

**EFFECT OF LIVE MICROBES AS DIETARY ADJUNCT ON
RUMINAL FERMENTATION, NUTRIENT UTILIZATION
AND GROWTH OF CALVES**

**THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF**

**DOCTOR OF PHILOSOPHY
IN
ANIMAL NUTRITION**

**BY
RAMAN MALIK**

**DIVISION OF DAIRY CATTLE NUTRITION
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL-132001 (HARYANA), INDIA**

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TO
RAJIVE MALIK

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BY
RAMAN MALIK

A thesis submitted to the National Dairy Research
Institute (Deemed University), Karnal in partial
fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY
IN
ANIMAL NUTRITION

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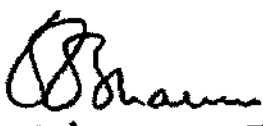
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DATE: _____

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CHAPTER - I

INTRODUCTION

1. INTRODUCTION

Productivity potential of livestock cannot be fully exploited without sufficient quantity of good quality feed. The feed resources available in the tropical parts of the world are comparatively lower in nutritive value than temperate regions. On diets having low digestible feeds, first limitation is lower feed intake associated with lower rate at which fibrous material digested and removed from the rumen (Weston, 1983). The situation is particularly acute in South Asia where chronic annual feed deficits and

annual increase of the animal population are common, thus making the problem a continuing saga (Devendra, 1988).

Achievement of maximal feed utilization by the provision of optimal rumen conditions is a highly cost effective method. Traditionally, it has involved manipulating such parameters as ammonia concentration, pH or the level and type of supplementary fat to stimulate and increase the population of desirable organisms. Only recently, the addition of live organisms to the rumen has been shown to be a highly promising approach. The microbial supplements have shown to improve feed conversion efficiency and health status of ruminants, and known as probiotics. The term "probiotic" was coined by Parker in 1974 and was derived from the Greek words meaning "for life" which contrast with more familiar word antibiotic meaning "against life". The term probiotic is used to describe a product composed of live microbial cells which are fed directly to the animals.

Until recently it was not generally accepted that microorganisms previously considered to be totally aerobic could grow and multiply in the anaerobic environment present in the rumen and gut. This situation has now dramatically changed following the discovery and identification of microorganisms able to colonise in the anaerobic environment of the rumen and intestine. Strains of S.cerevisiae, L.acidophilus and S.thermophilus are facultative anaerobes and used as dietary adjunct in the diets of ruminants to improve nutrient utilization (Sharma and Malik, 1992). The use of

culture supplements has been associated with improved feed intake (Ruf et al., 1953; Phillips and Von Tungeln, 1985; Malcolm and Kiesling, 1986; Fallon and Harte, 1987; Gunther, 1989; Williams and Newbold, 1990b), improved milk production in dairy cattle and goats (Hoyos et al., 1987; Teh et al., 1987; Williams and Newbold, 1990a), alterations in milk composition (Hoyos et al., 1987; Huber et al., 1989; Gunther, 1989; Williams and Newbold, 1990b), improved weight gains and feed efficiencies in meat producing animals (Ruf et al., 1953; Greive, 1979; Phillips and Von Tungeln, 1985; Fallon and Harte, 1987; Hughes, 1988). Many of the culture supplements affect directly on microbial activities in the rumen. The effects are visible from decreased ruminal ammonia concentrations (Dawson and Newman, 1987; Harrison et al., 1988), altered VFA production (Teh et al., 1987; Grey and Ryan, 1988; Harrison et al., 1988; Martin et al., 1989), moderated ruminal pH (Malcolm and Kiesling, 1986; Teh et al., 1987; Williams, 1989a), decreased lactic acid concentration (Williams, 1989a), decreased soluble sugar concentration (Williams, 1989c, 1990a), reduced methane production (Williams, 1989c, 1990a), altered digestive patterns (Ruf et al., 1953; Gomez-Alarcon et al., 1987; Wiedmeier et al., 1987; Williams, 1989a; Chandemana and Offer, 1990), stabilised rumen fermentation (Harrison et al., 1988), increased concentration of anaerobic bacteria (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1990), and increased concentration of cellulolytic bacteria (Wiedmeier et al., 1987; Harrison et al., 1988;

Dawson *et al.*, 1990). Above results clearly indicate that supplementation of live culture improved the feed efficiency. This technique could be employed to improve utilization of the large quantities of ligno-cellulosic waste and low quality forages available throughout the world.

The increase in use of microbial cultures has been fueled, in part, by consumer demand for animal products that are produced with a minimum amount of antibiotics and other growth enhancing drugs. In the most European countries the use of antibiotic substances, anabolic agents, natural and recombinant growth hormones are banned as growth promoters. Furthermore, if India ban on the use of these products, then this new possibility will be available for the use of biological aids to improve animal production and increase profit margins. Internationally, a number of feed manufacturers have started to use an increasing amount of microbial cultures (Dennis, 1992).

During the past number of years, live cultures have been used with very encouraging results, however, a lot of questions are still to be answered. For instance, to what extent is there a stimulatory effect of live cultures on cellulolytic activity in the rumen on high roughage diets compared to high concentrate diets? What is the effect of live culture on ruminal dilution rate and on the ruminal balance of nutrients? Is there a noticeable increase in the amount of microbial mass, especially, of microbial protein? Can optimal effect be achieved through

combined supplementation with lactic acid bacteria? Are the indigenous strains exhibit equally on in vitro, in vivo and on growth performance? Furthermore it is widely accepted that not all strains of yeast and lactic acid bacteria have the same metabolic activities and growth characteristics. Similarly, a number of studies have suggested that the ability of different strains to stimulate the growth and activity of specific strains of ruminal bacteria is not the same (Dawson, 1989). These differences in strains provide an opportunity to further improve animal production by allowing for the selection of new strains for use in feed supplements. Therefore, there is an immediate necessity to further improve the probiotic supplement by selecting highly suitable strains to enhance the feed conversion efficiency and ultimately production. In the present investigation, efforts have been made to select the highly potential microbial strains to meet the following objectives:

- (i) Comparative performance of L.acidophilus, S.thermophilus and S.cerevisiae on rumen fermentation in vitro and in sacco fiber degradability.
- (ii) Their influences on rumen fermentation pattern,
- (iii) Their influences on growth of young calves and efficiency of feed utilisation.

CHAPTER - II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Metchnikoff (1908) formulated a theory correlating the beneficial effects of yoghurt and the longevity of Bulgarians. He speculated that the intestinal environment could be improved, through the ingestion of beneficial microorganisms (lactobacilli). This balancing would then improve health and increase life expectancy of man and animals. Thus, the concept of live microbial cells inoculation for prophylaxis called probiotic was born. Previously it was restricted to only lactic acid producing bacteria

and mono-gastric animals. However, in recent years rumen fermentation was also optimised using live microbial cells to improve ruminant productivity. The two fungal cultures have received considerable attention (S.cerevisiae, yeast culture and Aspergillus oryzae, a fungal additive) have been shown to increase production of ruminants when added in small quantities to the diet. The work done on supplementation of lactic acid producing bacteria alongwith S.cerevisiae have been reviewed in the ensuing pages.

2.1 INFLUENCE OF SUPPLEMENTING LACTIC ACID BACTERIA ON THE MICROBIAL ECOLOGY OF THE GUT AND THEIR MODE OF ACTION

2.2.1 Ability of lactic acid bacteria to survive and colonise in the gut

Not all microorganisms taken in the diet survive in the digestive tract through stomach, duodenum and small intestine due to acidic environment or the presence of bile salts (Renner, 1991). Gilliland et al. (1984) reported that L.acidophilus possessed several characteristics that enable it to survive and grow in the presence of bile salts. Cultures of lactobacilli identified as L.acidophilus from the intestine contents of young calves varied in their ability to grow in broth containing 0.3% oxgall compared with control broth. In a feeding trial involving new born calves, supplementation of bile-resistant strain of L.acidophilus increased population of facultative lactobacilli in the upper small intestines compared to a strain

exhibiting lower resistance to bile salts (Renner, 1991). It has been demonstrated in different species of animals that lactic acid bacteria survive gastro-enteric passage and are found in the faeces (Garvie et al., 1984; Ushe and Nagy, 1985;; Jonsson, 1986; Ratcliff et al., 1986; Conway et al., 1987; Binchi-Salvadori, 1986,1988).

2.1.2 Translocation of microorganisms from the gut

The 'Bacterial translocation' is the passage of viable bacteria through the gastro-enteric barrier into the mesentric lymph nodes and other organs. Translocation occur in cases of physical damage of the mucosa, immune deficiency, prolonged antibiotic treatment or bacterial hyperplasia.

Camaschella et al. (1988) had shown that E.coli was found in lymph nodes of all animals (100 percent translocation) which had been treated with this micro-organism. 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 (Bourlioux, 1986). However, S.thermophilus has never been found in the mesentric lymph nodes nor in other organs of the animals treated with this microorganisms (Camaschella et al., 1988).

In mice feeding of L.bulgaricus and S.thermophilus gave a reduction in the translocation of E.coli into the mesentric lymph nodes of 70 and 50 percent respectively (Binchi-Salvadori et al., 1988).

- Competition for nutrients
Wilson and Perini (1988),
Freter et al. (1983a,b);
Fredrickson (1977).
- Production of anti-bacterial substances
Marshall (1987), Axelsson
et al. (1987), Attaie et al.
et al. (1987); Bloksma et al.
(1979)

2.1.4 Antitumor activities of lactic acid bacteria

Epidemiological evidence and dietary studies have shown that supplementation of lactic acid bacteria may reduce the risk of colon cancer in both animals and human (Friend and Shahani, 1984). When mice were intraperitoneally inoculated with Ehrlich ascites tumor cells, feedings of lactic acid bacteria inhibited growth of tumor (Shackelford et al., 1983; Takano et al., 1985) found similar results with L.bulgaricus and S.thermophilus.

The anti-tumor properties of lactic acid bacteria may be due to:

- Inactivation or inhibition of carcinogenic compounds in the gastrointestinal tract
Keating (1985)
- Stimulation and enhancement of the immune system of the host
Friend and Shahani (1984)
- Reduction of activity of the faecal bacterial enzymes (like
Shackelford et al. (1983);
Cole et al. (1984);

β -glucoronidase, azoreductase, Goldin and Gorbach
 nitro-reductase) which (1984a); Cole and
 activates carcinogens Fuller (1987)
 Degradation of carcinogenic Shahani (1983)
 compounds

2.1.5 Interactions with immuno-competence

In the past few years interaction between dietary lactobacilli and immuno-competence have been studied extensively (Murofushi et al., 1983; Bourlioux, 1986; de-Simone et al., 1986b, 1988). These investigations have revealed that lactobacilli not only constitute an integral part of the healthy gastro-intestinal micro-ecology but also involved in the hosts protective mechanisms by increasing its specific and non-specific immune mechanisms (de Simone, 1986, de Simone et al., 1986a; de Simone, 1987). Live indigenous bacteria or their antigens can in fact penetrate the epithelial barrier of the intestine directly stimulating the immuno-competent cells (Yasui et al., 1989). Perdigon et al. (1986a,b; 1987; 1988) found enhancement of the immune response in mice fed with lactic acid bacteria.

2.2 EFFECT OF LACTIC ACID BACTERIA ON METABOLISM

2.2.1 Mineral absorption

Supplementation of lactic acid bacteria increased the bioavailability of calcium, phosphorus, magnesium and zinc from all diets (Schaafsma et al., 1988). Experiments on rats resulted into 7 and 11 percent higher availability

of calcium and phosphorus respectively when yoghurt was one of the diet (Balasubramanya et al., 1984). During ageing of rats, a decrease of bone weight, ash and calcium content was noticed in the animals fed a control diet but not in animals supplemented with lactic acid bacteria (Dupuis et al., 1985). Consumption of lactic acid bacteria resulted into increased bone calcium and improved bone formation (Kaup, 1988).

2.2.2 Cholestrol metabolism

Number of studies suggested that lactic acid bacteria produced hypo-cholestromic effects in pigs (Gilliland et al., 1985), rabbit (Kiyosawa et al., 1984), rats (Dickinson et al., 1971; Rao et al., 1981; Pulusani and Rao, 1983; Ishida and Kubo, 1985; Grunewald, 1985; Uchida et al., 1986; Kaup et al., 1987; Abdei Gawad et al., 1988; Kim, 1988; Metwally et al., 1988) and in human (Bazzarre et al., 1983; Jasper et al., 1984; Gorbach et al., 1988;; Rajala et al., 1988). Hypocholestromic effects were reported on yoghurt culture with S.thermophilus and S.bulgaricus (Jasper et al., 1984).

2.3 EFFECT OF LACTIC ACID BACTERIA ON GASTRO- INTESTINAL DISEASES

Apart from growth, lactic acid bacteria also controls diarrhoea in young calves. A number of studies suggested that supplementation of lactobacilli reduced the incidence of diarrhoea in neonatal calves (Fettman et al.,

1987; Sissons, 1988), adult calves (Kimura, 1984; Bonaldi et al., 1986; Kopecny et al., 1989), steers (Waller et al., 1988), cattle (Guo et al., 1990) and in bulls (Liberša, 1991).

Diarrhoea symptoms disappeared at the rate of 95.8 percent when Lactobacillus sp. culture was added in the diet of cattle (Guo et al., 1990). Kimura (1984) observed that administration of a live preparation of bifidobacteria and lactobacilli reduced the scouring rate in calves. Bulls given a mixed probiotics (L.casei, L.plantarum, E.faecium, B.subtilis, B.pumilus) increased daily live weight gain by 100 g/d and had a better carcass classification (Liberša, 1991). Steven and Johnston(1988) reported that inclusion of lactic acid bacteria in the diet resulted in 36% decrease in scours. Havrevoll et al. (1988) also found that diarrhoea was less pronounced in calves supplemented with Streptococcus faecium.

2.4 EFFECT OF SUPPLEMENTATION OF LACTIC ACID BACTERIA ON GROWTH OF CALVES

Most of the studies on lactic acid bacteria supplementation are carried out on the rats and pigs (Pollman et al., 1984; Vass et al., 1984; Fuller et al., 1986; Mordenti, 1986; Pollman, 1986b; Camaschella and Bianchi-Salvadori, 1987, Camaschella et al., 1988). Whereas a limited number of studies are conducted on the calves (Kopecny et al., 1989; Vucetic etal., 1989). Supplementation of lactic acid bacteria significantly improve the weight gain and feed conversion efficiency in calves (Havrevoll et al., 1988;

Kopecny et al., 1989) and rats (Broussalian and Westhoff, 1983; Grunewald, 1985; McDonough et al., 1985; Wong et al., 1985, 1987; Hitchines et al., 1986).

Supplementation of S.bovis, Lactobacillus sp. and B.Fibrisolvans to one week old calves significantly improve body weight gain (0.81 Vs 0.64 kg/d). The effect decreased gradually and disappeared 70 days after weaning (Kopecny et al., 1989). Hutcheson (1990) also reported that probiotic eliminates the stress placed up on calves in early life. Vucetic et al. (1989) recorded higher body weight gain and a lower energy intake/kg of live weight gain in calves on supplementation of dietary Lactobacillus sp. culture. They also concluded that supplementation of this culture is beneficial for young calves upto 60 days of age. Supplementation of S.faecium (@ 1.8×10^6 cfu/g) in the diet of calves, increased dry matter digestibility, N-balance, feed conversion efficiency (2.63 Vs 2.43 Fu/kg), dry matter in faeces and body weight gain (Havrevoll et al., 1988). Steven and Johnston (1988) found 9 and 8 percent advantage in daily weight gain and feed efficiency respectively, in group supplemented with lactic acid bacteria. They also concluded that probiotics behaved as appetite stimulants and modulators of stress diarrhoea.

Bakshi and Langar (1990) used Lactobacillus sp. culture (@ 100g/100kg feed) in growing buffalo calves. The dry matter intake, nutrient digestibility, N-retention and availability of ME were improved in group of calves receiving Lactobacillus sp. culture. They also suggested that

altered rumen microbial population with modified rumen fermentation pattern in the presence of lactobacilli was beneficial for the host.

However, in veal calves supplementation of S. faecium did not improve dry matter intake or daily weight gain (Gutzwiller and Wyss, 1988). Waller et al. (1988) found that probiotic did not improve performance sufficiently to recover the cost of probiotic supplementation.

Probiotics work effectively when the calves are weaned at early age (Smith, 1965; Barrow et al., 1977; Tannock, 1983) or reared under unhygienic conditions (Thickett et al., 1991). Many studies are not well documented with regard to the culture used, the basis for selecting the culture and viability of the culture at the time of feeding (Gilliland, 1988). Surveys of the effectiveness of probiotics (Pollman, 1986a,b; Collington et al., 1988; Fox, 1988) also showed that the most consistent response was achieved when probiotics are fed to neonatal and young calves.

2.5 MODE OF ACTION OF S. CEREVISIAE

Work carried at Scotland Rowett Research Institute (Williams, 1988, 1989a,b,c,d, 1990a) threw more light on mode of action of S. cerevisiae resulting into altered rumen fermentation, gain in body weight, milk production and milk composition.

2.5.1 pH-lactic acid and mechanism

It is reported that rumen pH is mainly associated with concentration of lactic acid in the rumen (Williams, 1990a; Williams et al., 1991), which is clear from the earlier investigations (Malestein et al., 1981; Williams et al., 1991) that pH depression coincides with the concentration of lactic acid. Supplementation of S. cerevisiae decreased the lactic acid concentration which ultimately help in elevation of ruminal pH (Williams, 1989a, 1990a). Presence of yeast culture was 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 levels are low or in the region of 6.0 (Williams, 1989b, 1990a). Diets with readily fermentable carbohydrate or presence of starch and simple sugar depressed ruminal pH (Schneider and Flatt, 1975; Offer, 1990; Williams and Newbold, 1990b; Williams et al., 1991) lead to reduction of cellulolytic bacteria (Stewart, 1977; Thomas and Rook, 1981), impaired forage degradation (Williams, 1989a,b,c,d) and feed intake (Orskov et al., 1978).

In this mechanism ruminal pH is elevated via reduction in the concentration of lactic acid by S. cerevisiae supplementation. However, lactic acid is not a substrate used by S. cerevisiae (Panchal et al., 1984a). This effect must have resulted from either direct reduced lactic acid production (due to utilization of lactic acid precursor by S. cerevisiae) or by indirect stimulation of

lactic acid utilising bacteria in the rumen. Authors reported both direct or indirect mechanisms to lower the lactic acid concentration. Panchal et al. (1984a) found that yeast cells reduce the concentration of starch degradation products. However, Nisbet and Martin (1990) reported, S.cerevisiae stimulate the lactic acid utilising bacteria Selenomonas ruminantum for increased lactic acid uptake (almost four folds).

It was not likely that the modulation of rumen pH was the main mechanism where yeast culture affects cellulolysis (Williams, 1989b, 1990a). The reduction in the concentration of the simple sugar in the presence of yeast culture might be responsible for improved rumen fermentation (Hungate, 1966; Williams et al., 1991). Mould and Ørskov (1983-84) conclude from their work with high concentrate diets that it was not low rumen pH per se, but rather the presence of easily degradable starch that inhibit fibre degradation. Researchers (Panchal et al., 1984b; DeMot, 1987) reported that simple sugars were actively transported across the yeast cell wall, resulting in their removal from the external medium and internalisation into the yeast cell for their utilization.

2.5.2 Production of growth stimulating factors for rumen microbes

Another possibility is that yeast cells in the rumen 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 (Weidmeir and Arambel, 1985; Dawson, 1987; Chandemana and Offer, 1990). This mechanism could also explain the increased number of protozoa with S.cerevisiae treatment (Chung et al., 1989; Carro et al., 1992). Andries et al. (1990) found similar results when supplementing iso-acids in the diet. However, there is conflicting evidence as to the microbial need for such performed nutrients (Redman et al., 1980; Sriskandarajah et al., 1982).

2.5.3 Improved microbial population and digestibility

Many of the beneficial production responses associated with the use of yeast culture supplements can be directly related to their effects on the increased microbial population in the digestive tract. For the most part evidence for such effects are the result of intensive studies of microbial activities in the rumen of cattle and sheep (Dawson, 1990; Offer, 1990; Williams and Newbold, 1990b; Edwards, 1991), but is supported by studies of the effects of yeast culture on digestive activities in the hind gut of the horses (Glade and Biesik, 1986; Glade, 1991). Increased concentration of total anaerobic bacteria have been observed, but in a number of studies increased concentration of specialized bacteria associated with fibre digestion and lactic acid utilization have been observed (Dawson, 1992). Such increase in the number of bacterial cells suggest that live yeast supplements can stimulate cellulolytic bacteria in the rumen and alter cellulose digestion in the rumen.

Yeast culture supplementation resulted in higher cellulolytic bacteria (Dawson, 1987; Williams, 1989b), higher fibre degradation (Williams, 1989d; Chandemana and Offer, 1990) as well as dry matter intake (Hovell, 1986; Lopez et al., 1989). Offer (1990) suggested that S. cerevisiae stimulates the initial colonization of plant fragments in the rumen and therefore allowing rapid commencement of fibre breakdown.

2.5.4 Feed protein conversion

Rumen microbes upgrade the biological value of feed protein by converting it to microbial protein especially when dietary source of low and medium quality forage. The addition of yeast culture reduced the ammonia production in the rumen by 10 to 35% in the in vitro (Dawson and Newman, 1987, 1988; Carro et al., 1992) and in vivo studies (Adams et al., 1981; Dawson and Newman, 1987; Harrison et al., 1987a, 1988; Newbold et al., 1990; Chandemana and Offer, 1990). This results in an improved microbial capture of ammonia and increased microbial biomass (Dawson, 1987; Wiedmeier et al., 1987; Chandemana and Offer, 1990; Carro et al., 1992). A higher proportion of microbial nitrogen derived from ammonia-N with yeast culture supplementation support this suggestion (Carro et al., 1992). The cows kept on yeast culture exhibited a higher non-ammonia-N (Williams et al., 1990; Erasmus, 1991) and dry matter (Williams et al., 1990) flow due to increased microbial-N flow. Also significantly increased the absorption of dry matter and non-ammonia-N from small intestine

(Williams et al., 1990). Williams and Newbold (1990b) demonstrated that daily absorption of non-ammonia nitrogen in the intestine was increased by 23 percent when yeast culture supplements were provided in the diet. These investigators suggest that this represents an increase in the flow of useful microbial protein to the small intestine. Gomez-Alarcon et al. (1987) found yeast culture supplementation resulted in higher microbial yield (20.0 Vs 15.5g/100g of fermented OM) and resulted in more microbial true protein reaching the duodenum (635g Vs 355 g/d). In another investigation, Edwards (1991) demonstrated increased allantoin nitrogen in the urine and decreased urea nitrogen concentrations in plasma which also support a mechanism for enhancing the conversion of ammonia nitrogen in the digestive tract into microbial cell mass.

Yeast culture also selectively stimulate certain rumen microbial population which has an impact on the protein quality (Carro et al., 1992) which is indicative from the amino acid concentration of duodenal digesta. The amino acid concentration of serine, threonine, cystine and methionine was found higher (Erasmus, 1991; Lyons, 1992). Stimulation of protein synthesis has some important implications and may be used to explain many of the production responses associated with yeast culture supplementation. Rumen function does not always supply all the nutrients required for maximal growth or production in ruminant animals. Protein limitations are particularly important in young growing animals and in high producing dairy cattle.

The most beneficial responses to yeast supplementation would also be expected in animals receiving the low quality feeds (Dawson, 1992).

2.5.5 Integrated effect on mode of action

Based on observations made in ruminant animals and with cultures of isolated ruminal bacteria different models have been developed, which explain the action of yeast cultures on digestive processes and production traits (Newbold, 1990; Offer, 1990; Dawson, 1990, 1992; Lyons, 1992; Wallace, 1992; Wallace and Newbold, 1992). None of these explain its effect collectively. However, the effect of yeast on feed intake, ruminal and post-ruminal processes cannot be discussed in isolation. Lately, Lyons (1992) put forth that feed intake entirely depends on the changes occurring in the rumen which also in turn influence feed conversion efficiency (Fig 2.1). However, all models explained the effects of supplemented cultures on digestive processes. Dawson *et al.* (1990) reported increased concentration of anaerobic bacteria was closely related to the presence of live yeast cell in the rumen and was not observed on heat treated yeast or supplementing killed yeast cells.

Recently Lyons (1992) proposed an alternative model, in which all of the observed effect on rumen fermentation, and the consequent effects on nutrition, stem from the stimulation of growth and activity of specific bacteria by yeast culture supplementation.

