+AD)	10.24±0.00	9.12±0.12	9.23±0.07	9.22±0.18 ^a	9.45±0.26 ^a
E ₆ (SMP+S+GL)	10.24±0.00	10.01±0.11	9.99±0.17	9.50±0.05 ^c	9.93±0.16 ^b
CD				0.24	0.24

Means with different superscripts (a,b,c,d,e,f,g,h) differ significantly
 SMP = Skim milk power, S = Sucrose, AD = Adonitol, GL = Mono-sodium glutamate

Table 4.42 : Fortnightly changes of viable counts of preserved probiotic powders

Treatment	Initial $\times 10^9$	1st fortnight $\times 10^9$	2nd fortnight $\times 10^9$	3rd fortnight $\times 10^9$
<u><i>Yeast (SC-B)</i></u>				
Y ₁ (SMP)	17.8	5.1	3.1	2.6
Y ₂ (SMP+AD)	17.8	7.7	7.0	6.5
Y ₃ (SMP+GL)	17.8	9.6	9.5	8.5
Y ₄ (SMP+S)	17.8	1.7	1.5	1.3
Y ₅ (SMP+S+AD)	17.8	8.2	6.8	8.6
Y ₆ (SMP+S+GL)	17.8	10.9	8.6	7.9
<u><i>L. plantarum (LP)</i></u>				
L ₁ (SMP)	17.3	4.8	4.2	3.7
L ₂ (SMP+AD)	17.3	3.9	5.0	4.5
L ₃ (SMP+GL)	17.3	13.0	12.7	11.6
L ₄ (SMP+S)	17.3	3.3	2.4	1.8
L ₅ (SMP+S+AD)	17.3	3.5	4.2	2.1
L ₆ (SMP+S+GL)	17.3	14.8	14.6	11.3
<u><i>E. faecium (EF)</i></u>				
E ₁ (SMP)	17.0	7.2	5.7	5.8
E ₂ (SMP+AD)	17.0	9.4	7.7	7.5
E ₃ (SMP+GL)	17.0	7.8	7.8	7.0
E ₄ (SMP+S)	17.0	1.2	1.4	1.7
E ₅ (SMP+S+AD)	17.0	1.4	1.7	1.8
E ₆ (SMP+S+GL)	17.0	10.6	10.5	3.2

SMP = Skim milk power, S = Sucrose, AD = Adonitol, GL=Mono-sodium glutamate

Table 4.43: Survival per cent of preserved probiotics power consisting sucrose, adonitol and mono-sodium glutamate

Treatment	Initial	1st fortnight	2nd fortnight	3rd fortnight
<u>Yeast (SC-B)</u>				
Y ₁ (SMP)	100.00	28.65	17.42	14.61
Y ₂ (SMP+AD)	100.00	43.26	39.33	36.52
Y ₃ (SMP+GL)	100.00	53.93	53.37	47.75
Y ₄ (SMP+S)	100.00	09.55	08.43	07.30
Y ₅ (SMP+S+AD)	100.00	46.07	38.20	48.31
Y ₆ (SMP+S+GL)	100.00	61.24	48.31	44.38
<u>L. plantarum(LP)</u>				
L ₁ (SMP)	100.00	27.75	24.28	21.39
L ₂ (SMP+AD)	100.00	22.54	28.90	26.01
L ₃ (SMP+GL)	100.00	75.14	73.41	67.05
L ₄ (SMP+S)	100.00	19.08	13.87	10.40
L ₅ (SMP+S+AD)	100.00	20.23	24.28	12.14
L ₆ (SMP+S+GL)	100.00	85.55	84.39	65.32
<u>E. faecium(EF)</u>				
E ₁ (SMP)	100.00	42.35	33.53	34.12
E ₂ (SMP+AD)	100.00	55.29	45.29	44.12
E ₃ (SMP+GL)	100.00	45.88	45.88	41.18
E ₄ (SMP+S)	100.00	07.06	08.24	10.00
E ₅ (SMP+S+AD)	100.00	08.24	10.00	10.59
E ₆ (SMP+S+GL)	100.00	62.35	61.76	18.82