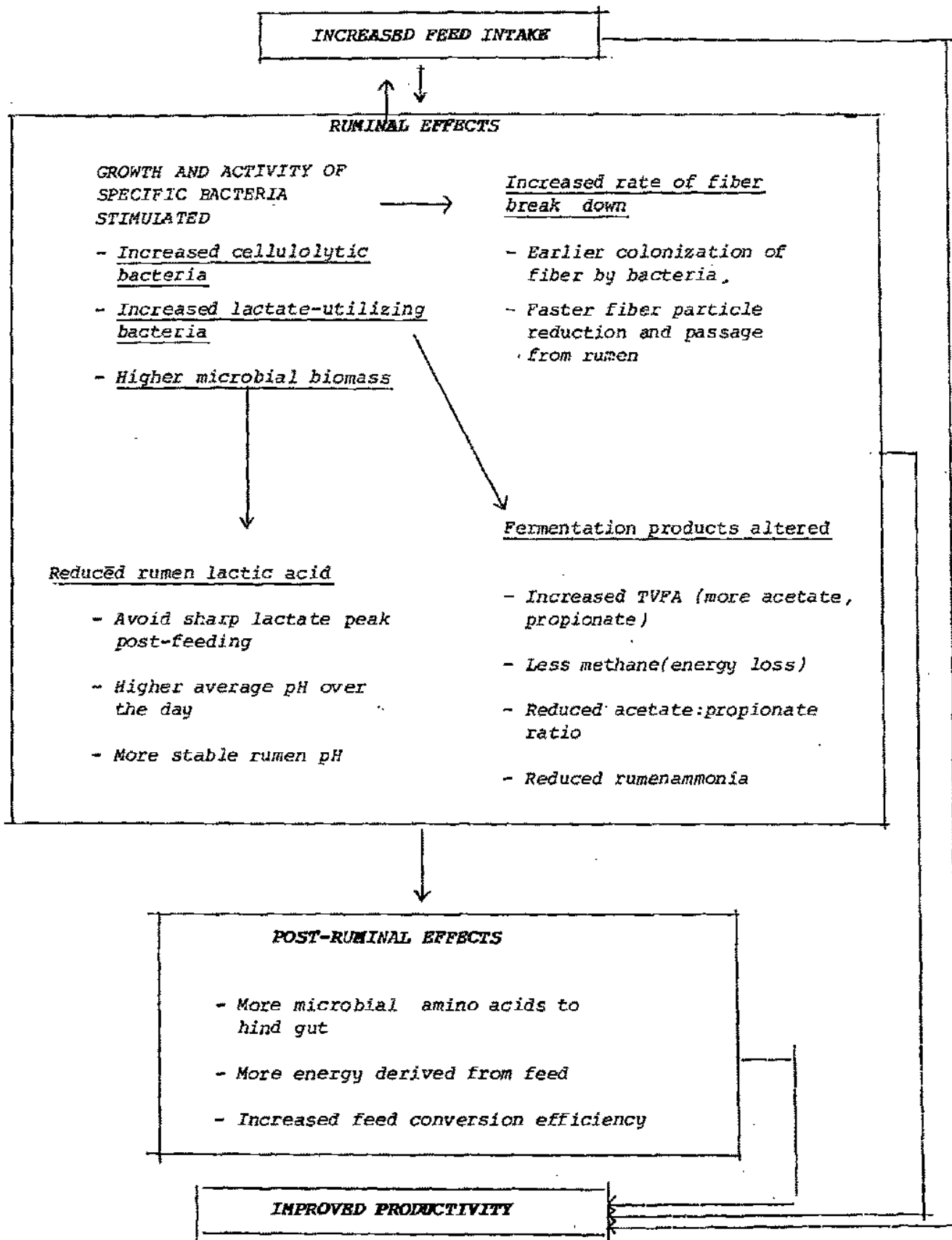


Fig 2.1 Proposed mode of action of yeast culture in the rumen

2.6 EFFECT OF S.CEREVISIAE ON GASTRO- INTESTINAL DISEASES

Another important function of yeast cells may be related to their ability to influence animal health by preventing gastro-intestinal diseases as with lactic acid bacteria (Maffeo and Castiglioni, 1983; Bonaldi *et al.*, 1986; Dawson, 1992). Shityi *et al.* (1990) and Dawson (1992) suggested the addition of yeast cells to the gastro-intestinal tract can have a significant impact on animal health. Some of these effects have been related to the ability of the yeast cell wall to bind toxins and pathogenic bacteria (Dawson, 1992). Unlike some of the other beneficial effects, the yeast cells in the intestinal tract may stimulate the immune system and provide for better protection against invading pathogens (Dawson, 1992). Currently little published informations support the use of yeast culture for improving animal health by preventing gastro-intestinal infections (Lyons, 1992).

2.7 EFFECT OF S.CEREVISIAE ON RUMINAL FERMENTATION

2.7.1 Bacterial population

One of the common features of yeast culture supplements was its ability to stimulate the growth of cellulolytic bacteria in the rumen (Harrison *et al.*, 1987a, 1988; Wiedmeier *et al.*, 1987; Dawson, 1990). It is particularly pronounced when forage containing diets are fed (Dawson, 1990).

Addition of yeast culture in dairy cows increased total viable bacteria and cellulolytic bacteria 1.3 and 1.5 fold (Wiedmeier et al., 1987), 12.2×10^9 Vs 7.76×10^9 and 12.0×10^7 Vs 6.6×10^7 MPN/ml (Harrison et al., 1987a), respectively.

In fermenter system yeast culture addition increased counts of anaerobic bacteria (1.3 to 5.0 times) and cellulolytic bacteria (2.0 to 2.5 times) (Dawson and Newman, 1987; Williams, 1990a).

When a pure culture of S.cerevisiae and a mixed culture with lactobacilli and enterococci supplemented with roughage diet to the steers, concentration of cellulolytic bacteria in both were 5 to 40 times greater (Dawson et al., 1990).

The concentration of anaerobic and cellulolytic bacteria increased on supplementation of live yeast cells but there was no increase when supplements were heat treated (autoclaved 121°C for 15min) to kill the yeast (Dawson and Newman, 1987; Dawson, 1990). A number of studies indicating influence of yeast culture on rumen microorganisms varying with different feed combinations have been reported on the next page.

2.7.2 pH and lactic acid concentration

One important effect of yeast culture in high concentrate rations has been related to its ability to decrease ruminal lactic acid concentrations and moderate ruminal pH

Summary of studies describing responses of ruminal microbial population
to yeast cultures supplementation

Diet	Bacterial group	Condi- tion	Conc. in control group (CFUx10 ⁹ /ml)	Conc. in yeast group (CFUx10 ⁹ /ml)	Increase	References
50% concentrate	Total anaerobes	<u>In vivo</u>	19.6	25.5	30%	Wiedmeier <u>et al.</u> (1987)
	Cellulolytics	<u>In vivo</u>	2.50	3.98	56%*	
40% silage 60% concetrate	Total anaerobes	<u>In vivo</u>	7.76	12.3	58%	Harrison <u>et al</u> (1988)
	Cellulolytics	<u>In vivo</u>	0.066	.120	81%*	
77% hay	Total anaerobes	<u>In vitro</u>	3.8	43.6	11-fold*	Dawson <u>et al.</u> (1990)
	Cellulolytics	<u>In vitro</u>	.105	3.98	38-fold*	
77% hay	Total anaerobes	<u>In vivo</u>	.708	7.41	10-fold	Dawson <u>et al.</u> (1990)
	Cellulolytics	<u>In vivo</u>	.019	.158	8-fold*	
40% hay, 60% concentrate	Total anaerobes	<u>In vitro</u>	104.0	157.0	50%*	Newman and Dawson (1987)
	Cellulolytic	<u>In vitro</u>	13.4	16.0	19.4%	
Barley based finished diet	Total anaerobes	<u>In vivo</u>	.641	1.39	116%*	Edwards (1991)
	Cellulolytics	<u>In vivo</u>	.018	.035	89%	
	Lactate-utilizers	<u>In vivo</u>	.0083	.0277	3fold*	

*Indicates statistically significant increases (P/0.10) with yeast culture supplements

(Williams, 1989a). Williams et al. (1991) demonstrated consistently lower ruminal lactic acid concentrations in the rumen of steers fed a hay plus barley diet when yeast culture supplements were used. This decrease in lactic acid concentration was associated with greater ruminal pH and lower concentrations of Oligosaccharides (Malestein et al., 1981; Williams, 1990b; Williams et al., 1991). Martin et al. (1989) have demonstrated that extract from yeast cultures reduce the lactic acid concentration by stimulating the activities of the lactic acid-utilizing bacterium Selenomonas ruminantium. Edwards (1991) has also demonstrated greater concentrations of lactic acid-utilizing bacteria in the rumen of cattle receiving yeast culture supplement. In this study, the use of yeast culture complemented the effects of an ionophore which can also be useful in controlling lactic acid production.

2.7.3 Total volatile fatty acid and their proportions

Several investigators have used increased volatile fatty acid production by ruminal bacteria in batch cultures to measure the stimulatory effects of yeast cultures on ruminal fermentations (Gray and Ryan, 1990; Martin et al., 1989). However, it has not been possible to consistently associate similar increase in VFA production with yeast culture supplementation in the rumen or in rumen stimulating cultures (Malcolm and Kiesling, 1990; Wagner et al., 1990). Dawson et al. (1990) reported that yeast culture enhance the relative production of propionate and decrease the ratio of acetate to propionate in the rumen on silage or forage based rations.

In sheep, yeast culture supplementation 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). In a similar study with cows yeast culture supplementation reduced acetate (53.1 to 48.2 M%) and acetate:propionate ratio (2.00 to 1.65) due to higher propionate production (27.4 Vs 30.0 M%). The TVFA concentration was increased from 172.2 to 184.5 m mole/l (Harrison etal., 1987a). Such changes are indicative of improved fermentation efficiencies and decreased methane production in the gastro-intestinal tract, and could provide additional energy for production (Williams and Newbold, 1990^{a,b}). In contrast, other studies have demonstrated increase or non-significant changes in the ruminal acetate to propionate ratio when straw or high concentrate rations were fed to ruminants (Wiedmeier et al., 1987; Williams and Newbold, 1990^{a,b}; Edwards, 1991; Carro et al., 1992). 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. Differences in the ability of yeast culture to alter fermentation patterns may be explained by differences in dietary treatments. Review of the available literature suggest that there is currently not enough data available to identify specific dietary situations where yeast can be used as effective agents for altering fermentation pattern and end products formation in the rumen.

2.7.4 Methane production

The carbohydrates (cellulose, starch, sugars) are fermented in the rumen into acetate, propionate, butyrate with the intermediates like hydrogen and succinate. The removal of hydrogen by methanogenesis has been reported to increase the production of acetate (Wolin and Miller, 1983). Production of methane in the rumen resulted to a waste of 10% dietary energy since 2 to 3 percent of total bacterial population in the rumen represent methanogenic bacteria. Both propionic acid and CH_4 formation compete for the available hydrogen in the rumen as a result of fermentation. Yeast cell walls are known to possess a large proton buffering capacity (Cartwright et al., 1986) and offers a highly alternative from hydrogen transfer to a production of methane. The molecular hydrogen channeling into microbial system, and also increase in the efficiency of cellulolysis resulting into increased acetate production were observed due to supplementation of yeast culture. Yeast thus resulted into reduction of methane production in steers by 28% (Williams, 1990a).

2.7.5 Ammonia production and microbial protein synthesis

One of the common observations associated with the use of yeast culture in ruminants and rumen-stimulating fermentors has been the reduction of ruminal ammonia concentrations (Dawson and Newman, 1988; Harrison et al., 1988; Newbold et al., 1990; Williams and Newbold, 1990; ^{atb} Edwards, 1991).

It is suggested that inclusion of yeast culture in the diet reduced the ammonia production by 10 to 35 percent in vitro (Dawson and Newman, 1987, 1988; Carro et al., 1992) and in vivo (Adams et al., 1981; Harrison et al., 1987a, 1988; Chademana and Offer, 1990; Newbold et al., 1990). In steers supplemented with yeast culture, rumen ammonia concentration was slightly lower (18.1 Vs 20.3 mg/100 ml) (Adams et al., 1981). However, in sheep ammonia was significantly reduced by 35 percent (Newbold et al., 1990). Cow supplemented with yeast culture also shows a significantly lower ammonia concentration (87.5 Vs 12.6 mg/l) (Harrison et al., 1987a).

(Reduced ammonia levels have not been associated with decreased protein degradation or deamination (Williams and Newbold, 1990). The reduced amount of ammonia was utilized for microbial protein synthesis in the rumen (Dawson, 1987; Wiedmeier et al., 1987; Chademana and Offer, 1990; Carro et al., 1992), which was confirmed by greater microbial yield and microbial true protein reaching the duodenum (Gomez-Alarcon et al., 1987; Erasmus, 1991). They suggested that yeast may have selective stimulatory effects on specific rumen bacteria which result in a shift in the microbial population and would be reflected in increased protein synthesis and amino acid profiles. It is also supported by studies which indicate that yeast culture supplementation can change the amino acid and nitrogen metabolism in horses (Glade, 1991).

2.8 EFFECT OF S.CEREVISIAE ON FEED UTILIZATION AND GROWTH

Production response to yeast culture supplementation vary with species, diet and type of yeast added to diet. Some of the beneficial production responses associated with the use of yeast culture supplements have been summarized in the Table given below:

Some effects of yeast culture supplements on animal production

<u>Production response</u>	<u>Animal</u>	<u>References</u>
Increased intake	Dairy cattle beef cattle, and sheep	Ruf <u>et al.</u> (1953), Phillips and VonTungeln (1985); Malcolm and Kiesling (1986), Fallon and Harte (1987), Gunther (1989), Williams and Newbold (1990 ^{orb}), McLeod <u>et al.</u> (1990), Williams <u>et al.</u> (1991)
Increased milk production	Dairy cattle and goats	Hoyos <u>et al.</u> (1987), Teh <u>et</u> <u>al.</u> (1987), Williams <u>et al.</u> (1991)
Altered milk composition	Dairy cattle and goats	Hoyos <u>et al.</u> (1987), Teh <u>et</u> <u>al.</u> (1987), Gunther (1989), Williams <u>et al.</u> (1991)
Improved weight gain and feed efficiency	Beef cattle	Ruf <u>et al.</u> (1953), Greive (1979), Phillips, and Von Tungeln (1985), Fallon and Harte (1987), Hughes (1988),

		McLeod <u>et al.</u> (1991),
		✓ Edwards (1991)
Improved diges- tion and weight gain	Dairy goats	Williams <u>et al.</u> (1991),
		✓ Bhoi (1992)

2.8.1 Feed intake

Yeast culture has been shown to improve nutrient supply to the animal at a fixed intake (Williams et al., 1990), but in farm trials, its effect on intake appears the most important cause of improved performance. In several studies it has been found that yeast culture supplementation increased the feed intake in calves (Bonaldi et al., 1986; Fallon and Harte, 1987; Hughes, 1988), steers (Adams et al., 1981); lactating cows (Malcolm and Kiesling, 1986; Quinonez et al., 1988, 1989; Williams, 1990a; Williams et al., 1991; Wohlt et al., 1991) and in bulls (Drennan, 1990).

In lactating cows yeast culture supplementation significantly increased the DM intake by 1.2 to 1.6 kg/d with a higher milk production (Williams, 1990a; Williams et al., 1991). These responses were the results of increased nutrient supply to the mammary gland as a result of increased dietary feed intake, 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). In steers yeast supplementation significantly increased the DM intake as compared to control (3.17 vs 2.07 kg/d/100 kg b.wt). However, for a unit body weight gain the feed intake

was lower (6.32 vs 6.42 kg/kg body weight gain) in a group of animals supplemented with yeast culture. This was reflected to higher feed efficiency (Adams *et al.*, 1981).

Williams (1989a) suggested that a direct stimulation of cellulolysis and increased rate of fiber digestion resulted in higher intake of feed. Yeast culture alters the shape of the degradation curve causing a reduction in lag phase before digestion commences. The model of Hovell (1986) showed how a change of this type can lead to a large increase in *ad libitum* forage intake even though overall feed digestibility is not increased. Evidence is provided by Lopez *et al.* (1989) which showed that voluntary intake of hay by sheep was related to the degradation constants of the hay measured *in sacco*. Intake was closely correlated to the magnitude of the soluble component and to the degradability rate constant but not to the size of the potentially degradable fraction.

2.8.2 Nutrient digestibility

A number of studies provided evidence indicating that live yeast cells enhanced the digestive processes in the gastrointestinal tract. These effects have directly stimulated growth and its activity. Such effects have been summarised in a table on the next page.

S. cerevisiae supplementation on digestibility showed a variable response (Arambel and Kent, 1990; Moloney, 1990; Wohlt *et al.*, 1991). Supplementation of *S. cerevisiae* increased the digestibility of protein (Wohlt *et al.*, 1991),

Summary of studies describing direct effects of yeast culture on
digestive processes in domestic animals

Animal	Diet	Observation	Reference
Cattle	Alfalfa Hay	<u>In vitro</u> dry matter tended to increase after adaptation to yeast culture	Arambel <u>et al.</u> (1987)
Cattle	Hay with barley straw, rolled oats, beet pulp and wheat bran	Increased dry matter, crude protein and hemicellulose digestion in the total tract	Wiedemeier <u>et al.</u> (1987)
Calves	Barley/soya or corn gluten/barley diets	Enhanced dry matter, nitrogen, and organic matter digestion in the total tract	Fallon and Harte (1987)
Cattle	40% silage and 60% concentrate	No effects of dry matter, fiber, hemicellulose or starch digestibility in the total tract	Harrison <u>et al.</u> (1988)
Cattle	Hay with rolled barley	Dry matter digestion in the rumen was enhanced at 12h but not at 24h	Williams <u>et al.</u> (1991)
Sheep	High, medium and low forage:concentrate ratios	Increased initial rates of organic matter digestion in the rumen but did not alter the ultimate extent of digestibility	Chademana and Offer (1990)
Goat	Concentrate and <u>ad lib</u> lucerne as fodder	Increased dry matter, crude fiber, crude protein, NDF and ADF digestion in the total tract	Bhoi (1992)

cellulose (Gomez-Alarcon, 1987; Wohlt et al., 1991) as well as that of fiber (Wiedmeier et al., 1987; Gomez-Alarcon et al., 1990) but not always (Arambel and Kent, 1990). Carro et al. (1992) 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 digestibility. However, on high forage diet yeast culture had no effect on DM, NDF and cellulose digestibility. Similarly in vivo studies where DM digestibility was not changed by addition of yeast culture (Harrison et al., 1988; Arambel and Kent, 1988; Williams, 1989a,b; Chademana and Offer, 1990) suggested that the effects of yeast on digestion may be very subtle and cannot 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). Gomez-Alarcon (1987) reported that yeast culture increased the cellulose digestibility of DM in total tract was similar.

Some authors (Dawson et al., 1987; Williams, 1989a; Chademana and Offer, 1990; Williams et al., 1991) have reported yeast culture to increase the rate of degradation of DM and fibre in short term experiments (under 24h). However, with an extended period of incubation (48h) there was no difference in DM degradability (Dawson et al., 1987; Williams, 1989a; Chademana and Offer, 1990; Williams et al., 1991). In goat supplementation of a mixed culture of S.cerevisiae and L.acidophilus resulted in a higher digestion rate of DM, CP, NDF and ADF (Bhoi, 1992).

In addition, the initial stages of fibre digestion by cultures of individual ruminal bacteria has been shown to be enhanced by certain strains of yeast (Dawson, 1990). In these studies with isolated cellulolytic bacteria, the time required to initiate fibre digestion was decreased by 30 percent in the presence of live yeast cells. The yeast could not grow on the fibrous substrates provided in these cultures and concentrations of yeast cells in these symbiotic cocultures increased only after substrates were supplied through the action of the filter paper discs. These studies indicate that yeast culture supplementation may have a significant effect on the time and course of digestive processes in the rumen. Such changes in digestive function could increase the availability of nutrients in the rumen and could have a significant impact on intake (Williams and Newbold, 1990^A).

- Supplementation of yeast culture non-significantly increased the liquid dilution rate (Adams et al., 1981; Harrison et al., 1987b; Malcolm and Kiesling, 1990). However, Wiedmeier et al. (1987) found that yeast culture produced a small increase in the rate of particulate passage (5.11 Vs 4.52 percent/h) without any change in liquid outflow rate, which suggested an increase in the rate of fibre degradation.

It is clear from these studies that yeast culture supplements do not have equal beneficial effects with all types of diets. Loyns (1992) described different diets where yeast culture might be used.

(i) Mixed forage/concentrate diet: The rumen environment on this diet typically has a pH low enough to suppress cellulose digestion. Lactic acid, especially when high lactic acid silage is in the diet, can create low rumen pH and subclinical acidosis problems. Yeast strains selected for this diet must stimulate fibre degradation and lactate utilization.

(ii) High concentrate diet: As there is little digestible fibre in this diet yeast that stimulate cellulose degraders would have little value. Strains that stimulate lactic acid utilization, however, would be useful in fighting subclinical acidosis and maintaining feed intake.

(iii) High forage diet: Intake capacity often limits performance on this type of diet, especially when forage quality is poor. Rumen modifiers for this diet must maximize fibre degradation and conversion of feed protein to microbial protein. Increased population of cellulolytic species speed up degradation rate and allow increase intake.

(iv) High soluble nitrogen diets: Urea based diet have more soluble-N than carbohydrate available for microbial utilization. Excess ammonia crossing the rumen wall must be converted to urea by the liver, an energy requiring process. When rumen NH_3 levels are in excess of microbial needs, addition of the yeast culture should be beneficial.

2.8.3 Effect of *S.cerevisiae* on calves growth

Studies on young calves responded well to dietary supplement of *S.cerevisiae* alone (Quinonez et al., 1989; Hudyma et al., 1990; Wagner et al., 1990) and alongwith

Lactobacilli (Bonaldi *et al.*, 1986; Hughes, 1988; Vucetic *et al.*, 1989). Supplementation of *S.cerevisiae* with or without *Lactobacilli* increased the live weight gain significantly with higher DMI (Fallon and Harte, 1987; Hughes, 1988) and with a lower energy consumption (Vucetic *et al.*, 1989).

In a recent trial conducted at National Dairy Research Institute, Karnal on goat kids both crude protein and crude fibre were digested at higher rate on supplementation of *S.cerevisiae* compared to these two excelled over individual addition. The addition of individual organisms increased growth rate over the control but combination of both exhibited better results. The feed conversion efficiency was also better when a combination of both organisms was supplemented. The control of diarrhoea was more effective on *L.acidophilus* supplementation. The data is briefly summarized in the table presented below:

Feed conversion efficiency of kids on
supplementation of probiotics

Parameters	Treatment groups			
	A	B	C	D
DMI/KgW (^{0.75} 0.75)/d (g)	68.5	68.5	71.1	71.1
CP Dig. (%)	76.8	75.5	80.1	82.6
CF Dig. (%)	44.1	45.7	55.2	64.3
N-retained to N-intake (%)	54.2	57.8	61.5	65.7
Weight gain (g/d)	64.3	87.2	89.6	94.9

....contd.

Feed consumed/kg weight gain (kg)	7.7	5.6	5.6	5.4
Diarrhoea cases observed (No.)	10	3	6	4

 A, Control; B, L.acidophilus; C, S.cerevisiae;
 D, L.acidophilus + S.cerevisiae

Studies with veal calves showed that inclusion of S.cerevisiae and L.acidophilus significantly improved the daily body weight gain, feed intake and meat yield (Bonaldi et al., 1986; Hughes, 1988) without affecting carcass characteristics (Kimenai, 1990). The supplemented groups were found to be more economical (Hughes, 1988; Kimenai, 1990). When S.cerevisiae was supplemented at 1 percent or 2 percent of DMI to calves after stress, DMI increased without any difference between the levels of supplementation (Phillips and Vontungeln, 1985). However, body weight gain did not increase consistently. Similarly in some studies neither feed intake nor weight gain was affected by inclusion of S.cerevisiae (Quinonez et al., 1989; Seymour et al., 1989).

CHAPTER - III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

In order to achieve the objectives delineated for the current project, the investigations were carried out, firstly establishing and production of various microbial cultures to be used and secondly to evaluate the effect of these cultures on feed degradability and feed utilization for growth. The evaluation of the cultures is carried in four different phases. In the first and second phases the cultures were evaluated through in vitro and in vivo studies based upon several parameters.

In the third phase response of probiotic supplement on the growth of pre-ruminant and post-ruminant calves was studied. In the fourth and final phase a metabolic trial was conducted to study the effect of probiotic on digestibility and nutrient utilization.

The research methodology used in present study has been detailed separately for production and maintenance of microorganisms, and procedure followed during each those of the investigations.

3.1 PRESERVATION AND PRODUCTION OF PROBIOTIC SUPPLEMENTS

3.1.1 Source and type of probiotics

Six different standard strains of microorganisms taken from National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division of National Dairy Research Institute, Karnal have been listed as under:

- (i) Lactobacillus acidophilus (R)
- (ii) L.acidophilus(1)
- (iii) Streptococcus salivarius subsp.
thermophilus (HST) (New name of for
S.thermophilus)
- (iv) S.salivarius subsp. thermophilus(CH)
- (v) Saccharomyces cerevisiae (522)
- (vi) S.cerevisiae (B)

3.1.2 Maintenance of standard cultures

The standard strains of L.acidophilus and S. salivarius subsp. thermophilus were maintained on the litmus milk, however, strains of S.cerevisiae were maintained on the malt extract agar slants.

The cultures were aseptically inoculated in their respective medium and incubated for 24 to 48h depending upon the growth (L.acidophilus and S. salivarius subsp. thermophilus at 37°C and S.cerevisiae at 25°C). After optimum growth mouth of each test tube were wrapped in aluminium foil and kept at 4°C for further use upto a fortnight. At a time each strain was inoculated in two test tubes. One test tube was used for sub-culturing the medium for cell harvesting and another for sub-culturing other two test tubes and so on.

3.1.2.1 Preparation of litmus milk:

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

3.1.2.2 Composition of litmus milk

Fresh cow skim milk	100 ml
Litmus solution	1.0 to 1.5 ml/100 ml of skim milk
CaCO ₃	1.0 to 2.0g/test tube

3.1.2.3 Litmus solution:

5g of litmus powder was dissolved in 100 ml of distilled water and heated upto boiling. Solution was filtered through cotton and kept at 4°C for further use.

3.1.2.4 Preparation of malt extract slants:

About 1.5 to 1.8 percent agar-agar powder was added in the malt extract broth and dissolved by heating. 10 to 12 ml of this solution was added in test tube and sterilized at 121°C/15 psi for 30 minutes. After sterilization these test tubes were put in the incubator at 25°C for 10 minutes in the inclined position for setting of agar in slant form. Mouth of these test tubes were wrapped with aluminium foil and kept at 4°C for further use upto a month.

3.1.3 Production and preservation of probiotics

For harvesting the live cells of L.acidophilus, S.salivarius subsp. thermophilus and S.cerevisiae, the cultures were grown in respective media like MRS, LYPA and malt extract broth.

3.1.3.1 Composition of media:

The composition of the media used is given as under:

<u>MRS</u>	
<u>Ingredients</u>	<u>g/l</u>
Peptone	10.00
Meat extract	10.00
Yeast extract	5.00
K_2HPO_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>LYPA</u>	
<u>Ingredients</u>	<u>g/l</u>
Peptone	5.0
Yeast extract	5.0
Beef extract	10.0
Na_2HPO_4	3.0
Lactose	20.0
pH	No need to adjust

siae at 25°C). After having final growth the biomass was centrifuged at 3000 rpm for 15 minute. 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 percent sterilized glycerol solution which act as a cryoprotecting agent (Satadholders et al., 1979). These cells were preserved in sterilized small tubes placing in the liquid nitrogen (Gilliland, 1985) for further use. At the time of using the culture for feeding animals, the tubes were thawed in luke' warm water till it attain a room temperature.

3.1.4 Purity of cultures

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

3.1.5 Counting of live cells

For estimation of microbial population, standard plate count technique was used before and 1 month after keeping in liquid nitrogen.

Standard plate count:

Procedure:

1. Culture tube was vigorously shaken by rotating between the palms to disperse the cells uniformly.

2. Several dilutions with normal saline water were prepared in the following manner:

(i) 1 ml of well mixed culture was transferred to 9 ml of sterilized saline water. Mixed well by rotating between the palms.

(ii) Transferred 1 ml of this suspension to second test tube containing saline water and repeated the procedure to 3, 4, 5, 6, 7 and 8th test tubes. The concentration of the bacterial cells in each of these successive dilution were in multiple of 1/10. For plating purpose, 6, 7 and 8th dilution of cultures were used.

3. 1 ml of solution from 6th, 7th and 8th dilutions were transferred into sterilized petri-dishes in duplicate using separate sterilized pipettes.

4. Six test tubes of agar medium were melt placing in boiling water and cooled to 45°C in a water bath. The contents of each test tube were poured into one of the petri-dish.

5. Mixed the agar with inoculum gently by rotating the plates in opposite directions.

6. Allowed the agar to set in about 5 to 10 min, invert and incubate at appropriate temperature for 48h in the incubator. Colonies of each petri-dish were counted.

7. The petri-dishes having 30-250 clearly visible colonies in a similar dilution were considered for count-

ing. The average of two plate counts of the same dilution was used for calculating number of viable microorganisms. The total number of organisms in the culture to be used for feeding was calculated by multiplying the average count with the dilution factor.

PHASE-I

3.2 IN VITRO EVALUATION OF PROBIOTICS

Six individual strains of microorganisms were supplemented in the in vitro system (@ 10^9 cfu/flask) using a 60:40, wheat straw:concentrate ratio (on DM basis). The incubations were carried out for 24 and 48 hours. The following parameters were considered to select the two microorganisms each from bacteria and yeast.

1. Dry matter degradability
2. Total volatile fatty acid production
3. Total gas production
4. Ammonia nitrogen production
5. Total microbial protein nitrogen
6. Bacterial counts.

3.2.1 Feeding of doner animals

Adult rumen, fistulated crossbred male calves (Brown Swiss x Sahiwal) were fed ad lib on roughage and concentrate in the proportion of 60:40. The roughage consists of wheat straw and concentrate contained ground-

nut cake 30, maize 30, wheat bran 37, mineral mixture 2% and salt 1%. The feed was offered to the animals in the morning at 8 A.M. and the animals were given water at 12 A.M. and 4 P.M.

3.2.2 Collection of rumen liquor

Rumen liquor was collected from the donor animals from all parts of the rumen into a clean, thermos flask. 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.

3.2.3 In vitro technique

In vitro studies were conducted in quadruplicate according to the method described by Tilley and Terry (1963). The artificial saliva was prepared as per the method of McDougalls (1948). The samples were incubated for 24 and 48 h at 39°C.

3.2.3.1 Feed and probiotic supplementation:

In each flask 1g of feed, wheat straw:concentrate (60:40) on DM basis was taken as substrate. The ingredient composition of concentrate mixture is detailed in Table 3.1. Individual microorganisms were added @ 10^9 cfu per flask except control.

Table 3.1 Composition of concentrate used for substrate
for in vitro studies

Ingredients	Parts (kg)	CP (kg)	TDN (kg)
Maize	30.0	3.6	25.5
Groundnut cake	30.0	13.5	22.5
Wheat bran	20.0	2.4	14.0
Rice bran	17.0	1.7	11.9
Mineral mixture	2.0	0.0	0.0
Common salt	1.0	0.0	0.0
Total	100.0	21.2	73.9

3.2.3.2 Estimation of in vitro dry matter, crude fiber and organic matter degradability:

After in vitro incubations of 24 and 48h, the contents of the flasks were filtered through G-1 crucibles. The dry matter, crude fiber and organic matter were estimated according to AOAC (1984) in the sample of feed and in residues obtained in the G-1 crucibles. In vitro degradability of dry matter, crude fiber and organic matter were determined by subtracting the quantity of respective nutrients remained after incubation from the quantity of respective nutrients in the sample of feed.

3.2.3.3 Estimation of total volatile fatty acid concentration:

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

One ml of incubated rumen liquor was taken in Markham's distillation apparatus and one ml of Scarisbrick buffer (5% oxalic acid solution, 10% potassium oxalate solution mixed in the ratio of 1:1) was added. Hundred ml of the steam distillate was collected in a conical flask and titrated against standard 0.01N sodium hydroxide using phenolphthlein as indicator. The total volatile fatty acid concentration was calculated by using the formula:

$$TVFA \text{ mM}/100\text{ml} = \frac{\text{Volume of 0.01N NaOH used}}{A} \times 100$$

Where,

A = Volume of incubated rumen liquor taken

3.2.3.4 Estimation of total gas production:

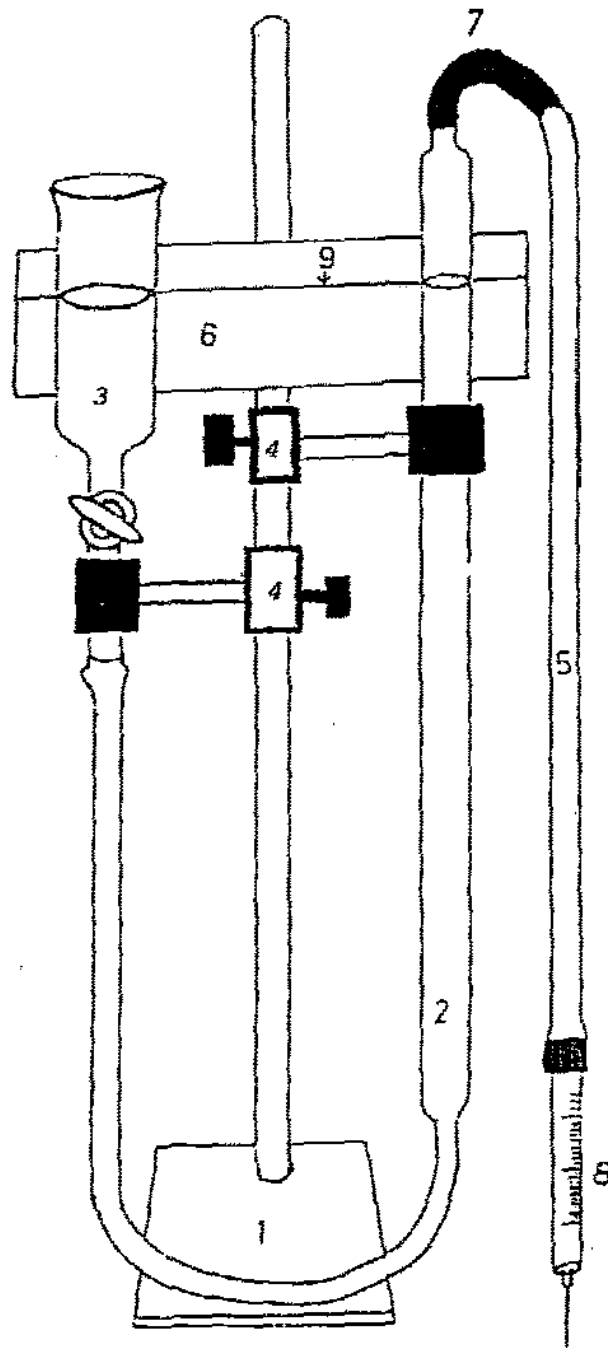
Total gas production was measured according to Prins (1987) using artificial saliva as per McDougalls (1948).

Rumen liquor was drawn from the different animals maintained on a diet of wheat straw:concentrate in the proportion of 60:40 (DM basis). One g of substrate (60:40 wheat straw:concentrate on DM basis) was taken in a 500 ml bottle with a standard joint, and 100 ml of McDougall's (1948) buffer solution was added to each of the above bottle. The bottles were fitted with a standard joint glass cone provided with a rubber cork at the top. These bottles were incubated in a shaker water-bath maintained at temperature so that buffer solution attained $39 \pm 1^{\circ}\text{C}$. To this, 100 ml of freshly drawn strained rumen liquor (SRL) was added and bottles were flushed with carbondioxide gas to create anaerobic environment, after removal of the cone which was immediately replaced after the addition of SRL. The gas production (ml) was measured at 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 hours post-incubation by puncturing the rubber stopper by a fine needle. This needle was attached to one of the two upper arms, a 'U' shaped manometer (Fig 3.1). Total gas production was indicated by level of water displacement.

3.2.3.5 Estimation of ammonia-nitrogen($\text{NH}_3\text{-N}$):

$\text{NH}_3\text{-N}$ was estimated according to Conway (1962).

FIG. 3.1 WATER MANOMETER FOR MEASURING GAS PRODUCTION
 IN in vitro RUMEN INCUBATIONS



1. STAND, 2. BURETTE, 3. FUNNEL, 4. CLAMPS 5. FLEXIBLE TUBING,
 6. GRAPH PAPER, 7. IRON WIRE, 8. PLASTIC SYRINGE WITHOUT
 PLUNGER AND WITH NEEDLE ATTACHED, 9. LINE FOR WATER LEVEL READING.

Two ml of 2% boric acid solution mixed with composite indicator was put in the inner chamber of dish. One ml of each of SRL and saturated sodium carbonate was put in opposite direction in outer chamber of dish. Dish was covered with its lid and the contents of outer chamber was mixed by gently tilting. Care was taken so that the contents of both the chamber were not mixed. Dish was incubated at 37°C for one hour. The released ammonia was absorbed in boric acid. The contents of the inner chamber were titrated against 0.01N H₂SO₄. A blank sample was also kept simultaneously. Ammonia-N concentration was calculated as per following formula:

$$\text{Ammonia-N (mg/100ml SRL)} = \frac{\text{Volume of 0.01N H}_2\text{SO}_4 \text{ used} \times 0.14 \times 100}{\text{Volume of SRL}}$$

3.2.3.6 Estimation of microbial protein:

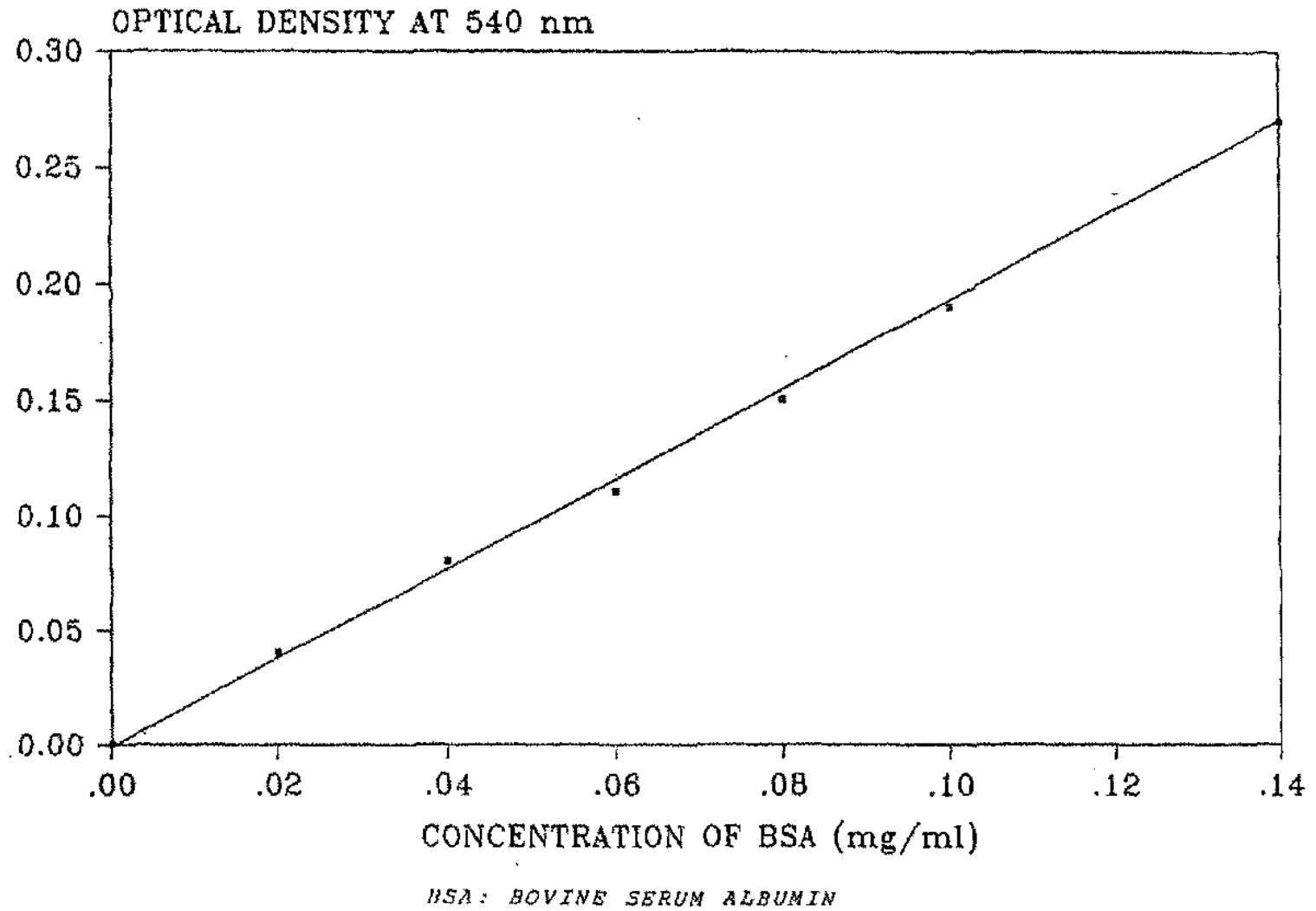
Microbial protein was precipitated by tungstic acid (Shultz and Shultz, 1970) and dissolved in 0.1N NaOH to make up volume 100 ml. The concentration of microbial protein was estimated according to Lowery *et al.* (1951) by preparing standard curve (Fig 3.2).

3.2.3.7 Counting of bacterial population:

Ruminal bacteria population was counted according to Gall *et al.* (1949) using nigrosin stain.

The rumen liquor samples were diluted in distilled water so that there was about 10 to 40 microorganisms per field. This dilution usually was 1:1000. A 1:10

Fig 3.2 : STANDARAD CURVE OF BSA
CONCENTRATION (IN VITRO)



or 1:100 dilution was made first and shaken vigorously for 3 minutes. Further dilutions were made from this and shaken by hand vigorously.

After shaking, 0.01 ml of the properly diluted sample was transferred by special Breed's pipette to the centre of the 1 square cm round area of the Breed's slide. The diluted sample was mixed with a 3 mm loopful of saturated methyl alcohol of water solution nigrosin and spread and the material in a thin film over the 1 square cm area of Breed's slide. This slide was dried quickly on a very hot electric plate.

Usually it was best to end by rocking the slide from side to side to effect a very even colour and to allow slight evaporation, so that there was not an excess of fluid when the slide is placed on the hot plate. The plate was very hot and the slide touched the plate at all points. Drying took 2 to 3 seconds at most and the slide was then removed from the hot plate.

Excess fluid often causes uneven drying. Too much or too concentrated dye causes cracking, while too slow drying causes a large area of shrinkage. An uneven plate causes ridges of dye and any foreign particle causes an unstained area which tends to be round.

Before counting, a survey of the slide was made to see that no part of the slide is unrepresentative. 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. There was a narrow zone of shrinkage near the border. This had a slightly lower count than the rest of the slide, but the inclusion of 1 field from this area in 10 fields helped for correct representation. Chains or pairs were counted as one. Any field having much debris or a large light area was discarded. The total microbial count was calculated by multiplying the average of fields with the factor obtained by calibrating the microscope (microscopic factor) with a certain set of oculars.

Calculation of microscopic factor:

(i) Diameter of the microscopic field was measured with a stage micrometer using 1.8 mm objective and 10X eyepiece.

(ii) Area of the field was calculated by the formula πr^2 (Where, 'r' is the radius of the field).

(iii) Microscopic factor is the number of microscopic fields per square centimeter (The area of the smear = $1/r^2$).

Number of bacteria/ml = $\frac{\text{Dilution factor} \times \text{Microscopic factor} \times \text{average number per field} \times 100}{1}$

Precaution:

The first essential to this technique was an absolutely clean slide. New slides were soaked in ethyl alcohol for at least half an hour, removed with forceps and dried with quick long motions with an absolutely clean "non-lintly" towel.

3.2.4 Statistical analysis

Statistical analysis of the data was carried out as per Snedecor and Cochran (1967).

PHASE-II

3.3 IN VIVO EVALUATION OF PROBIOTIC

Two microbes selected on the basis of in vitro studies were evaluated through in vivo studies for the selection of best treatment. The microorganisms were supplemented individually and in combination with control. The following parameters were considered to select the best treatment out of three:

- (i) In vivo DM and CP degradability
- (ii) Level of ammonia-N
- (iii) Total volatile fatty acids
- (iv) Proportion of volatile fatty acid
- (v) Total microbial protein
- (vi) Lactic acid concentration
- (vii) Bacterial count
- (viii) Rumen volume and flow rate of liquid digesta

3.3.1 Selection, Feeding and management of experimental animals

Twelve crossbred adult male animals of about 1½ to 2½ years of age were selected for in vivo studies.

The animals were operated upon to fix permanent fistulae in the rumen.

The particulars of the experimental animals and their distribution in different groups are given in Table 3.2. Thus twelve rumen fistulated animals were randomly divided into four groups of three animals each based on body weights. Animals in all treatment groups were fed ad lib (60:40 Green maize:concentrate, on DM basis).

Group T_4 was control with no supplementation of probiotic, however, the animals of group T_1 , group T_2 , and group T_3 were provided with L.acidophilus, S.cerevisiae and combination of both organisms, respectively. The limiting microbial cells were supplemented @ 10^9 cfu/kg of dry matter intake by the animal.

3.3.2 Dry matter and crude fibre degradability by Nylon bag technique

In vivo dry matter and crude fiber degradability were estimated as per Orskov and McDonald (1979).

3.3.2.1 Suspension and Removal of the Nylon bags:

The ground samples (5-6g) of 60:40 wheat straw:concentrate (on DM basis, Table 3.1) were put in each of the nylon bag. These were tied properly with nylon thread to an iron chain piece of 20 to 25 cm length and suspended in the rumen of fistulated animals. Each sample was placed in eighteen nylon bags and tied with three iron chains each having 6 bags tied to it which were then suspended in the rumen of three animals to serve as replicates.

Table 3.2 Distribution of rumen fistulated adult animals into treatment groups

Animal No.	Individual body weight (kg)	Date of birth
GROUP-T ₄ - Without supplement (CONTROL)		
KS 3891	318.0	1.05.88
KF 4942	305.0	4.05.88
KS 3880	327.0	26.03.88
Mean±S.E.	316.67±6.39	
GROUP-T ₇ - Supplemented with <u>L.acidophilus(I)</u>		
KS 3893	396.0	27.05.88
KS 3884	302.0	8.04.88
KS 3889	290.0	22.04.88
Mean±S.E.	296.00±3.46	
GROUP-T ₂ - Supplemented with <u>S.cerevisiae (B)</u>		
KS 3923	304.0	8.10.88
KF 4788	292.0	2.02.88
KF 4943	309.0	6.05.88
Mean±S.E.	301.67±5.04	
GROUP-T ₃ - Supplemented with combination of <u>L.acidophilus(I)</u> and <u>S.cerevisiae(B)</u>		
KF 5004	288.0	27.12.88
KS 3879	298.0	24.03.88
KF 4944	306.0	11.05.88
Mean±S.E.	297.33±5.21	

Nylon bags were taken out at 6, 12, 24 and 48 hours. The bags were washed thoroughly under running tap water until clear water was observed to be coming out of bags. These bags were dried to constant weight in the oven at 70°C. The bags were weighed after drying to determine DM retention at various hours. The contents of the bags were also analysed for crude fiber as per AOAC (1984). The percent disappearance of DM and CF from nylon bags at various hours was calculated which was indicative of the rate of degradation. From the DM and CF disappearance data, effective degradability in the rumen was calculated at 0.05%/h outflow rate using following equation (Orskov and McDonald, 1979):

$$P = a + b(1 - e^{-Ct})$$

Where,

- P = Degradability after 't' hours of incubation,
- a = Instantly soluble fraction,
- b = Potentially degradable fraction,
- C = Fraction rate constant at which the fraction 'b' would be degraded per hour, and
- t = time (hours)

Using DM and CF disappearance data at different hours, the values of 'a', 'b' and 'C' were calculated arithmetically using equation $P = a + b(1 - e^{-Ct})$. Effective degradability was calculated at 0.05%/h outflow rate

using following equation:

$$P = a + \frac{bc}{C + K}$$

Where, 'K' is the outflow rate

3.3.3 Estimation of ammonia-Nitrogen(NH₃-N), Total volatile fatty acids (TVFA) and their proportions, microbial protein, lactic acid and bacterial counts in the rumen

3.3.3.1 Sampling of rumen liquor:

Rumen liquor was drawn from all animals after giving adaptability period of 10 days. It was considered that optimum rumen environments will be attained within this period. To minimise the load and error, each day samples were drawn only from 4 animals (one from each group) at 0, 2, 4, 6, 8, 10 and 12 h post feeding. On each animal the samples were drawn for three days.

3.3.3.2 Estimation of ammonia-Nitrogen(NH₃-N):

As under section 3.1.3.5

3.3.3.3 Estimation of Total volatile fatty acids (TVFA):

As under section 3.1.3.3.

3.3.3.4 Estimation of proportion of volatile fatty acids(VFAs):

The TVFA were fractioned on 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. The pooled samples drawn at different hours were fractionated in triplicate and were prepared in accordance with the method of Ervin et al. (1961). For this 5 ml sample alongwith 1 ml (20 percent) metaphosphoric acid (prepared in 5N H_2SO_4) was kept overnight and centrifuged at 3500 to 4000 rpm for 15 to 20 minutes.

The supernatant was collected in small vials and kept in the refrigerator (at 4°C) for analysis. A fixed volume of supernatant was injected in Nucon gas chromatograph series 5500. The calculation of individual VFA was done as follows:

$$\text{Area of the peak} = \frac{1}{2} \text{ height} \times \text{width}$$

3.3.3.5 Estimation of microbial protein:

As under Section 3.3.6 (Standard curve Fig 3.3).

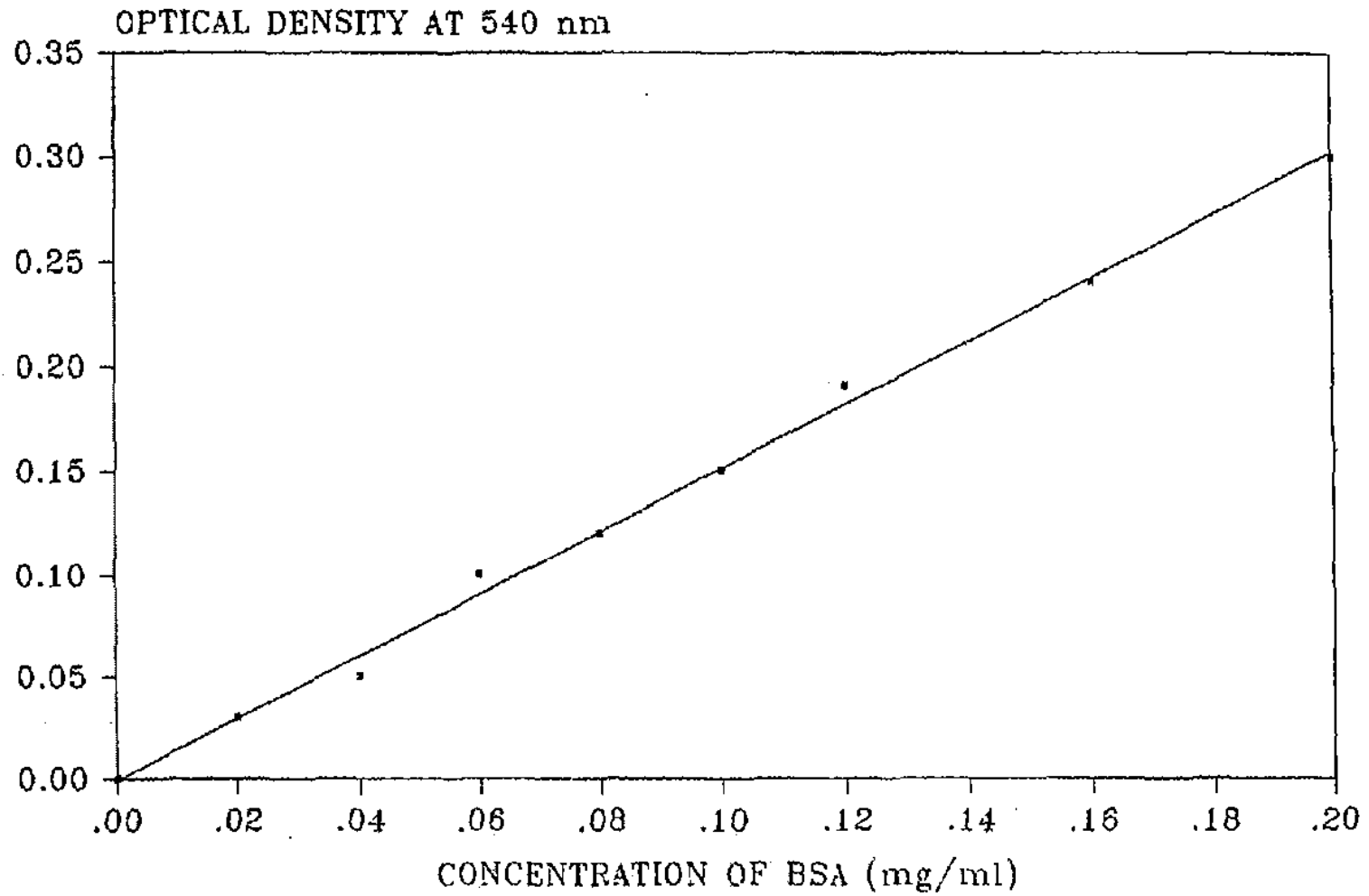
3.3.3.6 Estimation of lactic acid concentration:

The estimation of lactic acid was done as per the method of Barker and Summerson (1941).

Reagents:

- I. 10% sodium tungstate solution: 100g of sodium tungstate (reagent grade and carbonate free) dissolved in water and diluted to one litre. Solution was stable indefinitely.
- II. 0.66N H_2SO_4 solution: 19.5 ml concentrated H_2SO_4 was added in water and diluted to one litre and

Fig 3.3 : STANDARAD CURVE OF BSA
CONCENTRATION (IN VIVO)



BSA: BOVINE SERUM ALBUMIN

standardised it with alkali.

- III. 20% CuSO_4 solution: 200g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was added in about 500 ml water, heated to dissolve, cooled and diluted to one litre (stable indefinitely).
- IV. 4% CuSO_4 solution: 200 ml of reagent I. Diluted to 1 litre with water and mixed.
- V. Calcium hydroxide: $[\text{Ca}(\text{OH})_2]$ power (A.R. grade).
- VI. Concentrated H_2SO_4 (A.R. grade)
- VII. p-hydroxydiphenyl reagent: 1.5g p-hydroxydiphenyl added in 10 ml of 5% NaOH solution and about 10 ml of water. It was warmed with constant stirring to dissolve. It was diluted to 100 ml with water and stored in a brown bottle.
- VIII. Standard lactic acid solution: 0.213g of dry lithium lactate (A.R. grade) was dissolved in about 100 ml of water in a volumetric flask (1 litre). About 1 ml of concentrate H_2SO_4 was added to it and diluted to the mark with water. This solution contained 1 mg of lactic acid in 5 ml and was stable for an indefinite period, if kept in refrigerator.
- IX. Working standard lactic acid solution: Diluted the 5 ml of stock standard to 100 ml with water and mixed. This solution contained 0.01mg of

lactic acid per millilitre. Prepared the fresh standard solution at the time of analysis.