CHAPTER - V

SUMMARY AND CONCLUSIONS

5. SUMMARY

PHASE-I

5.1 TOTAL GASTROINTESTINAL TRACT SURVIVABILITY OF YEAST, LACTOBACILLI AND STREPTOCOCCI

Six promising probiotic cultures were selected to study their survivability status in total GI tract of crossbred calves. The cultures were as following:-

1. *Saccharomyces cerevisiae*-B
2. *S.cerevisiae*-522
3. *Lactobacillus acidophilus*
4. *L.plantarum*
5. *Lactococcus lactis*
6. *Enterococcus faecium* (*Streptococcus faecium*)

Doses of 1.08×10^9 cfu/kg of concentrate mixture of the above cultures were supplemented to groups of three calves for five days. Faecal counts in the respective groups were recorded during control (pretreatment), treatment and post treatment periods to observe the survivability status of the above cultures.

Following inferences can be drawn based on the study:-

- i) In crossbred calves least square mean value (LSM) of faecal yeast counts (Log_{10} cfu/g of faeces) increased non-significantly during *S.cerevisiae*-B supplementation period (2.919) than control (2.791) and post treatment period (2.803). The increase in faecal yeast counts during treatment period was 50 per cent over control.
- ii) Supplementation of *S.cerevisiae*-522 in the ration of calves resulted a increase in faecal yeast counts during treatment period (2.944) over control

(2.752) and post-treatment period (2.892). The differences were non-significant although there were 49 per cent higher faecal yeast counts during supplementation period over control period.

iii) It was observed that faecal acid producing bacilli counts were increased significantly ($P<0.01$) during treatment period with *L.acidophilus* (6.365) than control (6.092) and post treatment period (6.214). Acid producing bacilli were higher by 73 per cent during supplementation period thereby indicating that a portion of *L.acidophilus* survived in GI tract of calves.

iv) In *L.plantarum* treatment group of crossbred calves, no significant difference was observed among control, treatment and post-treatment period regarding acid producing bacilli counts in the faeces. Hence, it may be concluded that *L.plantarum* could not pass to the lower tract.

v) In *L.lactis* treated calves, faecal acid producing cocci were marginally, but non-significantly higher during treatment period (6.055) over control (5.953) and post treatment period (5.807). Faecal acid producing cocci counts were 2.6 times higher during the period when *L.lactis* was fed daily.

vi) In crossbred calves, *E.faecium* supplementation also non-significantly improved faecal count of acid producing cocci during treatment period (6.257) over control (6.070) but the number fell significantly ($P<0.01$) during post treatment period (5.666). During treatment period, faecal counts of acid producing cocci remained 1.75 times higher over control. Therefore, a fraction of *E.faecium* remained viable during GI tract passage.

(vii) During comparison of treatment periods of *L.acidophilus* and *L.plantarum*, it was observed that faecal acid producing bacilli counts were higher ($P<0.01$) in *L.acidophilus* supplemented animals than *L.plantarum* treated animals. This indicated that *L.acidophilus* has better survival in the digestive tract than *L.plantarum*. Whereas, no difference in survivability was observed between *S.cerevisiae*-B and *S.cerevisiae*-522 and also between *L.lactis* and *E.faecium* when compared during treatment period.

PHASE-II

5.2 *IN VITRO* RUMEN FERMENTATION AS INFLUENCED BY INDIVIDUAL, DOUBLE AND TRIPLE COMBINATIONS OF PROBIOTIC CULTURES:

Six probiotic cultures, namely, *S.cerevisiae*-B, *S.cerevisiae*-522, *L.acidophilus*, *L.plantarum*, *Lactococcus lactis* and *E.faecium* were taken in single, double and triple combinations. Yeast culture was kept common in all double and triple combinations. A total dose of 10^9 cfu of probiotic culture was supplemented in each *in vitro* bottle using oven dried oat fodder and concentrate mixture (70:30, DM basis) as substrate. In double combination of viable culture treatment 60:40 ratio was maintained in respect of yeast and lactobacilli or streptococci and the ratio in triple combinations was 60:20:20 in yeast, lactobacilli and streptococci. The incubation period was 48h at $39^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and following observations were recorded.