Procedure:

I. Preparation of protein free filtrate: 1 ml of strained rumen liquor (SRL) was taken in 10 ml graduated centrifuge tube in duplicate. To it 7 ml of water, 1 ml of 10% sodium tungstate and 1 ml of 0.66N H_2SO_4 was added. Shaked thoroughly and allowed to stand for 15 minutes. Centrifuged the contents at 3000 rpm for 15 minutes.

II. 2ml of protein free filtrate was taken in 10 ml graduated centrifuge tube.

III. 5 ml of standard lactic acid solution (No.IX) was taken in another centrifuge tube.

IV. In yet another tube a little water (blank) was taken.

V. 1ml of 20% $CuSO_4$ solution was added to each tube and made the volume to 10 ml. 1g of $Ca(OH)_2$ power was added to each tube, put stopper and was shaken vigorously until the solids were uniformly disappeared.

VI. Allowed to stand for 90 minutes with repeated shaking (at least once) in between.

VII. The contents were centrifuged at 3000 rpm for 10 minutes and transferred 1 ml of supernatant from each tube (in duplicate) to clean and dry wide test tubes.

VIII. 0.05ml of 4% CuSO_4 solution was added to each tube, followed by 6ml of concentrate H_2SO_4 (through burette) drop by drop with continuous mixing of the contents. (The tube contents became hot; it was not necessary to cool the tubes).

IX. Placed the tubes upright in boiling water for five minutes, then transferred the tubes to cold water (preferably running and cooled to 20°C or below).

X. After cooling the contents, 0.1 ml of p-hydroxydiphenyl reagent was added drop by drop to each tube (lower tip of pipette should not touch the wall of the tube). Mixed the reagent throughout the solution as quickly and uniformly as possible by using test tube shaker.

XI. Placed the tubes in a waterbath at 30°C for 30 minutes or longer. Shaked the tubes during this period (at least once).

XII. The tubes were kept in vigorously boiling water for exactly 90 seconds, removed and cooled in cold water to room temperature.

XIII. Recorded the optical density at 560 nm by setting the photometer at zero with blank.

Calculations:

$$\begin{array}{l} \text{Lactic acid} \\ \text{(g/100ml of} \\ \text{rumen liquor)} \end{array} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard sample}} \times 0.005 \times 50 \times 100 \times 10 \times \frac{1}{1000}$$

3.3.3.7 Counting of bacterial population:

As under section 3.3.7

3.3.3.8 Rumen volume and flow rate of liquid digesta

Rumen volume and flow rate of liquid digesta were measured using Polyethylene glycol (PEG) according to method described by Smith (1959).

(i) Collection of rumen liquor: During rumen volume and flow rate estimation, a sample of rumen liquor was collected prior to feeding and taken as zero hour collection. Then 100 ml of 25% PEG solution was infused into the rumen. For better mixing of PEG, the rumen contents were agitated manually by inserting an arm in the rumen and one hour was allowed as the mixing time of PEG in the rumen (Smith, 1959). The samples of rumen liquor were collected through especially made stainless steel probes having large number of small holes drilled in them and covered with fine nylon cloth. These probes were placed at four different sites in the rumen so as to get a representative sample of rumen liquor. About 100 ml of rumen liquor was collected at 0, 1, 2, 3, 4, 6 and 8 hours interval in plastic bottles containing 0.2 ml of 10N H_2SO_4 to stop microbial activity.

The samples of rumen liquor were analysed for PEG concentration by the method of Smith (1959).

(ii) Reagents:

(a) ZnSO_4 solution (5% w/v)

(b) 0.3N Ba(OH)_2 solution (4.7325g of Ba(OH)_2 was dissolved in 70 ml of hot distilled water and the volume was made to 100 ml).

(c) BaCl_2 solution (10% w/v)

(d) Trichloroacetic acid(TCA) - BaCl_2 solution:

TCA (75.0g) and BaCl_2 (14.75g) were dissolved separately into two beakers containing 100 ml distilled water each. The solutions were transferred to 250 ml volumetric flask and the volume was made upto the mark. This gave the concentration 30% and 5.9% w/v for TCA and BaCl_2 respectively. The mixture was kept overnight and filtered before being used.

(iii) Standard curve: Seven test tubes marked B(Blank), 1, 2, 3, 4, 5 and 6 were taken. One ml of SRL collected at zero hour was taken in all test tubes except blank.

To it 0, 1, 2, 3, 4, 5 and 6 ml of PEG solution (1g/100 ml of distilled water) was added. In each test tube 2 ml of 0.3N Ba(OH)_2 and 5% ZnSO_4 solution was added. To each test tube 0.5 ml of 10% BaCl_2 solution was added and the volume made to 10 ml in all test tubes.

The contents of the above test tubes were well mixed and centrifuged at 3000 rpm for 30 minutes. From the supernatant, 2 ml of filtrate was taken and 5 ml of TCA- BaCl_2 solution and 3 ml of distilled water was added

to make the volume to 10 ml. The tubes were left exactly for 30 minutes for the development of turbidity and O.D. readings were taken in digital spectrophotometer at 540 nm wave length. A standard curve was drawn for different concentrations of PEG against OD reading (Fig 3.4).

(iv) Calculation of PEG concentration in samples of rumen liquor:

Following the procedure of standard curve preparation, the concentration of PEG in the rumen liquor samples collected at different intervals was calculated from the O.D. obtained by taking one ml rumen liquor instead of standard solution. The value of PEG concentrations against time interval of collection, i.e., 1, 2, 3, 4, 6 and 8 hours were plotted on a graph paper and PEG concentration at zero hour was extrapolated.

(v) Rumen volume determination: Rumen volume was calculated at the time of infusion of PEG into the rumen by making use of the following equation:

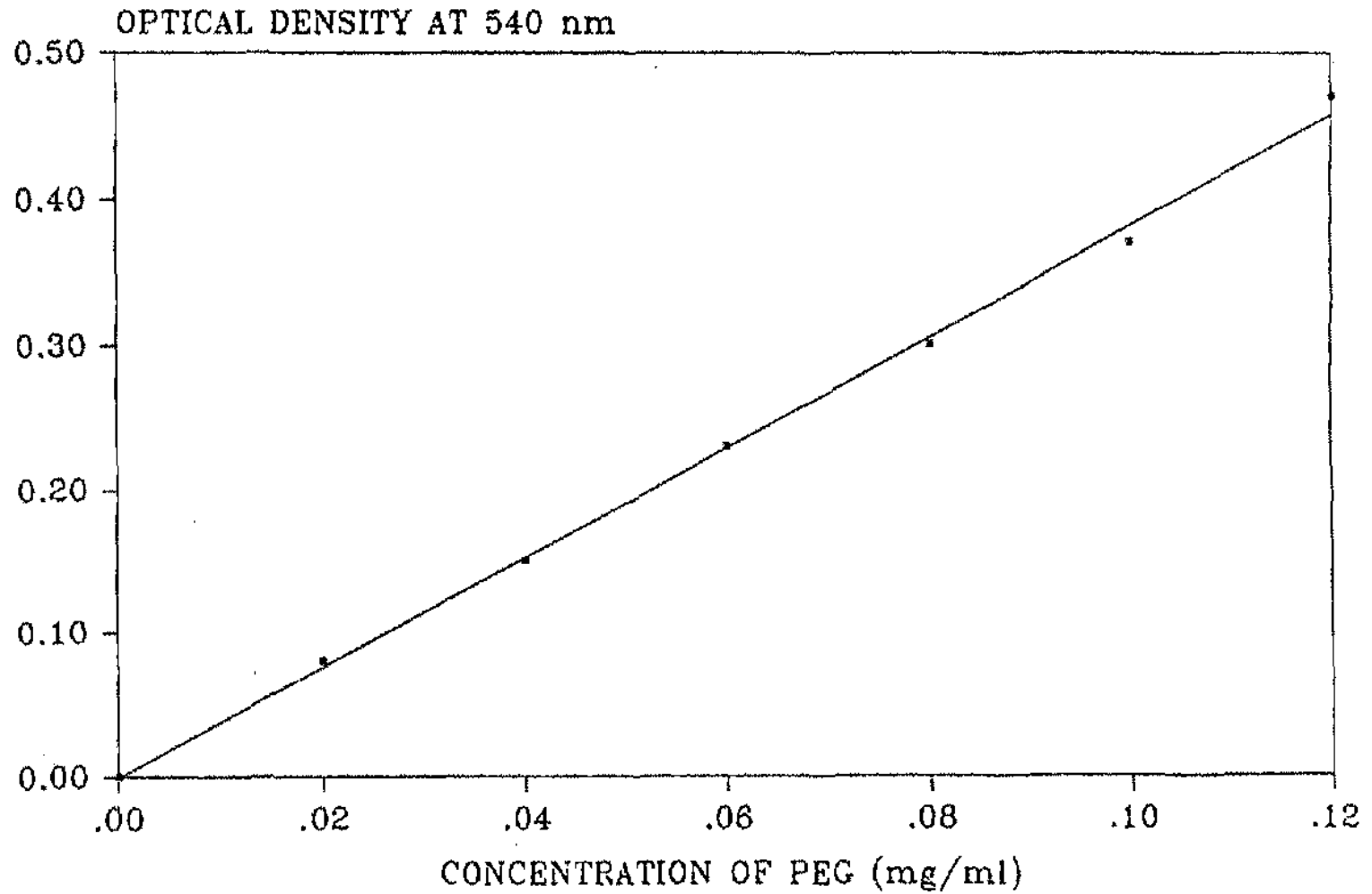
$$\text{Rumen volume (ml)} = b/A_0$$

Where,

'b' is the quantity of PEG infused into the rumen.

'A₀' is the zero hour PEG concentration (mg/ml of SRL).

Fig 3.4 : STANDARAD CURVE OF PEG
CONCENTRATION



PEG : POLYETHYLENEGLYCOL

(vi) Rumen fluid flow rate determination: The ruminal fluid flow rate (litres/day) was calculated from the values of ruminal volume, and exponential decline in PEG concentration by using the following equation:

$$\text{Flow rate(litres/day)} = \text{Rumen volume} \times b \times 1440$$

Where,

b is the exponential decline in the concentration of PEG.

3.3.4 Statistical analysis

Statistical analysis of the data was carried out as per Snedecor and Cochran (1967).

PHASE-III

3.4 RESPONSE OF PROBIOTIC SUPPLEMENT ON THE GROWTH OF PRE-RUMINANT AND POST-RUMINANT CALVES

Selected probiotic on the performance of in vivo studies was supplemented in the ration of pre-ruminant and post-ruminant calves @ 10^9 cfu/kg of DM intake. The growth and incidence of diarrhoea was observed.

3.4.1 Selection of pre-ruminant animals

Twenty healthy crossbred (Holstein Friesian x Tharparkar) calves of both sex at the age of 6 days were selected from the calf section of Cattle Yard, NDRI, Karnal.

3.4.2 Selection of post-ruminant animals

Twenty healthy crossbred (Holstein Friesian x Tharparkar) female calves at the age of 4 to 6 months were selected from calf section of Cattle Yard, NDRI, Karnal.

3.4.3 Allotment of animals into groups

Both types of animals were allotted randomly into two groups of ten animals each in a randomised block design (two groups for pre-ruminant and two groups for post-ruminant animals). While selecting the animals due care was taken to minimise the experimental error by narrowing the range of their age and live weights as far as possible. In pre-ruminant animals care was taken to equalise the sex in both groups. The particulars of both types of animals and their distribution to different groups are given in Tables 3.3 and 3.4.

3.4.4 Housing and management

Both type of animals were kept in loose housing system followed at Cattle Yard, NDRI, Karnal. However, post-ruminant animals were also individually fed for two months after the metabolism trial.

3.4.5 Watering

For both types of animals all the times clean and fresh drinking water was available ad lib. When the animals were individually fed, fresh clean tap water was

Table 3.3 Distribution of pre-ruminant crossbred (Holstein-Friesian x Tharparkar) calves for growth study

Animal No.	Sex	Date of birth	Initial body weight(kg)
GROUP-I			
(Supplemented with <u>S.cervisiae(B)</u> + <u>L.acidophilus(I)</u>)			
KF 5343	Male	6.11.91	33.0
KF 5344	Male	10.11.91	36.0
KF 5345	Male	15.11.91	30.0
KF 5341	Female	15.11.91	22.0
KF 5346	Male	22.11.91	29.0
KF 5347	Male	27.11.91	30.0
KF 5345	Female	1.12.91	34.0
KF 5347	Female	15.12.91	37.0
KF 5349	Female	21.12.91	25.0
KF 5350	Female	21.12.91	35.0
Mean ± S.E.			31.1±1.53
GROUP-II			
Without supplement (CONTROL)			
KF 5342	Female	17.11.91	29.0
KF 5343	Female	18.11.91	22.0
KF 5344	Female	22.11.91	28.0
KF 5348	Male	30.11.91	27.0
KF 5346	Female	12.12.91	30.0
KF 5349	Male	13.12.91	44.0
KF 5348	Female	19.12.91	29.0
KF 5352	Male	23.12.91	33.0
KF 5353	Male	24.12.91	28.0
KF 5354	Male	24.12.91	32.0
Mean ± S.E.			30.2±1.80

Table 3.4 Distribution of post-ruminant crossbred (Holstein-Friesian x Tharparkar) calves (female) for growth and metabolism trial

 Animal No. Date of birth Initial body weight (kg)

GROUP - I
 (Supplemented with S. cerevisiae(B)+L. acidophilus(I))

KF 5306	26.06.91	90.0
KF 5307	7.07.91	91.0
KF 5310	17.07.91	97.0
KF 5311	19.07.91	90.0
KF 5312	23.07.91	79.0
KF 5314	1.08.91	78.0
KF 5318	7.08.91	84.0
KF 5319	9.08.91	78.0
KF 5320	12.08.91	81.0
KF 5327	28.08.91	63.0

Mean ± S.E.

83.1 ± 3.03

GROUP - II
 (CONTROL)

KF 5309	13.07.91	86.0
KF 5315	3.08.91	75.0
KF 5316	4.08.91	80.0
KF 5317	6.08.91	91.0
KF 5322	20.08.91	81.0
KF 5323	25.08.91	82.0
KF 5325	27.08.91	81.0
KF 5328	3.09.91	83.0
KF 5329	12.09.91	87.0
KF 5330	12.09.91	83.0

Mean ± S.E.

82.9 ± 1.37

given ad lib to all the animals two times a day, i.e., 10 A.M. and 4 P.M.

3.4.6 Feeding of animals

3.4.6.1 Feeding of Pre-ruminant animals:

The calves were fed whole milk/separated milk/concentrate mixture as per schedule given in Table 3.5 and were offered green fodder ad lib. All the animals were having free access to water.

In the treatment group probiotic supplement was well mixed in the whole milk of ten animals. The feeding schedule was same as followed at Cattle Yard, NDRI, Karnal.

3.4.6.2 Feeding of post-ruminant animals:

During group feeding, each day quantity of probiotic needed for the animals was added in small amount of concentrate (approx. 1 kg). This amount was well mixed in whole concentrate (Table 3.6) offered to treatment group. The nutrients requirement was met from green fodder (Berseem, maize, sorghum and oats), dry roughage (wheat straw) and concentrate (1.5kg/d/head) as per NRC (1984) standard. During individual feeding each day probiotic supplementation was made for individual animals in small amount of concentrate (approx. 250g). This concentrate was fed in the morning before offering the remaining concentrate and fodder.

Table 3.5 Feeding schedule of pre-ruminant calves

Body weight (kg)	Whole milk (1/15th of body weight) (kg)	Skim milk (1/25th of body weight) (kg)	Concen- trate (kg)	Green foóder (kg)
21-25	1.5	1.0	0.12	<u>ad lib</u>
26-30	2.0	1.5	0.12	<u>ad lib</u>
31-35	2.5	1.5	0.12	<u>ad lib</u>
36-40	2.5	1.5	0.12	<u>ad lib</u>
41-45	3.0	2.0	0.12	<u>ad lib</u>
46-50	3.5	2.0	0.12	<u>ad lib</u>
51-55	3.5	2.0	0.12	<u>ad lib</u>
56-60	4.0	2.5	0.12	<u>ad lib</u>
61-65	4.5	3.0	0.12	<u>ad lib</u>
66-70	4.5	3.0	0.12	<u>ad lib</u>
71-75	5.0	3.0	0.12	<u>ad lib</u>
76-80	5.0	3.0	0.12	<u>ad lib</u>
81-85	5.5	3.5	0.12	<u>ad lib</u>
86-90	6.0	3.5	0.12	<u>ad lib</u>

Table 3.6 Composition of concentrate offered to the crossbred calves at pre- and post-ruminant age'

<i>Ingredients</i>	<i>Parts (kg)</i>	<i>CP (kg)</i>	<i>TDN (kg)</i>
<i>Groundnut cake</i>	10	4.5	7.1
<i>Soybean cake</i>	10	4.5	7.0
<i>Mustard cake</i>	6	3.7	7.0
<i>Cottonseed cake</i>	5	3.7	7.0
<i>Maize</i>	20	1.0	8.4
<i>Barley</i>	10	1.0	8.0
<i>Wheat bran</i>	30	1.4	6.5
<i>Molasses</i>	6	0.4	5.5
<i>Mineral mixture</i>	2	0.0	0.0
<i>Common salt</i>	1	0.0	0.0
<i>Total</i>	100	20.2	56.5

3.4.7 Weighing of the animals

The animals were weighed at fortnight intervals on a weighing bridge and also before and after the metabolism trial. Each time an average of weighing on two consecutive days was considered. Weighing was always done in the morning before offering the feed or water. The growth rate of the animals was determined from the change in body weights.

3.4.8 Incidence of diarrhoea

Every day a strict and regular vigilance on pre-ruminant calves was kept to observe symptoms of diarrhoea. A loose and watery faeces was considered to be a diarrhoea condition. Incidence of diarrhoea was recorded in number of days in the diarrhoea condition of each calve.

3.4.9 Statistical analysis

Statistical analysis of the data was carried out as per Snedecor and Cochran (1967).

PHASE-IV

3.5 EFFECT OF PROBIOTICS ON FEED UTILIZATION

To determine the effect of probiotic supplementation on N-balance, digestibility and intake of nutrients a conventional metabolism trial was conducted on all the twenty post-ruminant animals, two months prior to end of growth experiment. Animals were given two days adaptability to the environments of metabolism shed with 5 days of collection period. The animals were weighed on two consecutive days before offering feed and water prior and at the end of metabolism trial.

3.5.1 Collection of feed, faeces and urine samples

Samples of feed offered and left over were collected each morning for DM estimation and chemical analysis.

The amounts of faeces and urine voided by the experimental animals during 24 hours were recorded each day in the morning. A composite sample of dung and urine of each animal was carried in separate bottle to the laboratory every day for aliquoting.

3.5.2 Aliquoting of dung and urine

For estimation of nitrogen, 1/200 aliquot of the total dung voided each day was preserved with 25 percent

H_2SO_4 in pre-weighed plastic bottles. At the end of collection period, the bottles were again weighed, the mixed thoroughly and 2 g sample was taken for digestion. For dry matter, proximate and Van Soest principles, the dung samples were dried daily at $100^\circ C$ in the oven and pooled for 5 days for each animal. At the end of collection period, the dried pooled dung samples were ground in a Willey mill for estimation of proximate principles and cell wall components.

Similarly for determining nitrogen in urine 1/300 part of total urine of individual animals was taken daily and pooled for five days in glass bottles containing 30 ml of 25 percent H_2SO_4 . The nitrogen estimation was done by taking a known quantity of sample from each bottle separately and digesting it with concentrate H_2SO_4 and digestion mixture.

3.5.3 Analytical Procedures

The samples of metabolism trial were analysed for proximate principles according to AOAC (1984) and for fibrous constituents (NDF, ADF) according to Goering and Van Soest (1970) methods.

3.5.3.1 Estimation of Proximate Principles of feeds and dung samples:

Samples of feed and dung were analysed for proximate principles as described in AOAC (1984).

3.5.3.1.1 Moisture: A known quantity of the sample was taken in a moisture cup and dried overnight in oven at 95 to $100^\circ C$. Loss in weight after drying gave the moisture content.

3.5.3.1.2 Total Ash: 4 to 5 g of sample was taken in a China crucible and ignited in a Muffle furnace at $600^\circ C$ for an hour. The increase in weight of China crucible over empty weight was taken as total ash and expressed percent of the sample.

3.5.3.1.3 Nitrogen:-

Kjeldahl method of nitrogen estimation was followed. A weighed quantity of the sample was digested in the Kjeldahl flask with concentrate sulphuric acid in the presence of small quantity of digestion mixture (sodium sulphate and copper sulphate in the ratio of 10:1). After digestion, the contents were cooled and transferred to 100 ml volumetric flask and total volume made up with distilled water. A 5 ml of aliquot was transferred to a micro-Kjeldahl apparatus and sufficient amount of 40 percent NaOH solution added to make the contents alkaline. The distillate was collected in a conical flask containing 10 ml of 2 percent boric acid with mixed Toshiro's indicator (80 mg methyl red + 20 mg methylene blue in 100 ml ethanol and 10 ml of it added to 1 litre of 2 percent boric acid solution). The distillate was titrated against standard 0.01N H_2SO_4 . The crude protein content in the sample was calculated by multiplying the nitrogen content with 6.25.

$$\text{Crude protein content (\% DM basis)} = \frac{\text{Volume of } 0.1N H_2SO_4 \text{ used} \times 0.00014 \times 20}{\text{Dry matter weight in the sample taken}} \times 100 \times 6.25$$

3.5.3.1.4 Ether Extract:-

A known quantity of ground and dried sample was taken in a Whatman thimble and extracted for 10h with petroleum ether (40-60°C) in Soxhlet apparatus having a

pre-weighed round bottom flask. The flask containing the ether extract was cooled after removal of excess of ether and re-weighed. Difference in weight gave the amount of ether extract in the sample and the same was expressed on percent basis.

3.5.3.1.5 Crude fibre:-

Samples after ether extraction were transferred to a spoutless beaker (1000 ml). To this, 175 ml of distilled water and 25 ml of 10 percent H_2SO_4 were added (to make it 1.25 % solution) and the contents boiled on a hot plate for 30 minutes. The sample was filtered on double layer of muslin cloth and again transferred to 1000 ml spoutless beaker and treated similarly with 1.25 percent NaOH solution. The residue after acid and alkali treatments were transferred quantitatively to a China crucible, dried, weighed and ignited in a Muffle furnace at $600^{\circ}C$ for one hour. Loss in weight after ignition was calculated as crude fibre and expressed on percent basis.

3.5.3.1.6 Nitrogen free extract:-

The ash, crude protein, ether extract and crude fibre contents were added up and subtracted from 100 to give NFE content in the sample.

3.5.3.1.7 Organic matter:-

It was determined by subtracting the ash content from 100.

3.5.3.2 Estimation of cell wall components:

Samples of feeds and dung were analysed for cell wall components as per the method of Goering and VanSoest (1970).

3.5.3.2.1 Neutral Detergent Fibre:-

Preparation of various solutions used in the procedure was as followed:

Reagents:

1. Sodium lauryl sulphate	30.90g
2. Ethylene diamine tetra-acetic acid(EDTA)	18.61g
3. Sodium borate decahydrate	6.81g
4. Disodium hydrogen phosphate	4.56g
5. 2-Ethoxy ethanol	10.00ml

EDTA and sodium borate decahydrate were put together in a large beaker, some distilled water added and the contents heated until dissolved. Sodium hydrogen phosphate was taken in another beaker and dissolved in distilled water by heating. Both the solutions were put together in a volumetric flask. Sodium lauryl sulphate and ethoxy ethanol were also added in the flask and the final volume was made upto one litre with distilled water.

Procedure:

Approximately 1g sample was taken in a spoutless beaker. Hundred ml of neutral detergent solution, 2ml of decalin and 0.5g of sodium sulphite were added and the contents boiled with retort condensers on a hot plate for exactly one hour. After boiling, the samples were filtered through a pre-weighed scintered glass crucible G1 grade using a vacuum pump. The sample was washed with hot distilled water and acetone. The sample was dried overnight in an oven (100°C) and weighed. The NDF was calculated as follows:

$$\text{NDF (\%)} = \frac{B - A}{\text{Weight of sample on DM basis}} \times 100$$

Where,

A = Weight of empty crucible, and

B = Weight of crucible + cell-wall
components

3.5.3.2.2 Acid Detergent Fibre:-

Acid detergent solution was prepared by dissolving 20g Cetyl trimethyl ammonium bromide (CTAB) in one litre of 1N H₂SO₄ (27 ml of H₂SO₄ GR grade in one litre of solution with distilled water).

Procedure:

After the determination of NDF, the content of crucible was placed in the spoutless beaker. To this 100 ml of acid detergent solution, 2 ml decalin and 0.5g sodium sulphite were added and the contents boiled with retort condensers on a hot plate for one hour. After boiling, the contents were filtered through the same crucible, the crucible and beaker thoroughly rinsed with hot distilled water to ensure complete shifting of contents to the crucible. The contents were washed with hot water and acetone. The sample was dried overnight in an oven (100°C) and weighed. The ADF was calculated as follows:

$$\text{ADF}(\%) = \frac{(\text{Wt. of crucible+ADF}) - (\text{Wt. of empty crucible})}{\text{Weight of sample on DM basis}} \times 100$$

3.5.4 Statistical analysis

Statistical analysis of the data was carried out as per Snedecor and Cochran (1967).

CHAPTER - IV

PHASE - I

IN VITRO RUMEN FERMENTATION

INFLUENCED BY THE SUPPLEMENTATION

OF INDIVIDUAL MICROORGANISM

PHASE - I

4. IN VITRO RUMEN FERMENTATION INFLUENCED BY THE SUPPLEMENTATION OF INDIVIDUAL MICROORGANISM

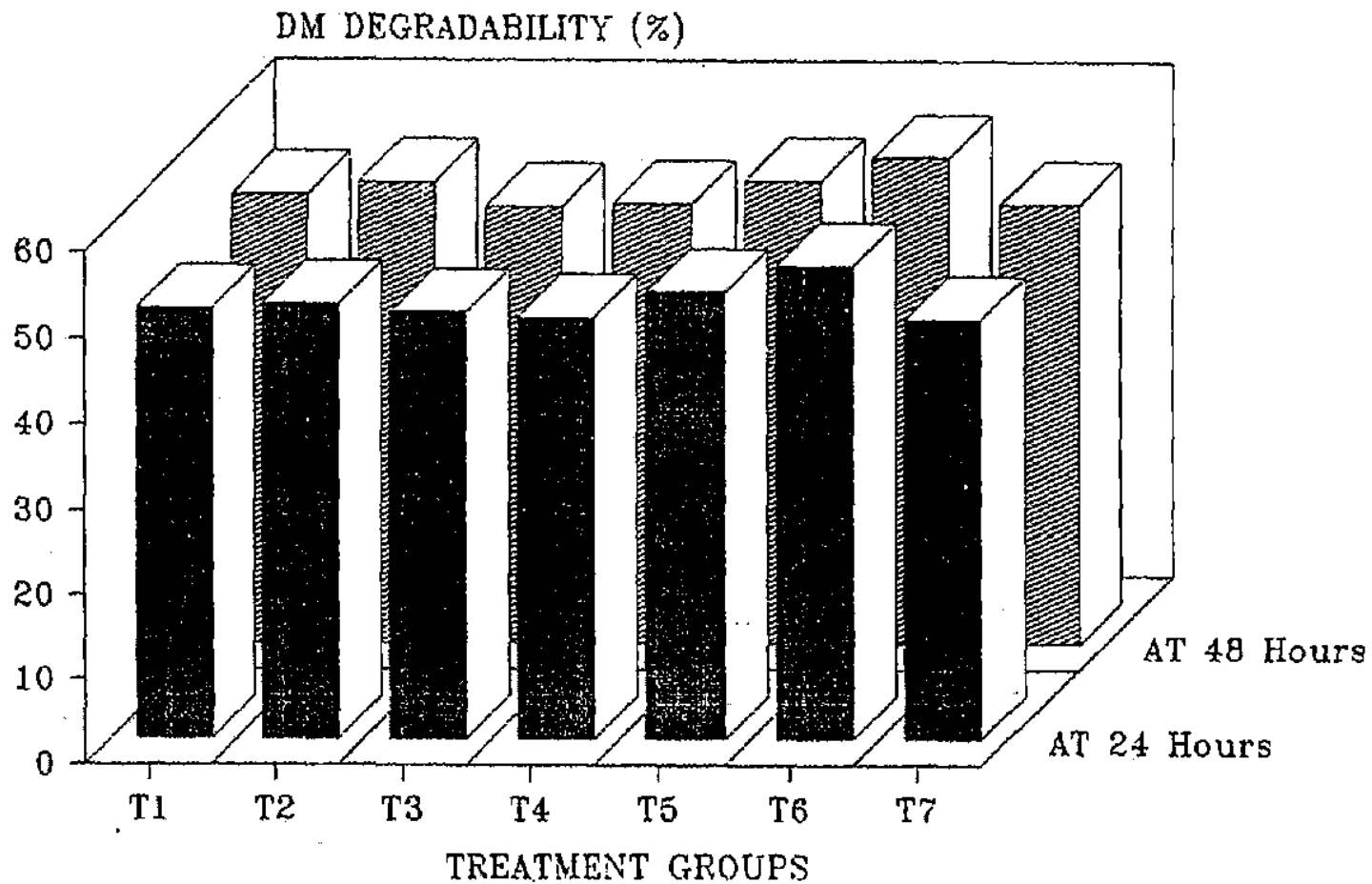
4.1 RESULTS

A mixed feed of wheat straw and concentrate (60:40 on DM basis, Table 3.1) was used as substrate for in vitro studies. The substrate was chemically analysed for proximate principles. The analytical values were 9.49%, 1.49%, 27.12%, 11.98% and 49.47% for CP, EE, CF, ash and NFE, respectively.

4.1.1. Effect of supplementation
on DM and OM degradability

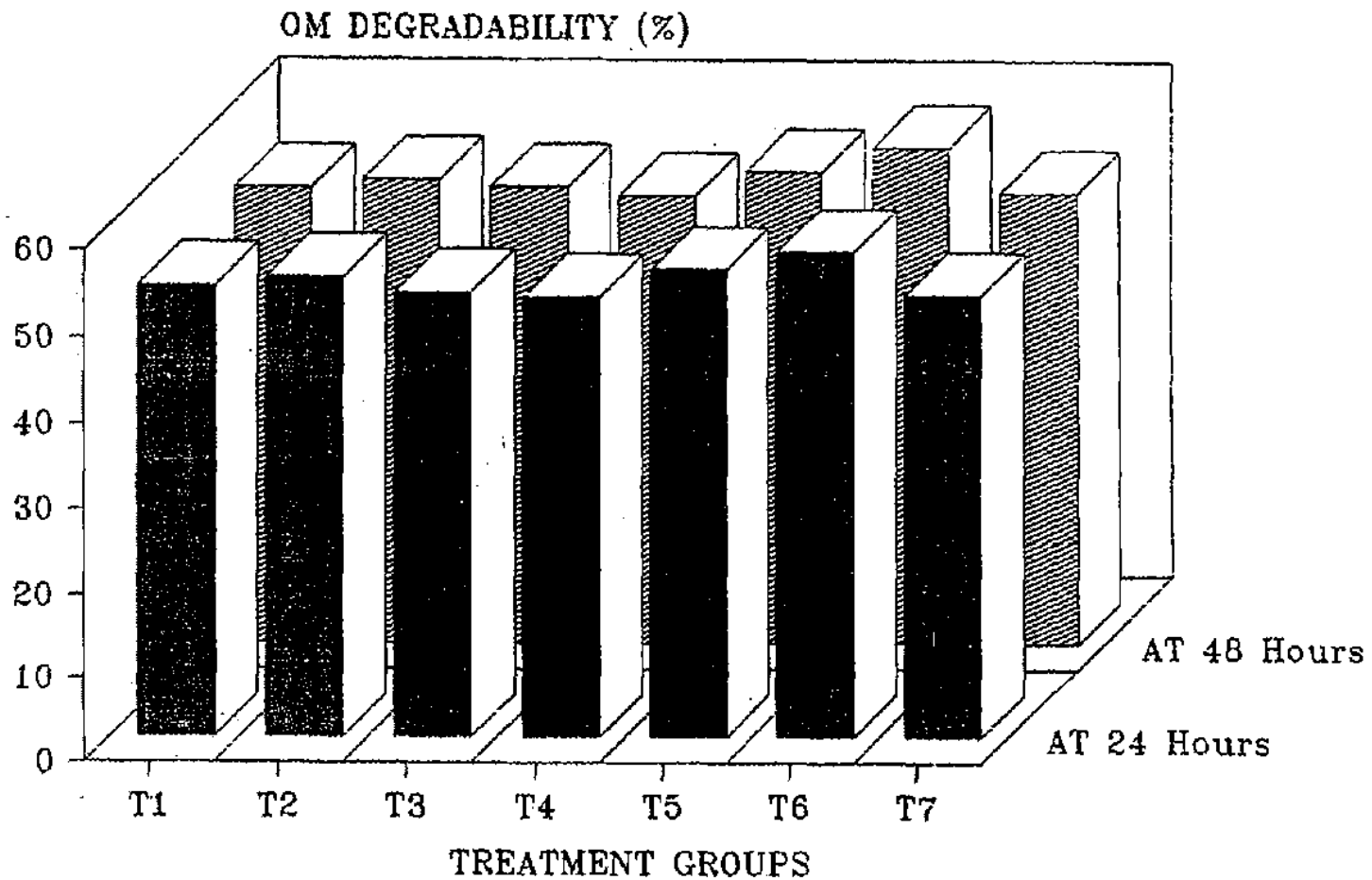
The substrate was analysed for DM (Fig. 4.1) and OM (Fig. 4.2) disappearance after incubation with rumen liquor and different microorganisms individually for 24 and 48 hours (Table 4.1). The DM degradability at 24 h incubation was 50.48, 51.02, 50.07, 49.47, 52.52, 55.57 and 49.30 per cent for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ treatment groups, respectively. DM degradability in group supplemented with S. cerevisiae (B) seems to be higher (55.57%) followed by the group supplemented with S. cerevisiae (522) (52.52%) when compared to other treatment groups including control (49.30%). However, in groups supplemented with strains of S. thermophilus (50.07 and 49.47%) and L. acidophilus (50.48 and 51.02%), the DM degradability was comparable within the strains of individual organism. Whereas supplementation of L. acidophilus (I) (51.02%) seems to have comparatively higher DM degradability. DM degradability (48h) ranged from 51.08 to 56.86 percent (Table 4.1). It was higher in the group supplemented with S. cerevisiae (B) (56.86%) followed by S. cerevisiae (522) (54.19%) than other groups including control (51.40%). It also appears (Table 4.1) that groups supplemented with strains of S. thermophilus exhibited similar DM degradability (51.08%) and (51.40%). However, the DM degradability (48h) in the groups supplemented with strains of L. acidophilus (52.34% and

Fig 4.1 : IN VITRO DM DEGRADABILITY OF
 FEED SUBSTRATE (WHEAT STRAW : CONC.
 60:40)



T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
 T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
 T₇, Control.

Fig 4.2 : IN VITRO OM DEGRADABILITY OF
FEED SUBSTRATE (WHEAT STRAW : CONC.
60:40)



T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
T₇, Control.

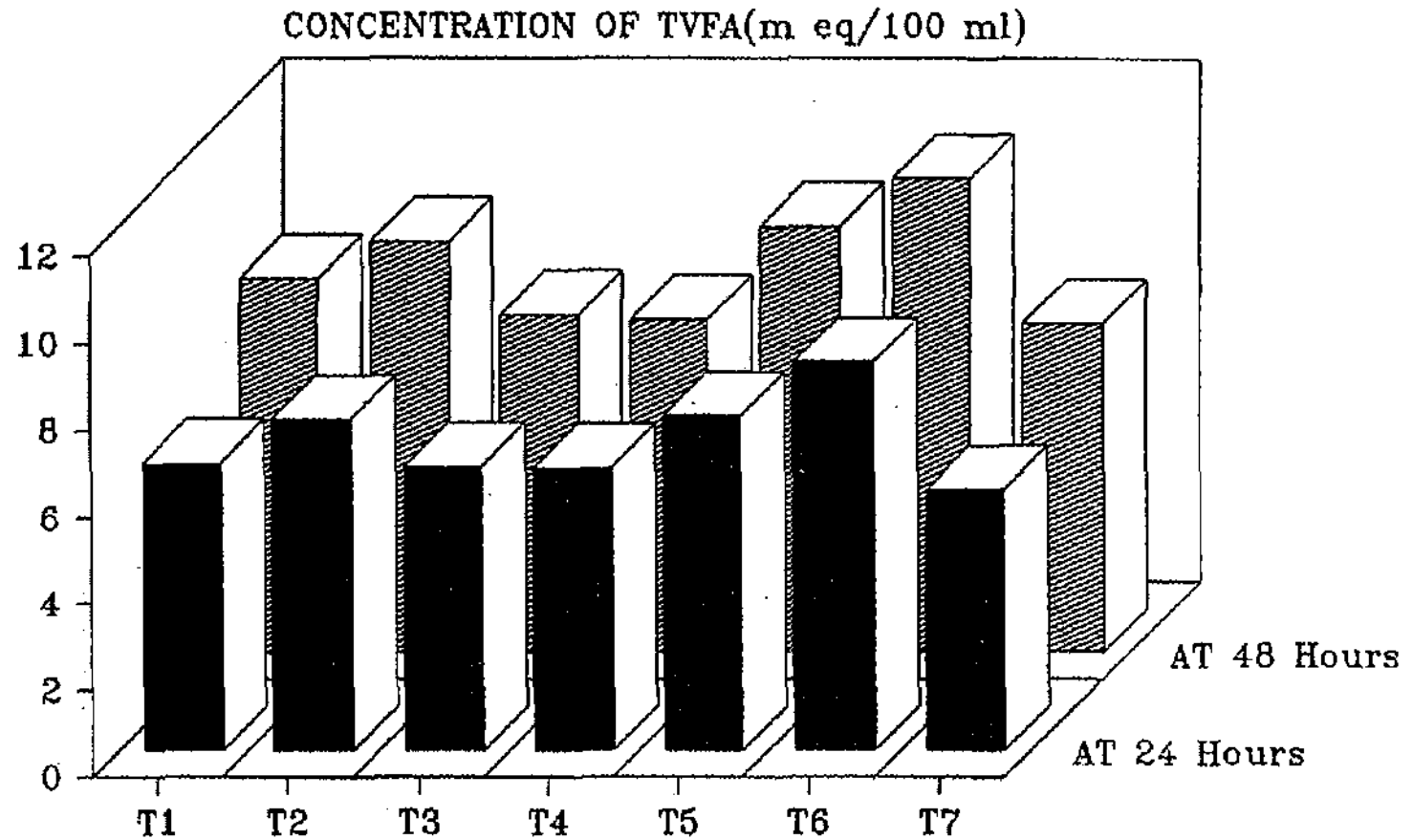
53.81% was not only different between the strains but also when compared to control group.

OM degradability (24h) ranged from the lowest of 51.69 percent in S.thermophilus (CH) to the highest of 57.08 percent in S.cerevisiae (B) supplemented group (Table 4.1) followed by S.cerevisiae (522). Organic matter degradability in the lactic acid bacteria supplemented groups as well as S.cerevisiae groups was different between the strains of each group. Same pattern was also observed at 48 hours of incubation. The OM degradability data (48h) were 53.16, 54.12, 53.32, 52.32, 55.17, 58.03 and 52.83 percent for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ treatment groups, respectively.

4.1.2 Effect on in vitro Total Volatile fatty acid (TVFA) Con-centration

Data on TVFA levels on different treatment groups and hours of incubation are presented in Table 4.2 (Fig. 4.3). TVFA concentration (24h) varied from the lowest in control group (6.03 meq/100 ml) to the highest in S.cerevisiae (B) supplemented group (8.96 meq/100 ml). The concentration of TVFA in the groups supplemented with strains of S.thermophilus (6.56 and 6.53 meq/100 ml) is similar but that of L.acidophilus (6.64 and 7.65 meq/100 ml) is different, however, these values are higher compared to control (6.03 meq/100 ml). TVFA concentration (48h) varied from the lowest in control group (7.59 meq/100 ml)

Fig 4.3 : IN VITRO TVFA CONCENTRATION ON
FEED SUBSTRATE (WHEAT STRAW : CONC.
60:40)



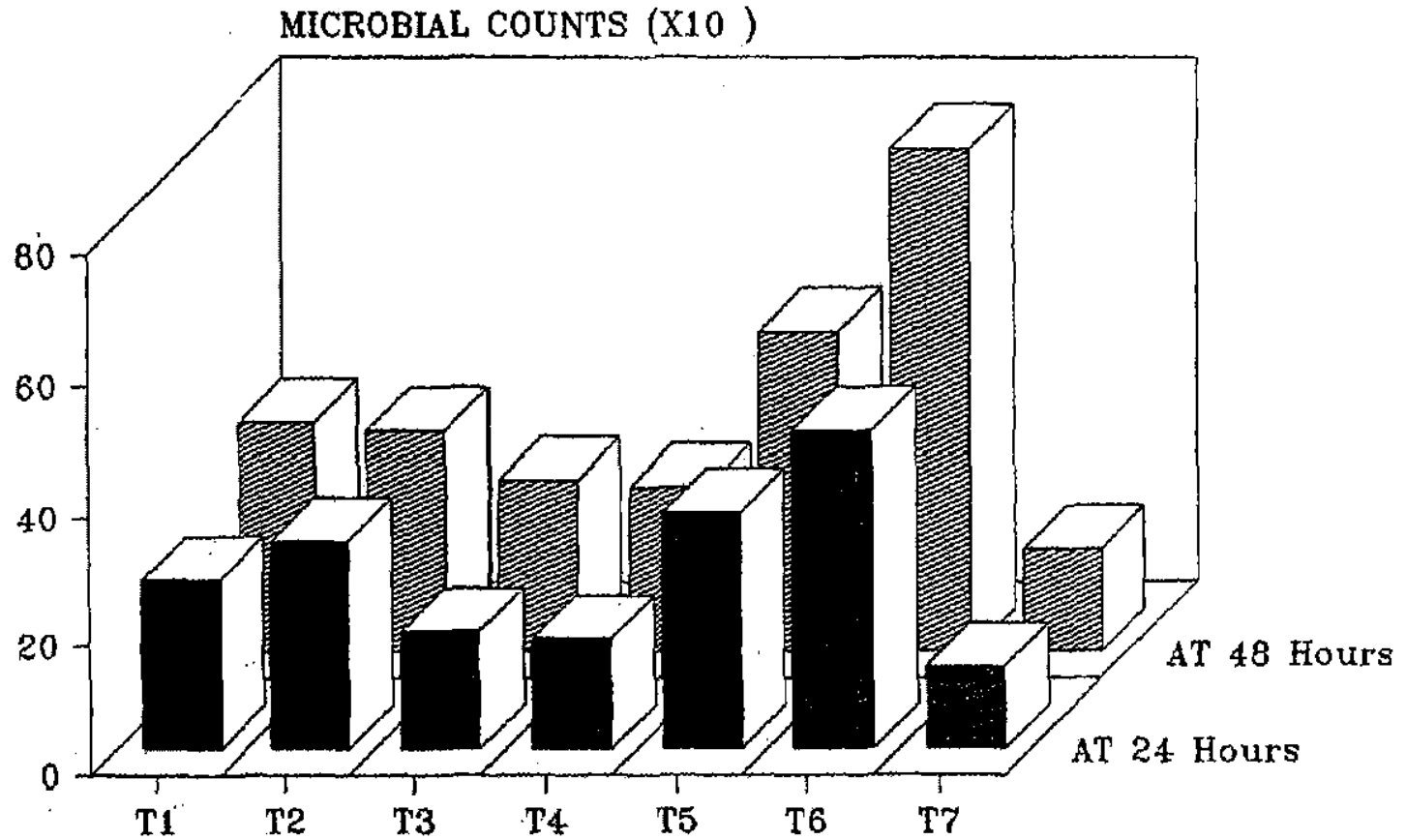
T_1 , *L.acidophilus* (R); T_2 , *L.acidophilus* (I); T_3 , *S.thermophilus* (HST);
 T_4 , *S.thermophilus* (CH); T_5 , *S.cerevisiae* (522); T_6 , *S.cerevisiae* (B);
 T_7 , Control.

to be highest in S.cerevisiae (B) supplemented group (10.89 meq/100 ml). The concentration of TVFA in the groups supplemented with strains of S.thermophilus (7.79 and 7.68 meq/100 ml) is similar but that of L.acidophilus (8.61 and 9.45 meq/100 ml) is different, however, the values of L.acidophilus are higher compared to control (7.59 meq/100 ml).

4.1.3 Effect on in vitro microbial population

Table 4.2 and Fig. 4.4 represent the microbial population during different hours and treatment groups. At 24 hours of incubation microbial counts ($\times 10^7$) were 26.35, 32.30, 18.25, 17.05, 36.70, 49.05 and 12.75 for T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7 treatment groups, respectively. The microbial counts were found higher in all groups as compared to control (12.75×10^7). However, it was the highest in group supplemented with S.cerevisiae strain B (49.05×10^7) followed by S.cerevisiae strain 522 (36.70×10^7), L.acidophilus strain-I (32.30×10^7), L.acidophilus strain-R (26.35×10^7), S.thermophilus strain-HST (18.25×10^7), S.thermophilus strain-CH (17.05×10^7). The count of S.therophilus stains on microbial population was lowest compared to other supplemented groups. At 48 h of incubation the microbial counts ($\times 10^7$) were 35.45, 34.10, 25.60, 24.55, 49.25, 77.00 and 15.98 for T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7 treatment groups, respectively. It was observed that microbial population increased in all groups at 48 h compared to 24 h incubation. Higher microbial

Fig 4.4 : IN VITRO MICROBIAL COUNTS ON
 FEED SUBSTRATE (WHEAT STRAW : CONC.
 60:40)



TREATMENT GROUPS

T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
 T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
 T₇, Control.

count was observed in the group supplemented with S.cerevisiae strain-B. The groups supplemented with L.acidophilus strains (35.45×10^7 and 34.10×10^7) exhibited higher microbial counts compared to the strains of S.thermophilus (25.60×10^7 and 24.55×10^7).

4.1.4 Effect on in vitro ammonia-nitrogen ($\text{NH}_3\text{-N}$)

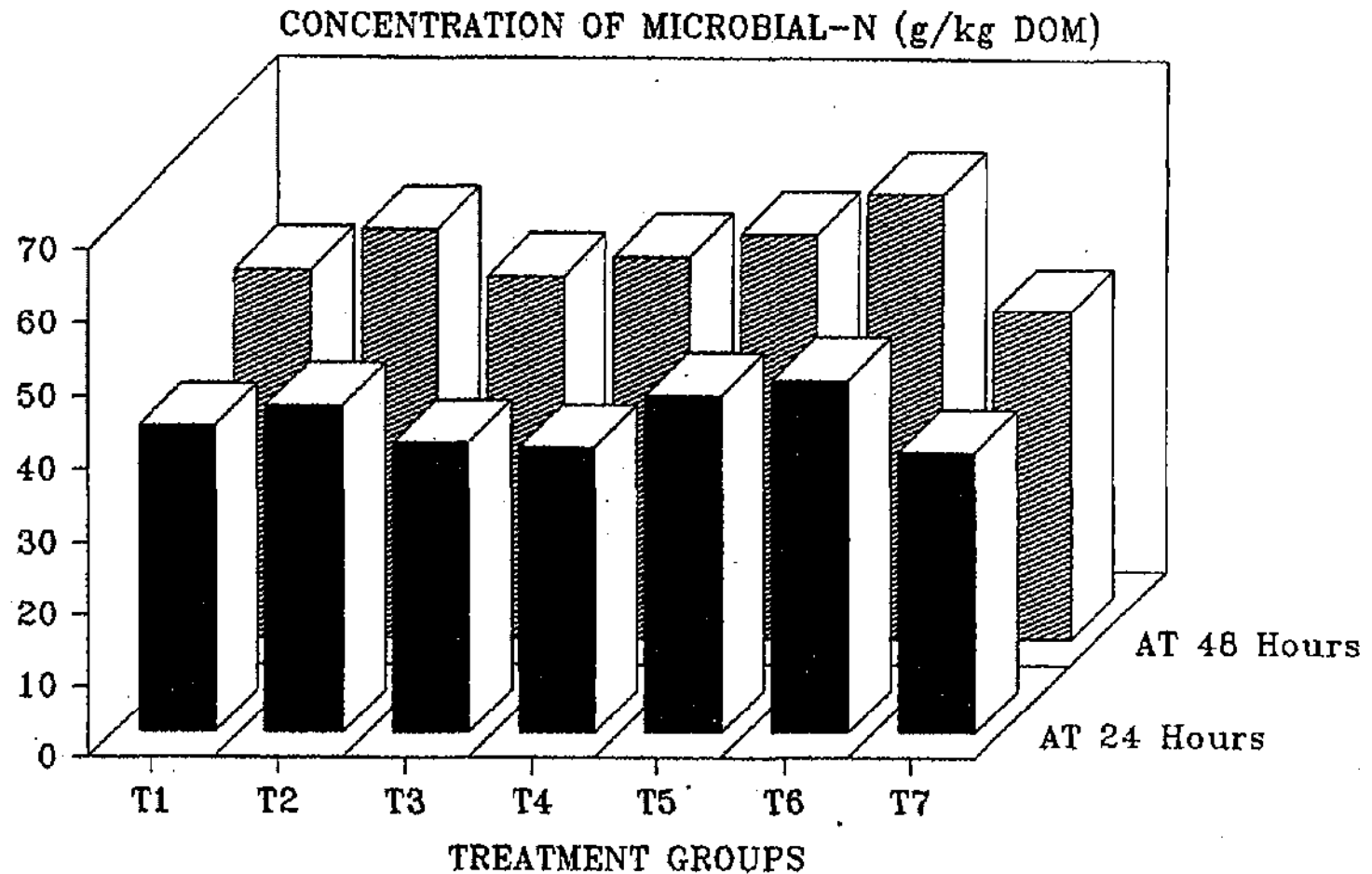
Concentration

The ammonia-N at 24 and 48 h in different treatment groups are presented in Table 4.3 (Fig. 4.5). At 24 h of incubation ammonia-N level varied between the lowest for S.cerevisiae strain B supplemented group (15.93 mg/100 ml) to the highest for control group (20.53 mg/100 ml). It was observed that levels of ammonia-N in groups supplemented with cultures of lactic acid producing bacteria did not differ with each other, the values ranged from 19.48 to 20.48 mg/100 ml. At 48 h of incubation levels of $\text{NH}_3\text{-N}$ were higher in all groups compared to 24 h incubation period. The levels of ammonia-N were 22.05, 21.70, 23.63, 22.40, 19.08, 16.45 and 23.15 mg/100 ml at 48 h incubation for T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7 treatment groups respectively. The concentration of ammonia-N was found lower at 24 and 48 h incubations in the groups supplemented with cultures of S.cerevisiae.