- i) In high fodder based substrate (Roughage to concentrate, 70:30) the *in vitro* DM degradability at 48h of incubation was significantly ($P < 0.01$) higher in all the probiotics supplemented groups (T_1 - T_{22}). Highest DM degradability was observed in T_3 (74.56), T_5 (74.85), T_7 (77.26), T_8 (75.87), T_9 (77.65), T_{10} (74.95), T_{11} (75.65), T_{12} (76.27), T_{13} (76.27), T_{14} (76.39), T_{15} (75.30), T_{17} (77.60), T_{18} (75.33), T_{19} (74.77), T_{20} (75.28), T_{21} (75.07) and T_{22} (77.03) treatment groups.
- ii) Total gas production (ml)(48h) was significantly ($P < 0.05$) increased in all the treatments (T_1 to T_{22}), probably due to increased fermentation. Higher range of total gas (ml) was estimated in the treatment groups of T_1 (117.67), T_2 (116.90), T_{15} (116.87) and T_{17} (125.70).
- iii) Due to culture supplementation, TVFA concentration (mmol/100 ml) was improved significantly ($P < 0.05$) in the treatment groups of T_{17} (9.68), T_{18} (8.70), T_{20} (9.70), T_{21} (9.23) and T_{22} (9.13). Among double combination cultures T_{12} (8.40) and T_{13} (8.05) showed non-significantly higher values of

TVFA.

iv) Addition of live microbial culture depressed ($P<0.05$) acetate proportion, and increased propionate level in only one single (T_4), one double (T_{11}) and all triple combinations (T_{15} to T_{22}). However, acetate to propionate ratio was depressed significantly ($P<0.05$) in T_{11} (1.67), T_{15} (1.41), T_{16} (1.35), T_{17} (1.28), T_{18} (1.24), T_{19} (1.19), T_{20} (1.26), T_{21} (1.21), T_{22} (1.44) groups. This can be ascribed to modification in type of microbial species and thereby change in fermentation pattern.

v) Butyrate proportion was similar among various treatments and control, except T_4 (15.93) and T_6 (14.48) wherein butyrate was significantly ($P<0.05$) increased.

Based on these data, following culture combinations appeared more promising, therefore, selected for further studies in Phase (III):

- 1) *S. cerevisiae*-B (SC-B)
- 2) *E. faecium* (EF)
- 3) *S. cerevisiae*-522 (SC-522) and *L. plantarum* (LP) combination.
- 4) *S. cerevisiae*-522 (SC-522) and *E. faecium* (EF) combination.
- 5) *S. cerevisiae*-522 (SC-522), *L. acidophilus* (LA) and *L. lactis* (LL) combination.
- 6) *S. cerevisiae*-522 (SC-522), *L. plantarum* (LP) and *E. faecium* (EF) combination.
- 7) *S. cerevisiae*-B (SC-B), *L. plantarum* (LP) and *E. faecium* (EF) combination.

PHASE-III

5.3 *IN VITRO* RUMEN FERMENTATION OF SELECTED PROMISING PROBIOTIC CULTURES

Based on the results of 2nd phase *in vitro* trials, promising cultures, two

each from single and double combinations; and three from triple combination treatments were selected for detailed studies. The selected treatments were:-

P ₁	=	SC-B
P ₂	=	EF
P ₃	=	SC-522+LP
P ₄	=	SC-522+EF
P ₅	=	SC-522+LA+LL
P ₆	=	SC-522+LP+EF
P ₇	=	SC-B+LP+EF

Total dose of probiotic culture and ratio was same as mentioned in the 2nd phase *in vitro* trial. A mixed feed consisting of roughage and concentrate was used as substrate in three ratios, i.e., 40:60, 60:40, and 70:30. Roughage portion contained oven dried maize fodder and wheat straw in the proportion of 60:40.