4.1.5 Effect on in vitro microbial protein-N

The data of microbial protein at 24 and 48 h of incubation are presented in Table 4.3 (Fig. 4.6). The level

Fig 4.6 : IN VITRO MICROBIAL-N
CONCENTRATION ON FEED SUBSTRATE
(WHEAT STRAW : CONC. 60:40)



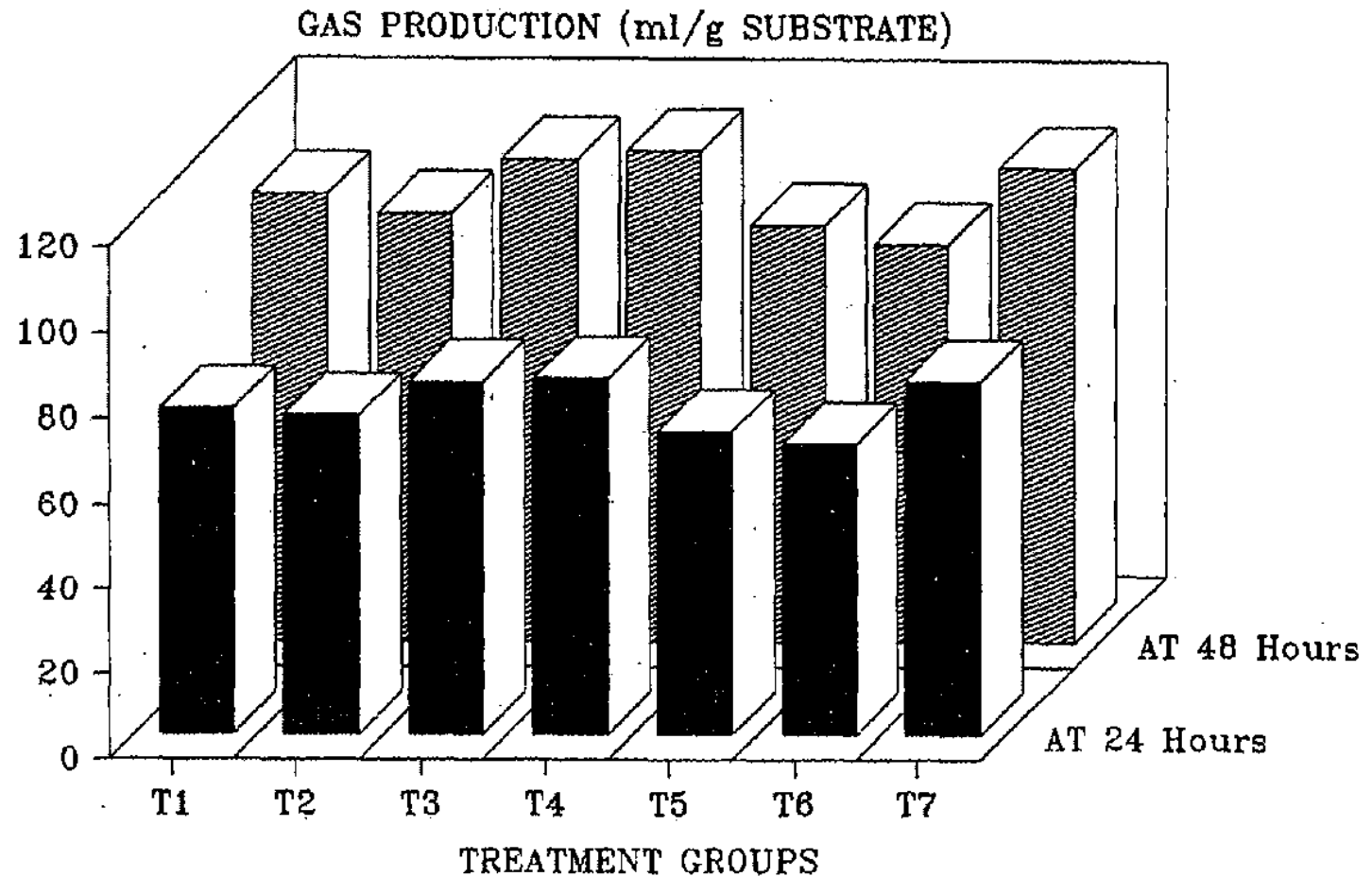
T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
T₇, Control.

of microbial protein-N g/kg of OM degraded were 42.35, 45.22, 40.11, 39.50, 46.67, 48.75 and 38.91 for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ treatment groups, respectively. Level of microbial protein-N was highest in groups supplemented with S.cerevisiae (B) (48.75 g/kg OM digested) followed by S.cerevisiae (522) (46.67 g/kg OM digested). The level of microbial protein was found higher in all the other groups compared to control. The cultures of lactic acid producing bacteria showed less effect compared to yeast culture. At 48 h incubation same trend was observed. The levels of microbial protein-N g/kg of OM degraded were 50.64, 56.09, 49.81, 52.49, 55.47, 61.08 and 45.15 for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ treatment groups respectively.

4.1.6 Effect on in vitro total gas production

Total gas production at 24 and 48 h incubation period in different groups are presented in Table 4.4 and 4.5 respectively (Fig. 4.7). Gas production (24h) varied between lowest in S.cerevisiae (B) supplemented group (68.4 ml/g of substrate) to the highest in S.thermophilus (CH) supplemented group (83.7 ml/g of substrate). Total gas production at 24 h in S.thermophilus strains supplemented groups were higher (82.9 and 83.7 ml) comparable to the control (83.2 ml/g substrate). However, on supplementation of L.acidophilus there was decrease in gas production (76.5 and 75.0 ml/g). At 48 h of incubation average total gas production were 104.4, 100.1, 112.5,

Fig 4.7 : IN VITRO GAS PRODUCTION ON
 FEED SUBSTRATE (WHEAT STRAW : CONC.
 60:40)



T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
 T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
 T₇, Control.

114.5, 97.4, 93.1 and 110.8 ml/g of substrate for T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7 treatment groups respectively (Table 4.5). It was observed that lowest total gas production was maintained in group supplements with S.cerevisiae strain B (93.1 ml/g of substrate) and the highest was in group supplemented with S.thermophilus strain CH (114.5 ml/g of substrate) followed by S.thermophilus strain HST (112.5 ml/g substrate). Supplementation of both strains of L.acidophilus and S.cerevisiae reduced the gas production.

4.2 DISCUSSION

The results of the effects of in vitro rumen fermentation influenced by supplementation of individual microorganisms are discussed on the basis of statistical analysis and available literature.

4.2.1 Effects of supplementation on DM and OM degradability

The statistical analysis revealed that the supplementation of both S.cerevisiae and L.acidophilus strains significantly ($P < 0.05$) increased the DM degradability at 24h incubation compared to control group. However, degradability also differed significantly ($P < 0.05$) between the species of these two cultures from each other. The DM degradability was higher in group supplemented with S.cerevisiae (B). The difference between both strains of L.acidophilus was non-significant. Supplementation of

S.thermophilus did not show any effect between strains on DM degradation and the difference was non-significant compared to control group. Similarly, there was significant increase of DM degradability (48h) in groups supplemented with both S.cerevisiae and L.acidophilus strains compared to control group. However, addition of S.thermophilus strain had no effect over control.

OM degradability (24 h) was significantly higher in groups supplemented with both S.cerevisiae and L.acidophilus strains compared to control group. The strains of S.cerevisiae increased the DM degradability to a higher level than strains of L.acidophilus. However, strains of S.thermophilus have a non-significant effect compared to control group. Similarly OM degradability at 48 h incubation was significantly enhanced in groups supplemented with S.cerevisiae and L.acidophilus compared to control group. The OM degradability (48 h) in S.cerevisiae supplemented groups were significantly higher than L.acidophilus groups. Whereas both strains of S.thermophilus did not effect OM degradability over control.

A higher DM and OM degradability observed in this experiment by supplementation of S.cerevisiae corroborated to earlier reports (Gomez Alaran et al, 1987; Chademana and Offer 1990 and Dawson, 1990). The improvement observed due to supplementation of S.cerevisiae maybe due to higher cellulosse (Dawson et al, 1987), hemicellulose (Glade and

Biesik, 1986; and Weidmmeier et al., 1987) or fiber degradability (Fallon and Harte, 1987). Dawson et al. (1987) observed 2 to 3 fold increase in cellulose degradation during first 96 hours of incubation. Dawson (1990) found that at least four times more cellulose was degraded during first 72 h of incubation with yeast cells. This short term stimulation of cellulose degradation appears to be related to a decrease in the amount of time required to initiate the digestion process (lag time). However, other workers observed a little or no effect on DM degradability due to differences in feed (Gray and Ryan, 1988; Dawson, 1989 and Williams, 1989a) and strains of S.cerevisiae (LeGrendre et al., 1957; Lassiter et al., 1958; Adams et al., 1981 and Harrison et al., 1988).

4.2.2 Effect on in vitro total volatile fatty acid (TVFA) concentration

TVFA concentration of substrate in all groups ranged from 6.03 to 8.96 and 7.59 to 10.89 meq/100 ml at incubation period for 24 and 48 h respectively. The lower concentration of TVFA observed in the present experiment might be due to wheat straw as a main component in the substrate (60%) or rumen liquor collected from the animals fed similar ration. The concentration of TVFAs in all the treatment groups were significantly higher over control projecting significantly higher values on S.cerevisiae (B) supplemented group.

However, differences between both strains of S.thermophilus and L.acidophilus (R) was non-significant. TVFA concentration (48 h) was significantly higher in groups supplemented with S.cerevisiae and L.acidophilus, however, groups supplemented with strains of S.thermophilus were not significantly different over the control group. The results of the present investigation on supplementation of S.cerevisiae are in total agreement with the findings of earlier studies (Weidmeir et al., 1987 and Williams, 1990a,b). However, other workers found a non-significant increase in TVFA concentration by supplementation of either S.cerevisiae alone (Harrison et al., 1988 and Carro et al., 1992) or with L.acidophilus (Dawson et al., 1990). A comparison of two levels of S.cerevisiae indicated that the effect on TVFA concentration was dose dependent (Williams, 1990a). The response of supplement on TVFA is also dependent on the composition of diet. Several authors (Dawson and Newman, 1988; Martin and Nisbot, 1989 and Chademana and Offer, 1990) have reported either small or no change in the rumen fermentation pattern when animals are kept on forage diet. Harrison et al. (1988) also reported that S.cerevisiae supplementation increased the molar proportions of total isoacids. Weidemeier et al. (1987) found that the acetate to propionate ratio remained unchanged when S.cerevisiae was included in the

diet but there was a tendency for the total pool of acetate to increase. However, majority of the reports suggested that addition of S.cerevisiae to either rumen fermenters or in vivo reduced the acetate to propionate ratio (Harrison et al., 1988; Newman and Dawson, 1988)).

4.2.3 Effect on in vitro microbial population

The microbial counts (24 and 48 h) were significantly higher in all groups over control showing highest count on S.cerevisiae supplemented group. At 24 h of incubation the differences between groups supplemented with S.thermophilus strains were not significant. The reason for increased microbial population in all groups may be due to supplementation of additional 10^9 cfu and their multiplication during incubation period. But unusually higher microbial population in the group supplemented with S.cerevisiae may be due to higher proliferation rate of this microflora for rapid incorporation of ammonia-N into microbial protein. This is also confirmed by a high level of microbial-N and a lower concentration of ammonia-N on S.cerevisiae supplemented groups in the present studies. The results obtained in this experiment corroborate with the findings of Weidmeyer et al. (1987), Harrison et al. (1988) and Dawson (1990). Dawson et al. (1990) observed that S.cerevisiae supplementation significantly increased the number of cellulolytic microorganism from 5 to 40 times. In the present experiment also the increased concentration of microbial population appears to be closely related to the

presence of live yeast in the supplement, which was not observed in the control group. This stimulation of microbial populaion has been observed in both rumen stimulating fermenter cultures (Dawson and Newman, 1987) and in the rumen of dairy cattle (Weidemeier et al., 1987 and Harrison et al., 1988).

4.2.4 Effect on in vitro ammonia-Nitrogen (NH₃-N) concentration

The level of ammonia-N (24 and 48 h) was significantly lower in groups supplemented with both strains of S.cerevisiae. However, strains of L.acidophilus and S.thermophilus did not effect ammonia-N concentration. It appears that reduced ammonia-N concentration might have resulted due to its incorporation into microbial-N. This has also resulted into higher level of microbial-N in the yeast supplemented groups. Adams et al (1981) observed a low level of ammonia-N (24.8 vs 32.8 mg/100 ml) with the supplementation of S.cerevisiae. With a high concentrate diet, Carro et al. (1992) found that S.cerevisiae supplementation reduced the ammonia-N production by 10 percent. Similar results have been reported in vivo by Harrison et al. (1988), Newbold et al. (1990) and Chademana and Offer (1990) and in vitro by Dawson and Newman (1988). However, Dawson et al. (1990) found that supplementation of S.cerevisiae along with L.acidophilus consistently

altered the relative concentration of ammonia in continuous cultures.

4.2.5 Effect on in vitro microbial protein-N

Microbial protein level (24 and 48 h) were significantly higher in all groups over control. However, the effect of S.ther mophilus at 24 h incubation was similar between the two strains, tested in the experiment through the microbial-N significantly increased by S.thermophilus (CH) after increasing the incubation period to 48 h.

Group supplemented with S.cerevisiae (B) showed the highest microbial protein level (24 and 48 h) followed by S.cerevisiae (522). The reason of higher microbial protein in all groups over control may be due to additional microbial protein through culture supplementation and their proliferation during incubation period. But unusually higher level of microbial-N in groups supplemented with S.cerevisiae might be due to synthesis of more microbial protein activated by supplement. This also confirmed by a higher level of microbial population with a lower ammonia concentration in the present investigation. Carro et al. (1992) also found a lower level of ammonia with a higher microbial protein synthesis with S.cerevisiae supplementation in Rusitech. Williams et al. (1990) also observed a higher non-ammonia nitrogen (NAN) at the duodenum in sheep with S.cerevisiae supplementation.

4.2.6 Effect on in vitro total gas production

Surprisingly less gas was produced in S.cerevisiae supplemented groups with a higher TVFA level. It appears that supplementation of S.cerevisiae suppresses the methane production, which need further confirmation. Williams (1989a) also observed a reduced methane production with the supplementation of S.cerevisiae. Carro et al. (1992) also observed a lower methane on medium and low concentrate diet. Harrison et al. (1987) also observed a lower gas production (56.8 Vs 64.2 ml) with the supplementation of S.cerevisiae. 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 ($P/0.01$) reduced after 12 h incubation. They further reported that the increase in level of propionate observed in vitro could have been the reason for the reduction in methane production. The production of propionate involves the utilization of metabolic hydrogen leading to the reduction in the synthesis of methane. This reason may be true in present investigation but require confirmation.

Table 4.1 Effect of different probiotics on *in vitro* DM and OM degradability of feed substrate (Wheat straw: concentrate, 60:40)

Treatment	DM degradability		OM degradability	
	24h	48h	24h	48h
(1)	(2)	(3)	(4)	(5)
T ₁	50.01	51.61	52.61	53.41
	51.86	53.60	53.78	53.39
	49.95	51.78	52.94	53.43
	50.11	52.37	52.27	52.41
Mean ± S.E.	50.48 ^{bc} ±0.46	52.34 ^b ±0.45	52.90 ^b ±0.32	53.16 ^b ±0.25
T ₂	50.37	53.05	53.98	54.41
	51.11	52.75	53.82	54.68
	51.55	54.68	53.86	54.36
	50.69	54.77	54.09	53.03
Mean ± S.E.	51.02 ^c ±0.20	53.81 ^c ±0.53	53.94 ^c ±0.06	54.12 ^c ±0.37
T ₃	49.17	50.82	52.82	53.31
	48.68	50.63	52.61	52.46
	50.53	51.48	51.82	53.91
	51.89	51.37	51.75	53.59
Mean ± S.E.	50.07 ^{abc} ± 0.72	51.08 ^a ± 0.21	52.25 ^{ab} ± 0.27	53.32 ^{ab} ±0.31
T ₄	49.28	50.38	51.84	52.82
	48.72	51.74	52.22	52.66
	50.68	51.73	51.80	51.47
	49.21	51.78	50.90	52.34
Mean ± S.E.	49.47 ^{ab} ±0.42	51.40 ^{ab} ±0.34	51.69 ^a ±0.28	52.32 ^a ±0.30

....contd.

(1)	(2)	(2)	(3)	(4)
	53.02	53.51	55.41	55.77
T_5	52.83	53.11	55.36	54.21
	52.78	55.19	54.41	55.43
	51.48	54.63	54.71	55.26
Mean \pm S.E.	52.52 ^d	54.19 ^c	54.97 ^d	55.17 ^d
	± 0.35	± 0.46	± 0.24	± 0.34
	55.39	56.71	56.63	57.81
T_6	55.49	56.95	57.28	57.29
	55.13	56.63	57.43	58.86
	56.27	57.13	56.96	58.17
Mean \pm S.E.	55.57 ^e	56.86 ^d	57.08 ^e	58.03 ^e
	± 0.25	± 0.11	± 0.18	± 0.33
	49.18	51.10	52.07	52.49
T_7	48.71	52.28	51.48	53.27
	50.23	50.39	52.49	53.49
	49.07	51.83	52.01	52.08
Mean \pm S.E.	49.30 ^a	51.40 ^{ab}	52.01 ^a	52.83 ^{ab}
	± 0.33	± 0.42	± 0.21	± 0.33

a, b, c, d & e figures with different superscripts in the same column differ significantly ($P/0.05$).

T_1 , L.acidophilus (R); T_2 , L.acidophilus (I); T_3 , S.thermophilus (HST);
 T_4 , S.thermophilus (CH); T_5 , S.cerevisiae (522); T_6 , S.cerevisiae (B);
 T_7 , Control.

Table 4.2 Effect of different probiotics on in vitro total volatile fatty acids concentrations and microbial counts on feed substrate (Wheat straw:Concentrate, 60:40)

Treatments (1)	TVFA(meq/100ml)		Microbial counts($\times 10^7$)	
	24h (2)	48h (3)	24h (4)	48h (5)
T ₁	6.45	8.30	24.8	34.0
	6.50	8.75	27.4	36.2
	6.60	8.45	28.0	36.2
	7.00	8.95	25.2	35.4
Mean \pm S.E.	6.64 ^b ± 0.12	8.61 ^b ± 0.15	26.35 ^c ± 0.79	35.45 ^c ± 0.52
T ₂	7.45	9.50	31.0	36.8
	7.85	8.85	32.2	34.2
	7.60	9.90	32.0	31.8
	7.70	9.55	34.0	33.6
Mean \pm S.E.	7.65 ^c ± 0.08	9.45 ^c ± 0.22	32.30 ^d ± 0.62	34.10 ^c ± 1.03
T ₃	6.45	8.25	17.8	30.0
	6.30	7.50	19.2	22.6
	6.50	7.80	18.2	25.4
	7.00	7.60	17.8	24.4
Mean \pm S.E.	6.56 ^b ± 0.15	7.79 ^a ± 0.17	18.25 ^b ± 0.33	25.60 ^b ± 1.58
T ₄	6.15	8.05	16.2	25.8
	6.50	7.30	16.8	26.2
	6.85	7.55	18.2	21.8
	6.60	7.80	17.0	24.4
Mean \pm S.E.	6.53 ^b ± 0.15	7.68 ^a ± 0.16	17.05 ^b ± 0.42	24.55 ^b ± 0.99

....contd.

.....contd. Table 4.2

(1)	(2)	(3)	(4)	(5)
T ₅	7.50	9.65	37.6	43.6
	7.60	9.60	35.4	48.0
	8.00	9.70	36.2	47.6
	7.75	10.15	37.6	52.8
Mean ± S.E.	7.71 ^c	9.78 ^c	36.70 ^e	49.25 ^d
	±0.11	±0.13	±0.54	±1.20
T ₆	8.75	10.70	47.4	75.2
	8.95	10.75	45.4	77.4
	9.10	10.95	51.2	77.4
	9.05	11.15	52.2	78.0
Mean ± S.E.	8.96 ^d	10.89 ^d	49.05 ^f	77.00 ^e
	±0.08	±0.10	±1.60	±0.62
T ₇	6.47	7.48	12.1	15.0
	5.83	7.40	13.5	15.9
	5.88	7.80	12.2	16.5
	5.97	7.67	13.2	16.5
Mean ± S.E.	6.03 ^a	7.59 ^a	12.75 ^a	15.98 ^a
	±0.14	±0.09	±0.35	±0.35

a, b, c, d & e figures with different superscripts in the same column differ significantly (P/0.05).

T₁, L.acidophilus (R); T₂, L.acidophilus (I); T₃, S.thermophilus (HST);

T₄, S.thermophilus (CH); T₅, S.cerevisiae (522); T₆, S.cerevisiae (B);

T₇, Control.

Table 4.3 Effect of different probiotics on *in vitro* ammonia-nitrogen and microbial-nitrogen levels on feed substrate (Wheat straw:Concentrate, 60:40)

Treatments	NH ₃ -N (mg/100ml)		Microbial-N (g/kg of OM digested)	
	24h	48h	24h	48h
(1)	(2)	(3)	(4)	(5)
	20.3	21.7	40.99	48.95
T ₁	19.6	21.0	42.35	50.30
	21.0	23.1	43.72	51.66
	21.0	22.4	42.35	51.66
Mean ± S.E.	20.48 ^C	22.05 ^C	42.35 ^C	50.64 ^C
	±0.34	±0.45	±0.56	±0.65
	18.9	21.7	44.2	54.75
T ₂	19.6	21.0	45.56	56.09
	20.3	23.1	46.89	56.09
	19.6	21.0	44.22	57.43
Mean ± S.E.	19.48 ^C	21.70 ^C	45.22 ^d	56.09 ^f
	± 0.39	±0.49	±0.64	±0.55
	20.3	23.1	41.50	48.79
T ₃	21.0	23.1	38.73	48.79
	18.9	23.8	40.11	50.15
	20.3	24.5	40.11	57.50
Mean ± S.E.	20.13 ^C	23.63 ^C	40.11 ^b	49.81 ^b
	±0.44	±0.34	±0.57	±0.65
	20.3	21.7	40.55	51.11
T ₄	19.6	22.4	37.75	52.49
	20.3	24.5	40.55	53.88
	21.0	21.0	39.15	52.49
Mean ± S.E.	20.30 ^C	22.40 ^C	39.50 ^b	52.49 ^d
	±0.29	±0.76	±1.47	±0.57

....contd.

(1)	(2)	(3)	(4)	(5)
T ₅	18.2	18.2	46.01	55.79
	18.2	18.9	47.33	54.49
	18.2	18.9	44.70	55.79
	16.1	20.3	48.64	55.79
Mean ± S.E.	17.68 ^b ±0.53	19.08 ^b ±0.44	46.67 ^e ±0.85	55.47 ^e ±0.33
T ₆	16.1	16.8	48.12	61.03
	16.1	16.1	49.38	61.03
	16.1	16.1	48.12	62.27
	15.4	16.8	49.38	59.78
Mean ± S.E.	15.93 ^a ±0.18	16.45 ^a ±0.20	48.75 ^f ±0.36	61.03 ^g ±0.51
T ₇	19.8	22.6	37.52	43.78
	21.0	21.9	40.30	45.15
	20.3	24.8	37.52	46.51
	21.0	23.3	40.30	45.15
Mean ± S.E.	20.53 ^c ±0.29	23.15 ^c ±0.62	38.91 ^a ±0.80	45.15 ^a ±0.56

a, b, c, d & e figures with different superscripts in the same column differ significantly (P/0.05).

T₁, L.acidophilus (R); T₂, L.acidophilus (I); T₃, S.thermophilus (HST);
 T₄, S.thermophilus (CH); T₅, S.cerevisiae (522); T₆, S.cerevisiae (B);
 T₇, Control.

Table 4.4 Effect of different probiotics on *in vitro* gas production (ml/g substrate)(24h) on feed substrate (Wheat straw:Concentrate, 60:40)

Treatment	T ₁ ^{bcd}	T ₂ ^{abc}	T ₃ ^{cd}	T ₄ ^d	T ₅ ^{ab}	T ₆ ^a	T ₇ ^d
Hours							
2	13.6±0.29	13.1±0.38	15.4±0.45	15.6±0.52	12.2±0.24	12.2±0.25	15.1±0.10
4	12.8±0.32	11.8±0.37	13.4±0.36	13.9±0.47	10.7±0.26	9.8±0.52	13.3±0.26
6	9.0±0.31	9.0±0.28	9.0±0.26	10.7±0.46	7.8±0.29	7.9±0.32	10.1±0.45
8	7.9±0.23	7.8±0.40	8.6±0.16	9.2±0.27	7.7±0.21	7.4±0.05	8.7±0.06
10	7.4±0.22	7.2±0.31	8.2±0.24	8.2±0.31	7.4±0.23	6.9±0.29	8.2±0.06
12	7.3±0.08	7.1±0.20	7.6±0.31	7.6±0.28	6.6±0.21	6.6±0.29	7.8±0.09
16	6.7±0.26	6.9±0.23	7.5±0.15	6.7±0.29	6.5±0.20	6.2±0.18	7.2±0.17
20	6.0±0.21	6.4±0.33	6.7±0.42	6.1±0.12	6.5±0.09	6.0±0.27	6.6±0.18
24	5.8±0.23	5.7±0.25	6.5±0.47	5.7±0.30	6.0±0.19	5.4±0.05	6.2±0.20
Total	76.5 ^{bed}	75.0 ^{abe}	82.9 ^{ed}	83.7 ^d	71.4 ^{ab}	68.4 ^a	83.2 ^d

a, b, c, d & e figures with different superscripts in the same row differ significantly (P/0.05).

T₁, L.acidophilus (R); T₂, L.acidophilus (I); T₃,
S.thermophilus (HST);
T₄, S.thermophilus (CH); T₅, S.cerevisiae (522); T₆,
S.cerevisiae (B);
T₇ -

Table 4.5 Effect of different probiotics on *in vitro* gas production (ml/g substrate)(48h) on feed substrate (Wheat straw:Concentrate, 60:40)

Treatments	T ₁ ^{bcd}	T ₂ ^d	T ₃ ^d	T ₄ ^{ab}	T ₅ ^a	T ₆ ^c	T ₇ ^d
Hours	LAR	LI ^{abc}	STH ^d	STCH ^d	SC 522 ^{ab}	SC 13 ^a	Control
2	13.4±0.20	13.1±0.35	15.5±0.40	15.0±0.30	12.0±0.29	12.0±0.22	14.6±0.09
4	13.3±0.40	12.5±0.24	14.5±0.18	14.0±0.35	11.3±0.32	10.5±0.32	13.1±0.26
6	9.4±0.21	9.2±0.47	9.2±0.16	9.8±0.30	8.4±0.31	7.6±0.27	9.7±0.17
8	8.2±0.20	8.4±0.22	8.1±0.35	9.4±0.19	7.6±0.06	7.5±0.23	8.7±0.10
10	7.8±0.41	7.6±0.22	8.0±0.22	8.0±0.24	7.3±0.09	7.2±0.29	8.0±0.19
12	6.7±0.29	6.7±0.21	7.4±0.04	7.8±0.35	6.3±0.10	6.7±0.15	7.4±0.12
16	6.9±0.29	5.9±0.31	6.7±0.16	7.2±0.24	6.3±0.09	6.2±0.10	7.1±0.07
20	6.2±0.10	5.3±0.08	6.7±0.26	6.8±0.30	5.9±0.25	6.0±0.21	6.5±0.07
24	5.1±0.05	5.5±0.06	6.7±0.30	6.4±0.10	5.5±0.37	5.3±0.10	6.2±0.10
28	5.2±0.11	5.5±0.11	6.3±0.10	6.1±0.14	5.6±0.10	5.2±0.28	6.2±0.10
32	4.9±0.19	5.3±0.18	5.4±0.05	5.6±0.09	4.9±0.28	4.6±0.10	5.8±0.16
36	4.7±0.21	4.5±0.15	5.5±0.24	5.0±0.14	4.7±0.17	4.1±0.26	5.0±0.06
40	4.5±0.08	3.9±0.19	4.8±0.18	4.7±0.13	4.1±0.17	3.5±0.11	4.4±0.02
44	4.3±0.11	3.4±0.07	3.9±0.25	4.6±0.06	3.9±0.20	3.6±0.18	4.2±0.08
48	3.8±0.22	3.3±0.04	3.8±0.21	4.1±0.27	3.6±0.21	3.1±0.27	3.9±0.11
Total	104.4 ^{bcd}	100.1 ^{abc}	112.5 ^d	114.5 ^d	97.4 ^{ab}	93.1 ^a	110.8 ^d

a, b, c, d & e figures with different superscripts in the same row differ significantly (P/0.05).