The observations are summarised as following:-

- i) Inclusion of probiotics in high (60%), medium (40%) and low (30%) concentrate substrate resulted enhanced DM degradability. In high concentrate substrate P₁ (67.65), P₄ (68.37), P₆ (68.07) and P₇ (67.25) showed maximum DM degradability. In medium concentrate, although there was significant (P<0.05) increase in DM degradability, but, there was no difference among treatments. In low concentrate and high roughage groups, maximum IVDMD(%) was recorded in P₁ (52.16), P₂ (51.41), P₃ (52.09), P₄ (51.05) and P₅ (52.43) groups. Results depicted that probiotic cultures affected the *in vitro* system differently when different substrates were used.
- ii) In high concentrate substrate groups cumulative gas production (upto 24h) was highest (P<0.01) in P₁, P₂, P₃, P₄ and P₇. In medium concentrate P₇ showed significantly lower total gas production (upto 24h) as compared to P₁, P₂, P₃, P₄ and P₅. Almost similar trend was observed in low concentrate substrate where P₇ depicted significantly (P<0.01) lower total gas production

(upto 24h) over P_1 , P_2 and P_3 . But all the treatment groups under three substrate system showed significant increase in total gas production over control, probably due to higher DM degradability.

3) Methane production per cent remained unaffected upto 24h due to probiotic culture supplementation in high and in low concentrate substrate. Whereas, in medium concentrate methane per cent was enhanced significantly in all the treatment groups except P_3 over control upto 24h of incubation. In general, quantitative methane (ml) production was increased significantly due to probiotics supplementation in high and medium concentrate substrate groups, but in high roughage substrate methane (ml) was similar among control and treatment groups at 18h of incubation. Higher DM degradability could have reflected increase in methane production as well as total gas production.

iv) Due to addition of *S.cerevisiae*-B pH was increased significantly over control in high concentrate diet, in other substrates also increasing tendency was noticed. In medium concentrate significantly higher pH value was recorded in triple combination treatment groups (P_5 , P_6 and P_7). In low concentrate diet, pH value was found similar among control and treatment groups.

v) Inclusion of *S.cerevisiae*-B in *in vitro* resulted highest TVFA concentration (mmol/100 ml SRL) in high and low concentrate diet. In medium concentrate groups P_2 , P_3 , P_4 and P_6 depicted highest TVFA concentration. In general, addition of these cultures increased TVFA concentration under high, medium and low concentrate system.

vi) It was observed that addition of microbial cultures to *in vitro* system depressed acetate and increased propionate at three concentrate levels (60, 40 and 30 per cent). Maximum reduction of acetate was estimated in P_1 and P_7 (high concentrate) and P_7 in low concentrate substrate. Whereas in P_3 group maximum reduction of acetate was observed in medium concentrate substrate. Highest propionate level was estimated in same treatments.

Butyrate proportion was increased significantly in all the treatment groups under high and medium concentrate diet, whereas, P₆ and P₇ showed highest value of butyrate in low concentrate diet.

vii) Reduction of acetate to propionate ratio (A:P) was observed in all the treatment groups under high, medium and low concentrate (except P₂) substrate. Maximum reduction of A:P ratio was observed in P₁ (high concentrate), P₇ (high and low concentrate) and P₃ in medium concentrate. Combined culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium*(P₇) expressed maximum response on propionate production and reduction of acetate to propionate ratio which could probably be helpful for improving milk production in lactating cows.

viii) Due to supplementation of selected live cultures, there was significant reduction of NH₃-N concentration in high, medium and low concentrate substrate. Maximum reduction of ammonia-N was observed in mixed culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* (P₇) group in high and low concentrate rations. In medium concentrate diet maximum reduction of NH₃-N was estimated in P₁ and P₇ groups. The reduction of NH₃-N due to addition of probiotics was mainly due to incorporation of NH₃-N into microbial protein, hence, total bacterial counts was increased.