T₁, *L.acidophilus* (R); T₂, *L.acidophilus* (I); T₃, *S.thermophilus* (HST);
T₄, *S.thermophilus* (CH); T₅, *S.cerevisiae* (522); T₆, *S.cerevisiae* (B);
T₇, Control.

Table 4.6 Statistical analysis of different parameters of *in vitro* rumen fermentation.

Source of variation	d.f.	MSS					
		DM degrada- bility	OM degrada- bility	TVFA Conc.	Microbial counts	NH ₃ -N Conc.	Microbial protein-N
Between replicates	3	1.15	0.52	2.44	1.97	1.54	3.69
Between treatments	6	36.30*	30.37*	101.36*	2162.97	36.76	153.45
Between hours	1	45.64	4.29	390.28	1379.07	54.61	1366.41
Error	39	0.61	0.32	0.62	3.25	0.69	1.29

Significant at 5% level.

PHASE - II

5. SUPPLEMENTATION OF LIVE MICROORGANISMS ON IN VIVO RUMEN FERMENTATION

5.1 RESULTS

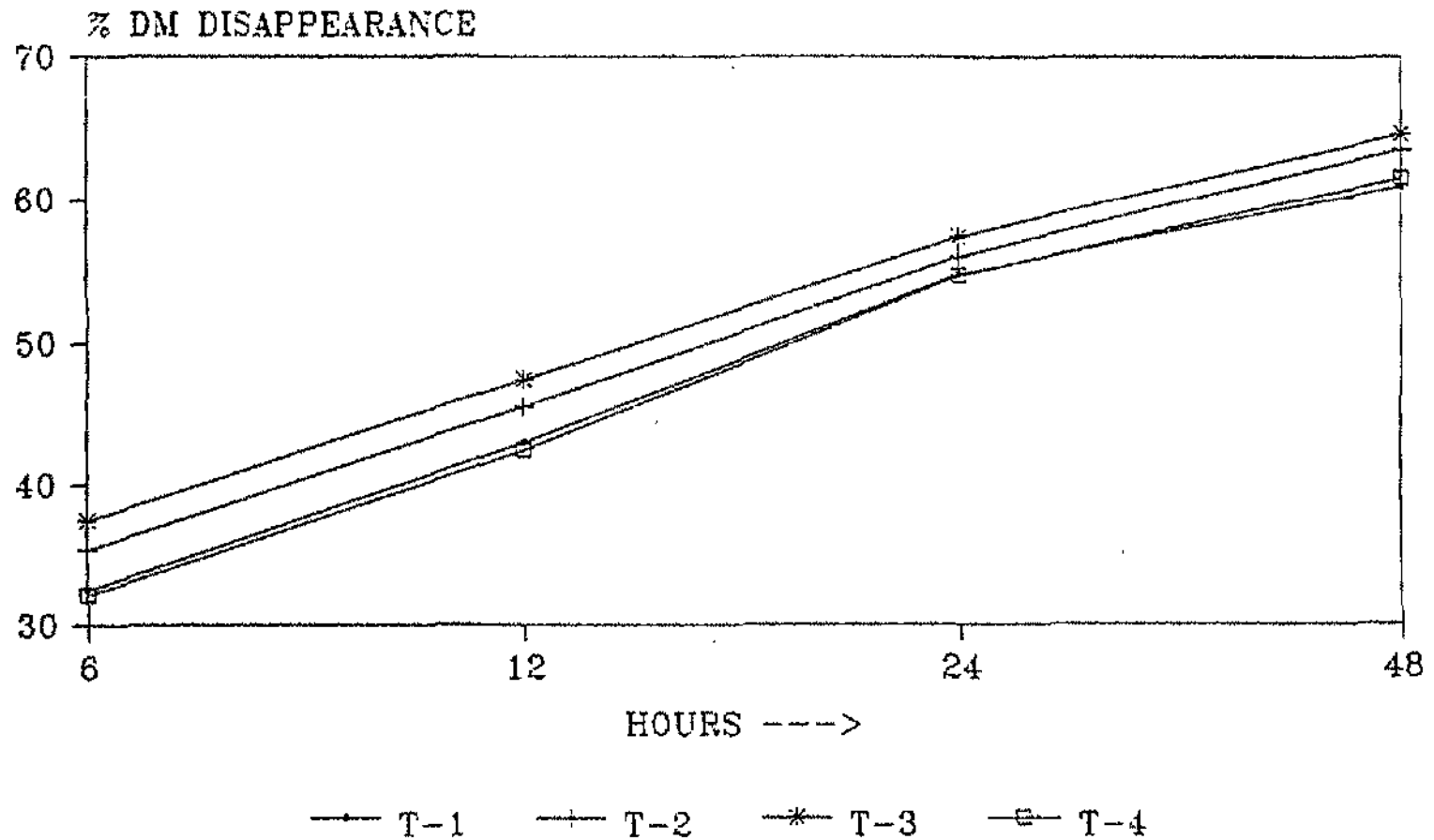
Out of the strains of lactic acid producing bacteria and yeast, one strain from each group, i.e. L.acidophilus (I) and S.cerevisiae (B) was selected on the basis of observations obtained during in vitro rumen fermentation. These microorganisms were supplemented in the diets of adult fistulated male cattle. The supplementation was made individually [L.acidophilus (I) (T₁), S.cerevisiae (B) (T₂)

and in combination (T_3) alongwith a group of control (T_4)] to study the effect on ruminal fermentation. On the performance of in vivo studies best supplement will be selected, which will further be used in growth experiments.

5.1.1 DM disappearance in Nylon bag

Measured and fitted values (Orskov and McDonald, 1979) of DM disappearance in different treatment groups and incubation periods are presented in Table & Fig. 5.1. At 6 h of post feeding DM degradability were 16.55, 10.24 and 1.18 percent higher in T_3 (37.46%), T_2 (35.43%) and T_1 (32.52%) treatment groups, respectively as compared to control group (32.14%). At 12 h of incubation DM degradability were 11.66, 7.21 and 1.20 percent higher in T_3 (47.37%), T_2 (45.48%) and T_1 (42.93%) treatment groups, respectively over control group (42.42%). This reduction also persisted during 24 and 48 h of incubations. DM degradability (24h) were 4.97, 2.49 and 0.26 percent higher in T_3 (57.28%), T_2 (55.93%) and T_1 (54.71%) treatment groups, respectively. At 48 h DM degradability was 5.24 and 3.40 percent higher in T_3 (64.63%) and T_2 (63.50%) treatment groups respectively over control group (61.41%). However, DM degradability (48h) in treatment group T_1 (60.86%) was slightly lower than control group. The supplementation of S.cerevisiae (B) alone and with a combination of L.acidophilus (J)

Fig. 5.1 : DM DISAPPEARANCE OF SUBSTRATE
AT DIFFERENT INCUBATION PERIODS AND
PROBIOTIC SUPPLEMENTS



T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

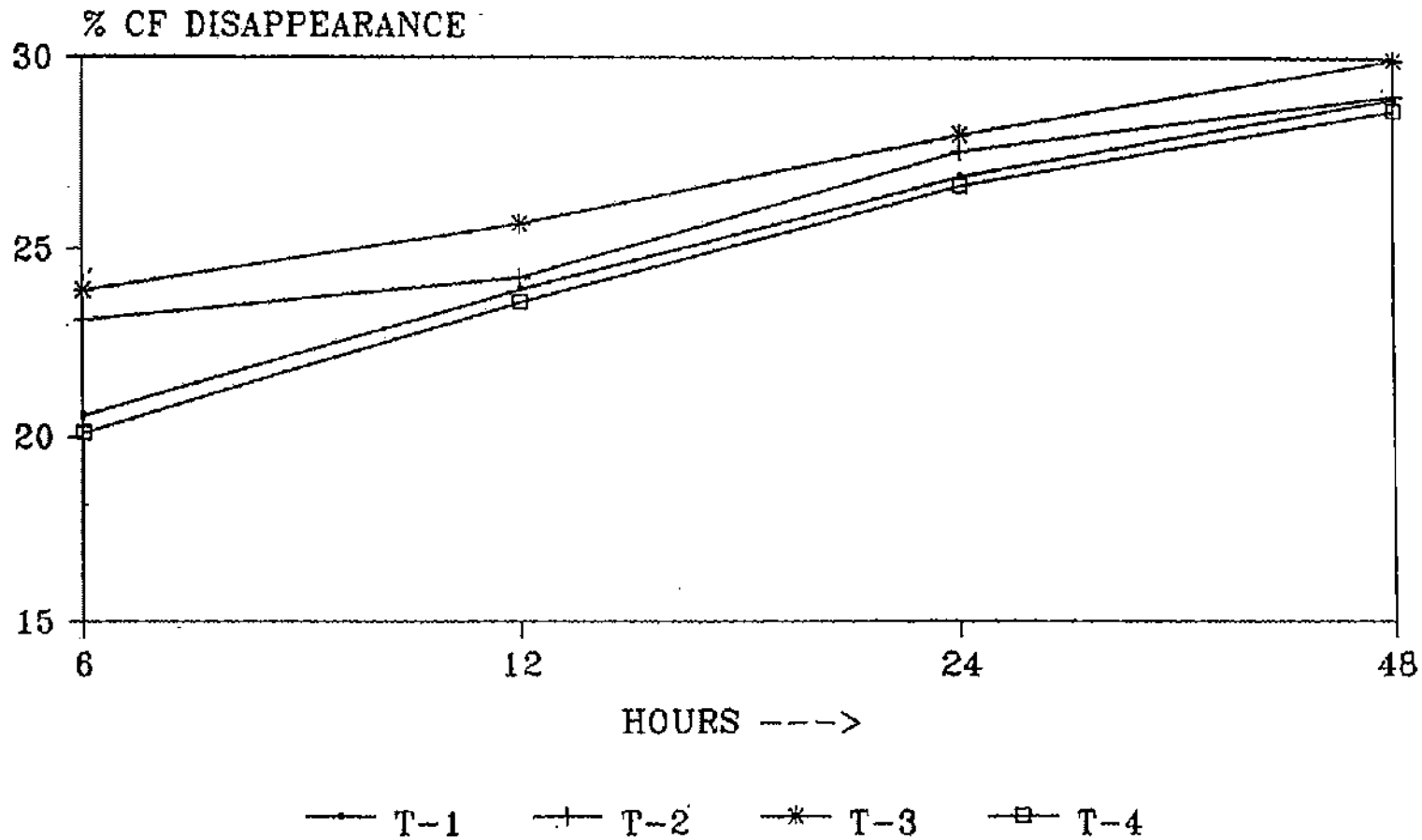
established increase in DM degradability over the control group. But as the time advanced, the increment in DM degradability was at a decreased rate. It also seems that supplementation of L.acidophilus alone had little effect on DM degradability. Whereas addition of S.cerevisiae (B) showed a substantial effect, and increased the DM degradability over S.cerevisiae supplementation alone upto 48h. Differences in DM degradability were wider for 6 and 12 h of incubations as compared to 24 and 48 h of incubations.

The coefficients of the DM degradability curves plotted according to the method of Orskov and McDonald (1979) are shown in Table 5.3. Intercept of the degradation curve (a value) at 0h was higher in treatment group T₃ (22.74) followed by T₂ (21.15) over T₁ (15.39) and T₄ (15.65) treatment groups. The rate constant for the DM degradation of the component with potential degradability (c value) was lower in T₃ (0.0693) and T₂(0.0657) treatment groups over T₁ (0.0752) and T₄ (0.0704) treatment groups. Effective degradability at a flow rate of 0.05 percent/hour was higher in T₃ (47.97%) and T₂(46.20%) treatment groups over T₁ (43.50%) and T₄(43.40%) treatment groups.

5.1.2 Fibre degradability

Table & Fig.5.2 showed fiber degradability during different

Fig. 5.2 : CF DISAPPEARANCE OF SUBSTRATE
AT DIFFERENT INCUBATION PERIODS AND
PROBIOTIC SUPPLEMENTS



T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B)
T₄, Control.

hours and treatment groups. Fiber degradability (6h) was 18.87, 14.98 and 2.28 percent higher in treatment groups T_3 (23.88%) T_2 (23.10%) and T_1 (20.55%) respectively over control group (20.09%). At 12 h of incubation fiber degradability were 8.87, 2.84 and 1.44 percent higher in treatment groups T_3 (25.65%), T_2 (24.23%) and T_1 (23.90%), respectively over control group (23.56%). At 24h of incubation the increase was 4.95, 3.34 and 0.86 percent in treatment groups T_3 (27.97%), T_2 (27.54%) and T_1 (26.88%), respectively over control group (26.65%). Similarly at 48h fiber degradability was 4.68, 1.36 and 1.11 percent higher in treatment groups T_3 (29.94%), T_2 (28.99%) and T_1 (28.92%), respectively over control group (28.60%). Similar pattern was followed by fitted fiber degradability calculated as per Orskov and McDonald (1979). Table 5.2 clearly indicates a higher fiber degradation in treatment groups supplemented with S.cerevisiae (B). Response was prominent after 6 to 12 h of incubations in S.cerevisiae (B) supplemented group. However, at 24 and 48h of incubations fiber degradability was comparable in all treatments.

Table 5.3 describe the fiber degradation rates in different treatment groups estimated as per Orskov and McDonald (1979). Intercept of the degradation curve at 0h (a value) was higher in T_3 treatment group (21.29) followed

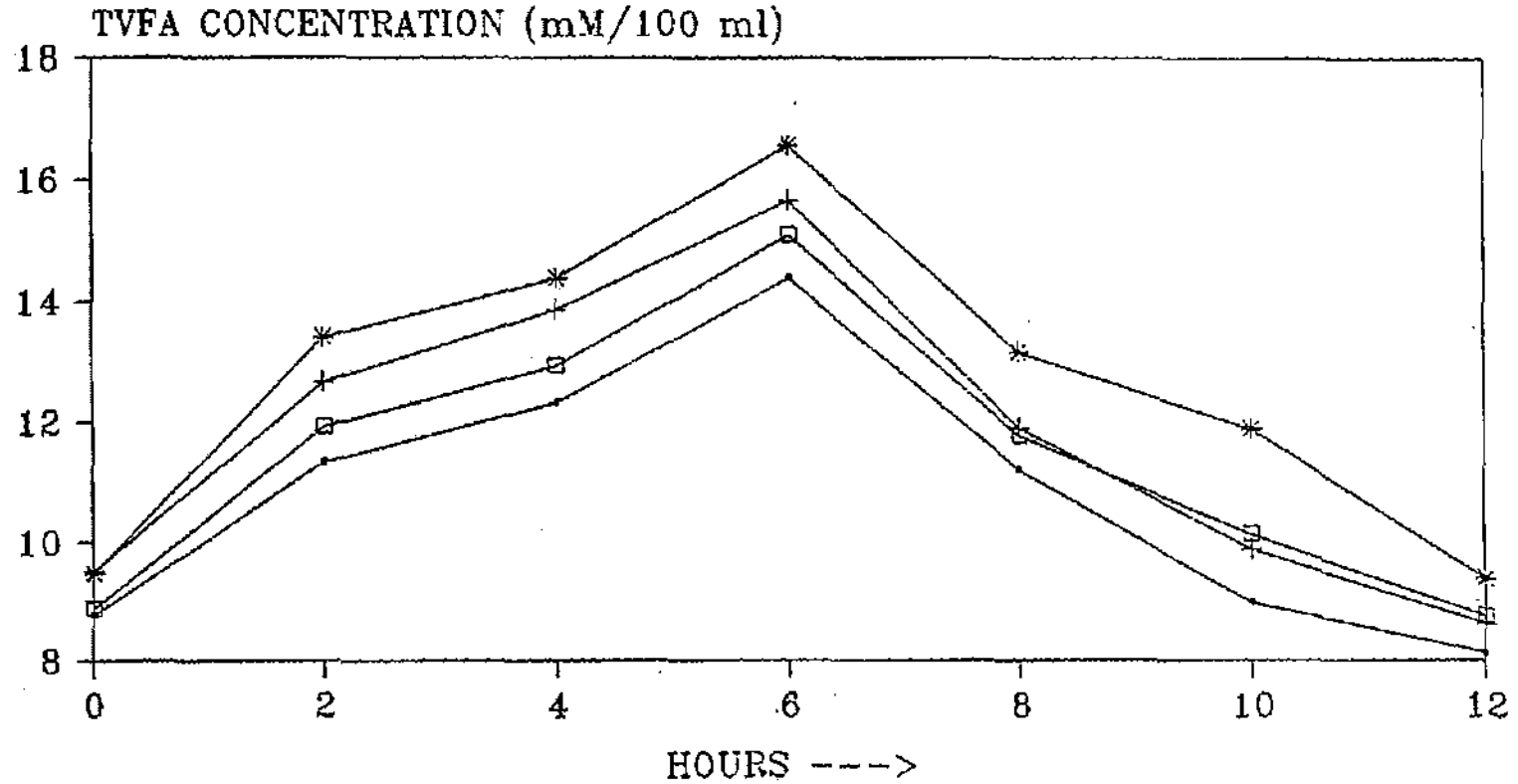
by T_2 (20.28) over treatment groups T_1 (14.43) and T_4 (14.53). The rate constant for degradation of the component with potential degradability (c value) were lower in treatment groups T_2 (0.0535) and T_3 (0.0484) as compared to treatment groups T_1 (0.0837) and T_4 (0.0814). Effective degradability at a flow rate of 0.05 percent/h was higher in T_3 (26.13%) and T_2 (25.20%) treatment groups over T_1 (23.80%) and T_4 (23.43%) treatment groups.

5.1.3 Ruminal total volatile fatty acid (TVFA) concentration

The data in respect of TVFA concentration in the rumen at different intervals and treatment groups are presented in Table 5.4 (Fig. 5.3). In all treatment groups TVFA concentration (mM/100 ml) reached to a maximum peak at 6h post feeding. The peak was highest in treatment group T_3 (16.56) followed by treatment group T_2 (15.64). Treatment group T_1 showed a lower TVFA concentration all the times as compared to control group. Treatment group T_3 showed a 6.64, 12.50, 11.13, 9.81, 11.80, 17.49 and 7.19 percent higher TVFA concentration at 0, 2, 4, 6, 8, 10 and 12 h post feeding respectively over control group.

Similarly treatment group T_2 showed 7.09, 6.29, 7.19, 3.71 and 1.10 percent higher TVFA concentration at 0, 2, 4, 6 and 8 h of post feeding respectively over control group. However, at 10 and 12 h of post feeding

Fig. 5.3 : TVFA CONCENTRATION AT
DIFFERENT HOURS OF POST-FEEDING
AND PROBIOTIC SUPPLEMENTS



— T-1 + T-2 * T-3 □ T-4

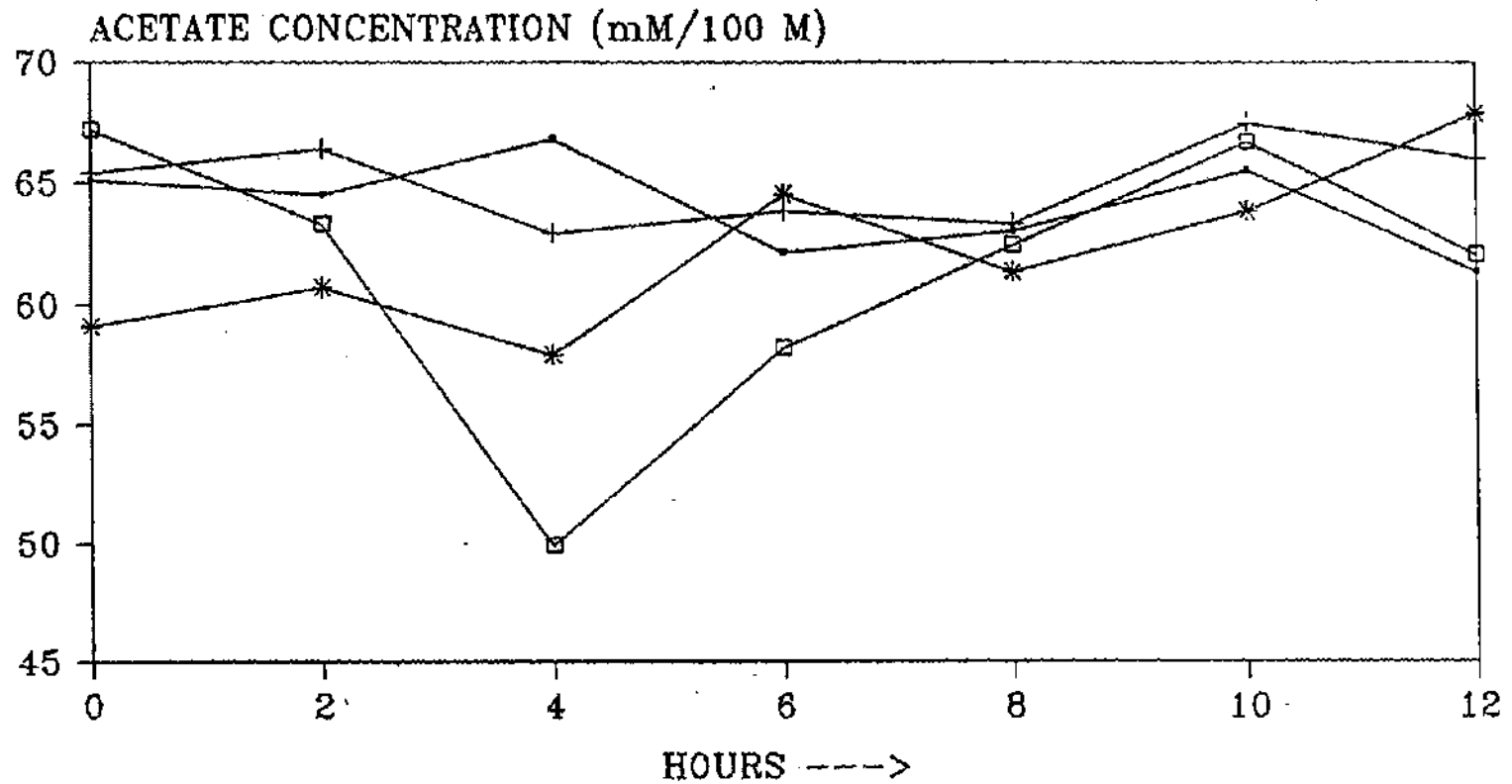
T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

concentration of TVFA in treatment group T_2 were 2.37 and 1.48 percent lower respectively over control group. Treatment group T_1 showed 1.23, 4.94, 4.71, 4.70, 4.84, 11.26 and 7.07 percent lower concentration at 0, 2, 4, 6, 8, 10 and 12 h respectively over control group. It seems that supplementation of L.acidophilus (I) alone had no effect on TVFA concentration. However, supplementation with S.cerevisiae (B) increased the efficiency.

5.1.4 Concentration of individual volatile fatty acids in the rumen

The concentration of acetate, propionate and butyrate during different hours and groups are presented in Table 5.4. The concentration of acetate (Fig. 5.4) in the treatment group T_1 was 1.90, 33.87, 6.70, 0.96 percent higher at 2, 4, 6 and 8h post feeding respectively compared to control group (T_4). However, at 0, 10 and 12h post feeding acetate concentration was lower by 3.13, 1.53 and 1.13 percent respectively. Similarly treatment group T_2 showed 4.90, 26.05, 9.62, 1.44, 1.20 and 6.45 percent higher acetate concentration at 2, 4, 6, 8, 10 and 12h post feeding, respectively except at 0h post feeding where it was 2.68 percent lower compared to control group (T_4). However, treatment group T_3 showed a reduced concentration of acetate by 12.05, 4.11, 1.76 and 4.35 percent at 0, 2, 8 and 10 h post feeding respectively compared to control group (T_4). Whereas at 4, 6 and 12h post feeding

Fig. 5.4 : ACETATE CONCENTRATION AT DIFFERENT HOURS OF POST-FEEDING AND PROBIOTIC SUPPLEMENTS



— T-1 + T-2 * T-3 □ T-4

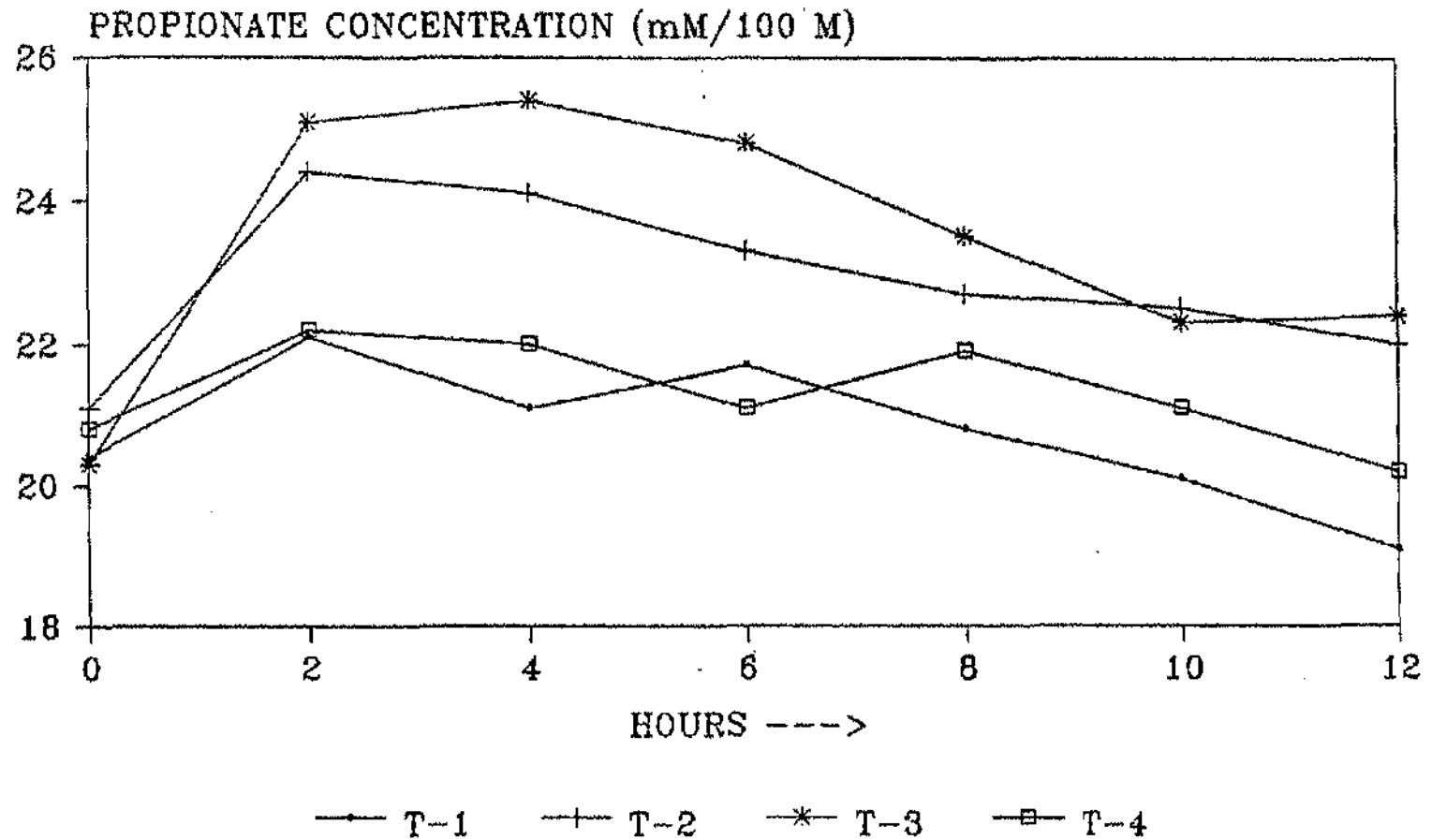
T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B)
T₄, Control.

concentration of acetate was higher by 16.03, 10.82 and 9.51 percent respectively over control group (T_4).