ix) Addition of live microbial culture significantly increased total bacterial population in the *in vitro* bottles having high or medium concentrate. Maximum bacterial population(Log₁₀ no/ml SRL) was observed in the mixed culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* treatment group in high (8.81), medium (8.76) and low (8.67) concentrate substrate. The difference among control and treatment groups in high roughage diet (70%) was non-significant.

x) Total protozoal population remained unchanged due to inclusion of probiotics in the *in vitro* system, but high concentrate had positive impact on total protozoa as well as holotrichs proportion. Viable yeast culture addition

increased holotrichs per cent in all three ration systems, whereas, combined cultures had variable response on holotrichs and spirotrichs population.

xi) In high concentrate diet, mixed culture significantly increased TCA-ppt-N (29.86) and ^{35}S incorporation rate (56.69%) over control (24.45 mg/100 ml SRL and 46.52%). Though there was 6.61 per cent increase in ^{35}S incorporation into microbial protein due to mixed culture supplementation in medium concentrate, but the difference was found non-significant.

xii) Overall values indicated that probiotics culture addition in high concentrate (60%) substrate showed better performance on *in vitro* fermentation than medium (40%) and low (30%) concentrate substrate.

Based on above observations, culture combination, namely, *S.cerevisiae*-B+*L.plantarum*+*E.faecium* (6:2:2), was selected to see its impact on lactation performance in crossbred milch cows.

PHASE-IV

5.4 EFFECT OF PROBIOTICS ON LACTATION PERFORMANCE AND NUTRIENT UTILIZATION IN CROSSBRED COWS

Effect of feeding probiotics culture in triple combination (*S.cerevisiae*-B, *L.plantarum* and *E.faecium*; ratio 6:2:2, total dose 10×10^9 cfu/animal) to crossbred lactating cows was studied on lactation performance and nutrient digestion. Following observations are summarised:-

i) There was increasing tendency of DMI per 100 kg b.wt due to inclusion of probiotics culture, the difference between control and treatment group was found significant in 2nd ($P < 0.10$), 4th and 6th ($P < 0.05$) fortnight. OMI per 100 kg b.wt followed same trend, whereas CPI per 100 kg b.wt was not affected due to probiotics supplementation.

ii) Due to addition of probiotics in the diet of crossbred cows milk yield (kg/day/animal) increased significantly ($P < 0.01$), average yield of 10.16 kg in control was increased to 10.60 kg in treatment group, thus the milk yield was

improved by about 40 kg/animal during 90 days experimental period. FCM (kg/day) yield also increased significantly ($P<0.10$) due to probiotics supplementation from 9.94 to 10.34 kg/day.

iii) The overall milk production efficiency (kg/kg DMI) was 0.88 in control and 0.90 in experimental group which was statistically higher ($P<0.10$) than control.

iv) Due to addition of probiotics, butter fat per cent was increased during 3rd to 5th fortnight, but average value of six fortnights was similar in both the groups. By and large, total milk solid, protein, lactose and milk ash per cent remained unaffected due to probiotics supplementation.

v) During digestibility trial addition of mixed viable culture to lactating cows had no impact on total DMI, DMI/100 kg b.wt, total CPI and CPI/100 kg b.wt.

vi) Whereas, DOMI/100 kg b.wt and TDN intake/100 kg b.wt were significantly higher in treatment group (2.19 kg and 2.29 kg) over control (2.04 kg and 2.14 kg). DCP intake/100 kg b.wt. followed the same trend. TDN per cent of the mixed ration was statistically higher ($P<0.05$) in treatment group than control, but DCP per cent was similar in both the groups.

vii) 4.25 MJ extra ME was taken by probiotics supplemented animals at similar level of total DMI in both the groups. The animals fed culture supplement also consumed 2.42 MJ more NE for lactation(NEL) which could be the reason for higher milk yield due to probiotics addition.

viii) NDF, ADF, hemicellulose and EE digestibilities were significantly increased due to viable culture feeding to lactating cows. But DM, OM, CP digestibilities remained unaffected.

ix) Economic analysis revealed that mixed probiotic culture supplementation to crossbred cows increased milk yield by forty kg and profit margin by about Rs.200 per animal during experimental period of 3 months.

PHASE-V

5.5 PRESERVATION OF PROBIOTICS CULTURE IN POWDER FORM AND EVALUATION OF SURVIVABILITY OF THE CULTURE IN POWDER

The most promising probiotic cultures, *S.cerevisiae*-B, *L.plantarum* and *E.faecium*, which were used during lactation trial were successfully converted to powder form, after using different basal materials and cryoprotective agents (Adonitol or mono-sodium glutamate), and preserved under refrigeration (4°C to 6°C). The observations were summarised as following:-

1. Highest survivability of SC-B cells preserved in powder form was obtained with sucrose + adonitol which was comparable to sucrose + mono-sodium glutamate and mono-sodium glutamate alone. Adonitol alone though found effective ($P < 0.01$) but was not comparable to above treatments.
2. For *L.plantarum* mono sodium glutamate was found most effective cryoprotectant alone or alongwith sucrose.
3. Whereas, survivability of EF was non-significantly improved due to adonitol and mono-sodium glutamate addition. With sucrose+mono-sodium glutamate, maximum protection was observed upto one month.

CONCLUSIONS

1. *Saccharomyces cerevisiae*-B and 522 feeding increased live yeast count in faeces by about 50 per cent.
2. During supplementation with *L.acidophilus*, 73 per cent higher acid producing bacilli were recorded in the faeces of calves.
3. During treatment period of *L.lactis* and *E.faecium*, there were 2.6 and 1.75 times higher acid producing cocci in the faeces of crossbred calves.
4. Probiotic culture supplementation, either in single, double or triple combination enhanced *in vitro* DM degradability and total gas production in most of the treatments.
5. Probiotic cultures were effective on all the three levels of concentrate (60, 40, 30 per cent) to enhance IVDMD and gas production.
6. Combined culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium* showed superior performance in respect of higher TVFA concentration with enhanced propionate level and reduction of acetate to propionate ratio.
7. Inclusion of promising probiotic culture in the *in vitro* system increased total bacteria (high and medium concentrate) which in turn reduced NH₃-N concentration. TCA-precipitable-N and ³⁵S incorporation into microbial protein was also increased. Maximum response was observed in mixed culture of *S.cerevisiae*-B, *L.plantarum* and *E.faecium*. Whereas, total protozoal counts remained unaffected.
8. Supplementation of mixed culture (*S.cerevisiae*-B, *L.plantarum* and *E.faecium*, Total dose, 10 x 10⁹, ratio 6:2:2) to crossbred lactating cows increased milk yield and FCM yield at similar level of total DM intake.
9. Although there was increasing tendency of butter fat per cent during 3rd to 5th fortnight of mixed culture feeding, but overall total solid, fat, protein, SNF and ash per cent in milk remained unaffected.

10. Addition of this combined culture to milch cows increased NDF, ADF, hemicellulose and EE digestibilities. Whereas, there was no improvement of DM, OM, CP and cellulose digestibilities in crossbred cows.
11. DCP and TDN intake per 100 kg body weight was enhanced in treatment group. ME and NEL intake was also marginally higher in probiotics added cows.
12. Inclusion of mixed culture to the ration of crossbred lactating cows increased milk yield by 40 kg and resulted in higher return of about Rs.200/animal during three months experimental feeding period.
13. Cultures were preserved in powder form using SMP as basal material, and viability of cells was not improved by inclusion of sucrose.
14. Both adonitol and mono sodium glutamate were effective to enhance viability of yeast (SC-B) and *E.faecium*.
15. Mono sodium glutamate appeared effective for protecting *L.plantarum* culture during freeze drying.

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