Concentration of propionate (Fig. 5.5) in treatment group T_1 was lower by 1.92, 0.45, 4.09, 5.02, 4.73 and 5.45 percent at 0, 2, 4, 8, 10 and 12h post feeding respectively except at 6 h post feeding where it was 5.02 percent higher compared to control group (T_4). However, treatment groups T_2 and T_3 showed a higher propionate proportion at all time of sampling. In treatment group T_2 propionate concentration was higher by 1.44, 9.91, 9.55, 10.42, 3.65, 6.64 and 8.91 percent at 0, 2, 4, 6, 8, 10 and 12 h post feeding respectively over control group (T_4). Similarly treatment group T_3 showed a higher concentration of propionate by 13.06, 15.45, 17.54, 7.31, 15.69 and 10.89 percent at 2, 4, 6, 8, 10 and 12 h post feeding respectively compared to control group (T_4). Whereas propionate concentration at 0h post feeding was 2.40 percent lower in T_3 treatment group.

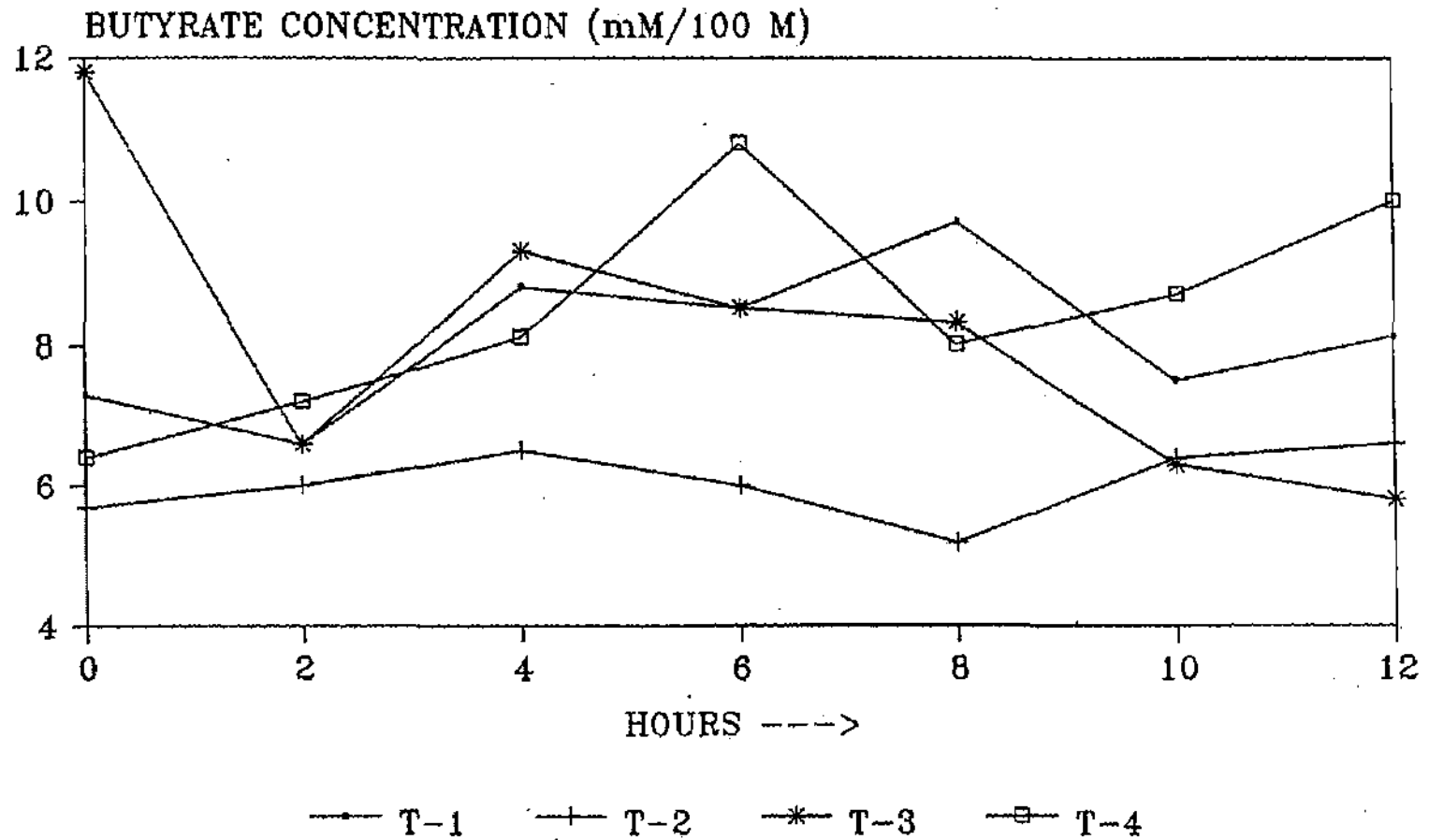
Ruminal concentration of butyrate (Fig. 5.6) in treatment group T_1 was lower by 8.33, 21.30, 13.79 and 19.00 percent at 2, 6, 10 and 12h post feeding respectively over control group (T_4). However, butyrate concentration at 0, 4, and 8h post feeding were higher by 14.06, 8.64 and 21.25 percent respectively compared to control group (T_4). In general treatment groups T_2 and T_3 showed a lower

Fig. 5.5 : PROPIONATE CONCENTRATION AT
DIFFERENT HOURS OF POST-FEEDING
AND PROBIOTIC SUPPLEMENTS



T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B)
T₄, Control.

Fig. 5.6 : BUTYRATE CONCENTRATION AT
DIFFERENT HOURS OF POST-FEEDING
AND PROBIOTIC SUPPLEMENTS



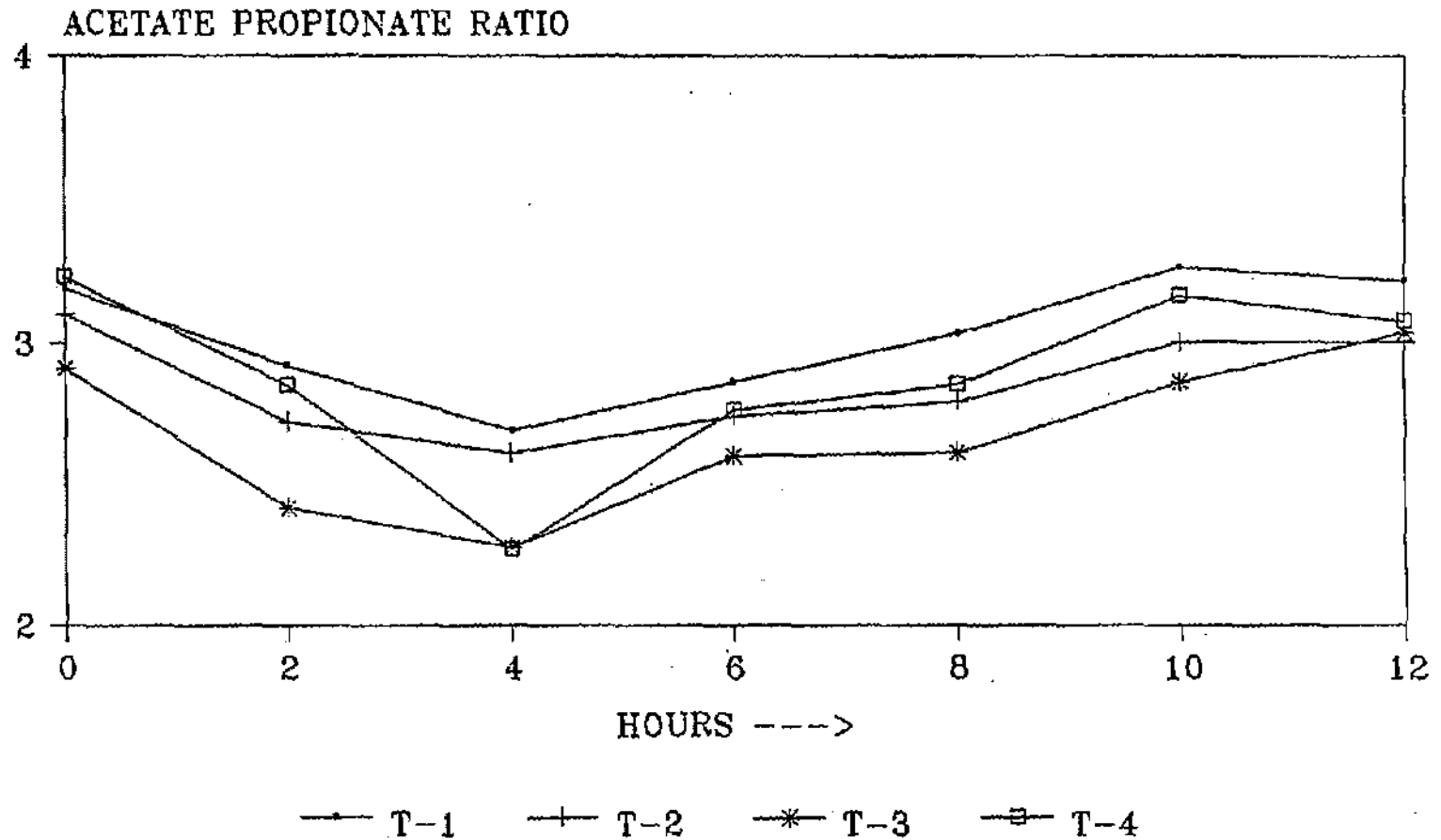
T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

concentration of butyric acid. In treatment group T_2 butyric acid concentration was lowered by 10.93, 16.67, 19.75, 44.44, 35.00, 26.43 and 34.00 percent at 0, 2, 4, 6, 8, 10 and 12 h post feeding respectively compared to control group (T_4). Similarly in treatment group T_3 butyric acid concentration was lowered by 8.33, 21.30, 3.75, 27.59 and 42.00 percent at 2, 6, 8, 10 and 12 h post feeding respectively compared to control group (T_4). However, butyric acid concentration at 0 and 4 h post feeding were 84.34 and 14.81 percent higher respectively.

5.1.5 Acetate:propionate ratio in the rumen

Acetate to propionate ratio in different hours and groups are presented in Table 5.4 (Fig. 5.7). The ratio dropped after feeding and was lowest at 4h post feeding. Thereafter, it rose gradually in all treatment groups upto 12h post feeding. In treatment group T_1 , except at 0h post feeding (where ratio was 1.23 percent lower) acetate:propionate ratio were 2.45, 18.50, 3.62, 6.31, 3.16 and 4.56 percent higher at 2, 4, 6, 8, 10 and 12h post feeding over control group due to a higher acetate proportions. Similarly in treatment groups T_2 and T_3 at 4h post feeding acetate to propionate ratios were 14.97 and 0.44 percent higher respectively. However, at 0, 2, 6, 8, 10 and 12h post feeding, acetate to propionate ratios in both groups (T_2 and T_3) remained lower (4.02, 4.56, 0.72, 4.56, 5.06 and 2.28 percent in treatment group T_2 and 9.90,

Fig. 5.7 : ACETATE PROPIONATE RATIO AT DIFFERENT HOURS OF POST-FEEDING AND PROBIOTIC SUPPLEMENTS



T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B)
T₄, Control.

15.08, 5.79, 8.42, 9.49 and 1.30 percent in treatment group T_3 respectively) due to a higher propionate proportion.

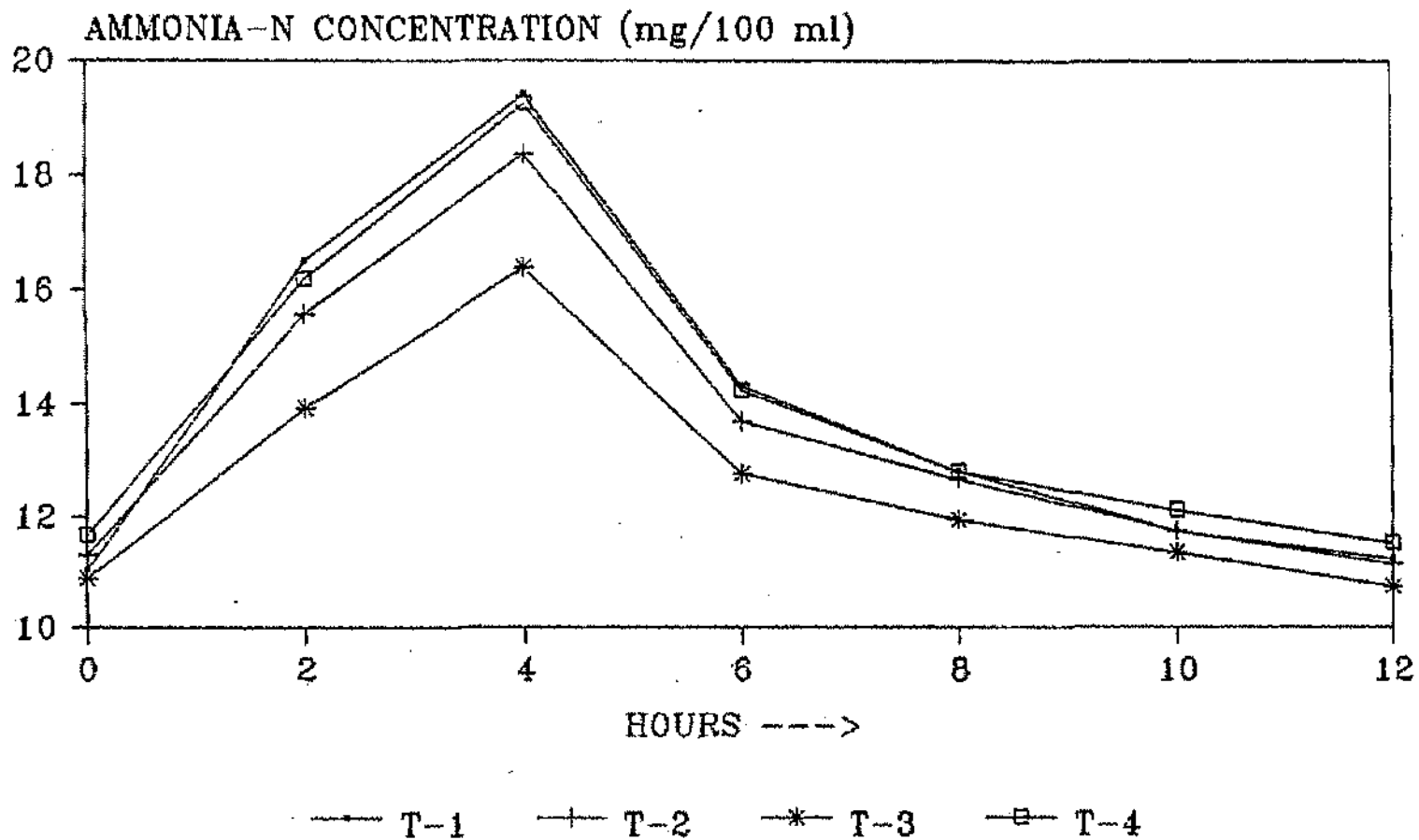
5.1.6 Levels of Ruminal ammonia-N

In all treatment groups ammonia-N (mg/100 ml) level increased after feeding and reached to peak at 4h post feeding (Table 5.4 and Fig. 5.8) in all treatment groups (T_1 19.44, T_2 18.39, T_3 16.38 and T_4 19.28). Level of ammonia-N in treatment group T_1 was 5.39, 2.89 and 2.51 percent lower at 0, 10 and 12h post feeding respectively. However, at 2, 4, 6 and 8h post feeding ammonia-N was 1.98, 0.83, 0.56 and 0.00 percent higher respectively over control group. Treatment group T_2 showed a lower ammonia-N concentration by 2.99, 3.77, 4.61, 3.79, 1.17, 2.89 and 3.20 percent at 0, 2, 4, 6, 8, 10 and 12h post feeding respectively as compared to control group. Similarly, treatment group T_3 showed lower concentration by 6.68, 13.91, 15.04, 10.40, 6.72, 6.03 and 6.85 percent at 0, 2, 4, 6, 8, 10 and 12h post feeding respectively. Supplementation of L.acidophilus (I) seems to have almost no effect. However, S.cerevisiae supplementation tended to lower the ammonia-N concentration particularly at the peak.

5.1.7 Levels of Microbial protein in the rumen

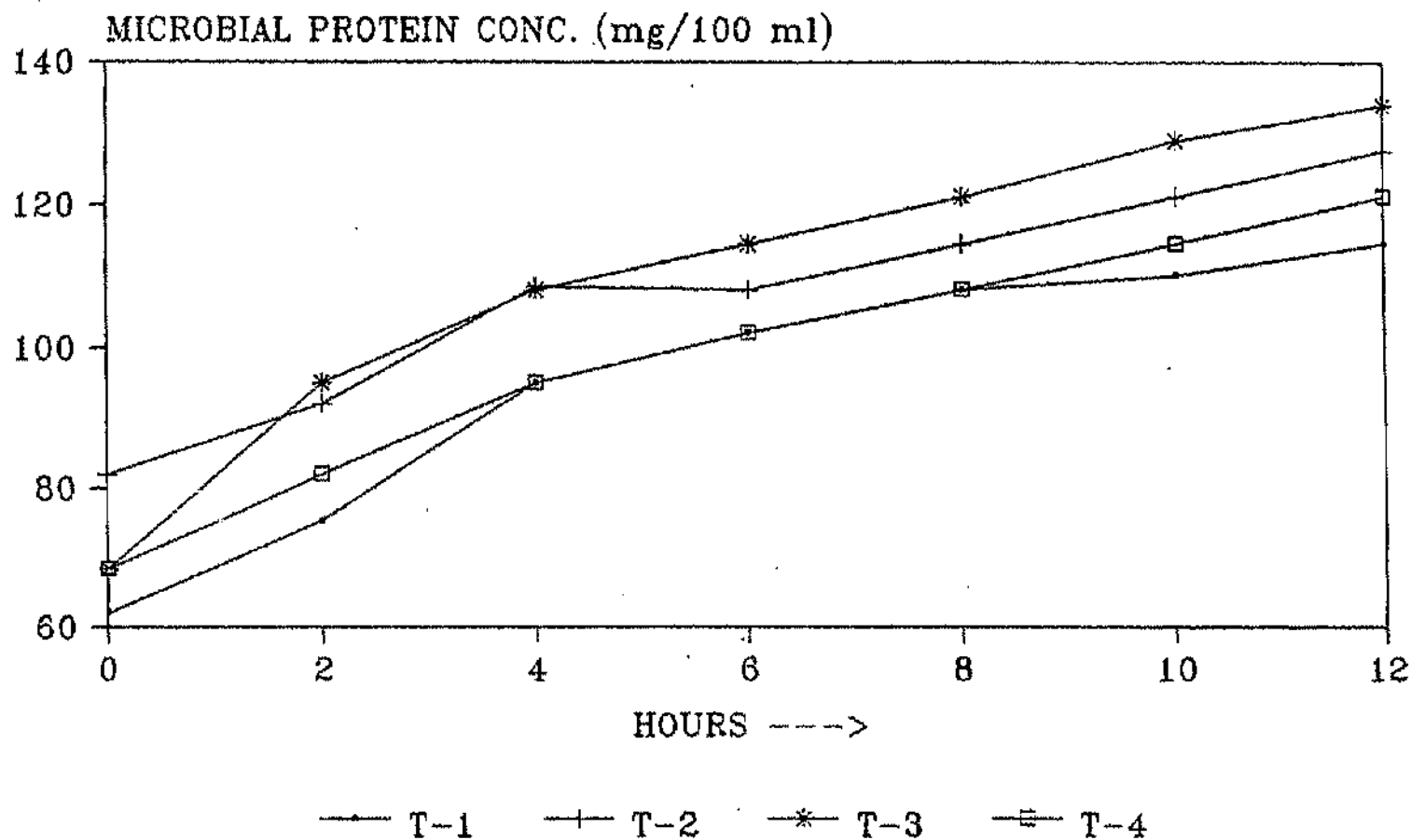
Microbial protein levels in the rumen liquor at different hours and treatment groups are presented in Table 5.5 (Fig 5.9). In all treatment groups level of

Fig. 5.8 : AMMONIA-N CONCENTRATION AT DIFFERENT HOURS OF POST-FEEDING AND PROBIOTIC SUPPLEMENTS



T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

Fig. 5.9 : MICROBIAL PROTEIN CONCENTRATION AT DIFFERENT HOURS OF POST-FEEDING AND PROBIOTIC SUPPLEMENTS



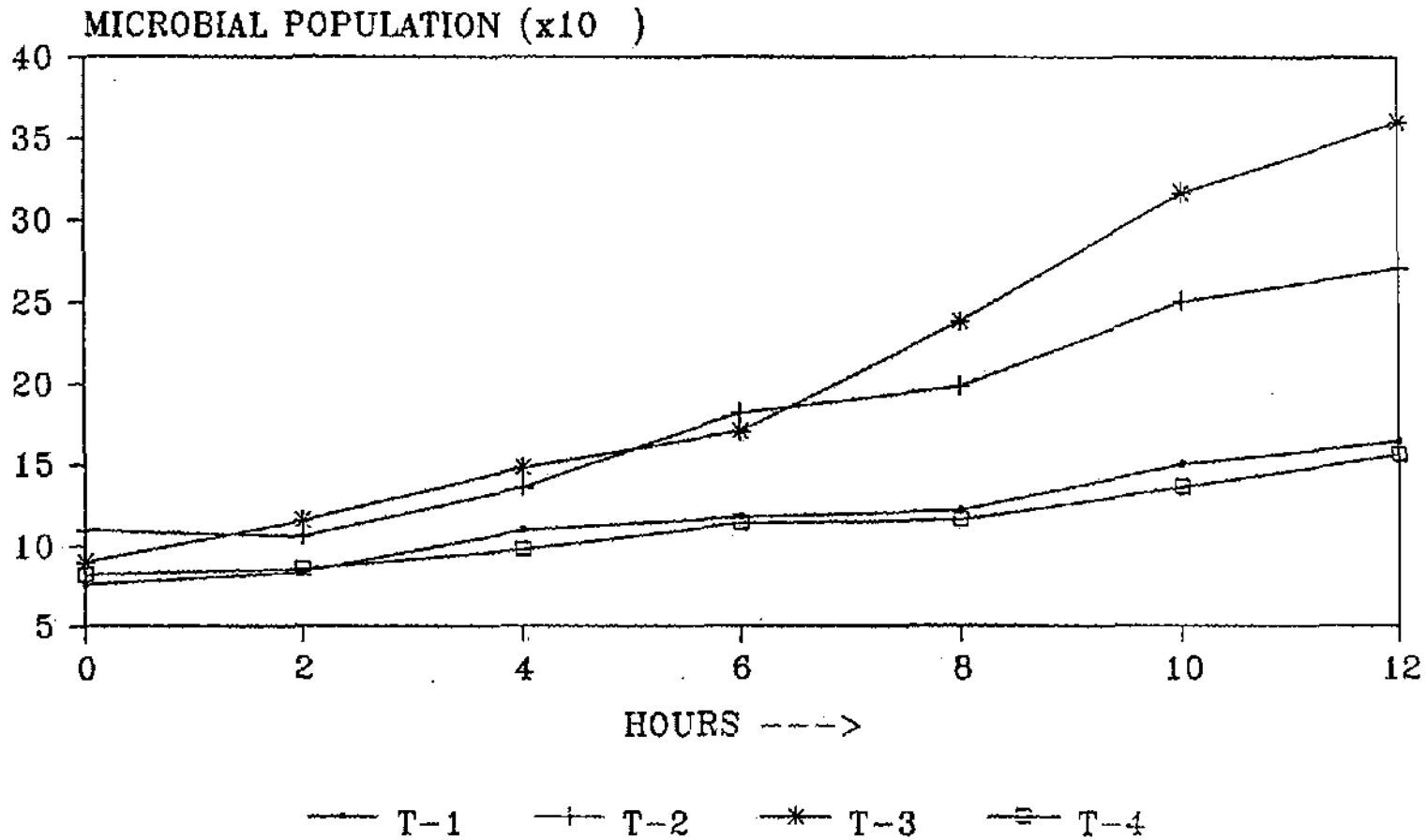
T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

microbial protein gradually increased after feeding. Treatment group T_1 showed less or almost similar microbial protein level compared to control group (T_4). The values were 9.48, 7.92, 0.00, 0.00, 0.00, 3.93 and 5.37 percent higher at 0, 2, 4, 6, 8, 10 and 12h of post feeding respectively. However, treatment groups T_2 and T_3 showed comparatively higher microbial protein levels in comparison to control group (T_4). In treatment group T_2 microbial protein levels were 12.19, 14.21, 5.88, 6.01, 5.67 and 5.37 percent higher at 2, 4, 6, 8, 10 and 12h of post-feeding, respectively. Similarly treatment group T_3 showed 0.00, 15.85, 13.68, 12.25, 12.03, 12.66 and 10.74 percent higher microbial protein respectively over control group (T_4).

5.1.8 Microbial population in the rumen content

Table 5.5 (Fig. 5.10) showed the microbial population in the rumen liquor at different hours and treatment groups. Microbial population increased gradually after feeding and reached to a maximum level at 12h post feeding in all treatment groups. An unexpected high microbial population was noticed in group T_3 followed by treatment group T_2 . In Treatment group T_1 microbial counts were 7.31 and 2.32 percent lower at 0 and 2h post feeding respectively over control group (T_4). However, 4h after feeding microbial population increased by 12.28, 3.50, 5.17, 10.29 and 5.12 percent at 4, 6, 8, 10 and 12h post feeding respectively compared to control group. Treatment group T_2 and T_3 also

Fig. 5.10 : MICROBIAL POPULATION AT DIFFERENT HOURS OF POST-FEEDING AND PROBIOTIC SUPPLEMENTS



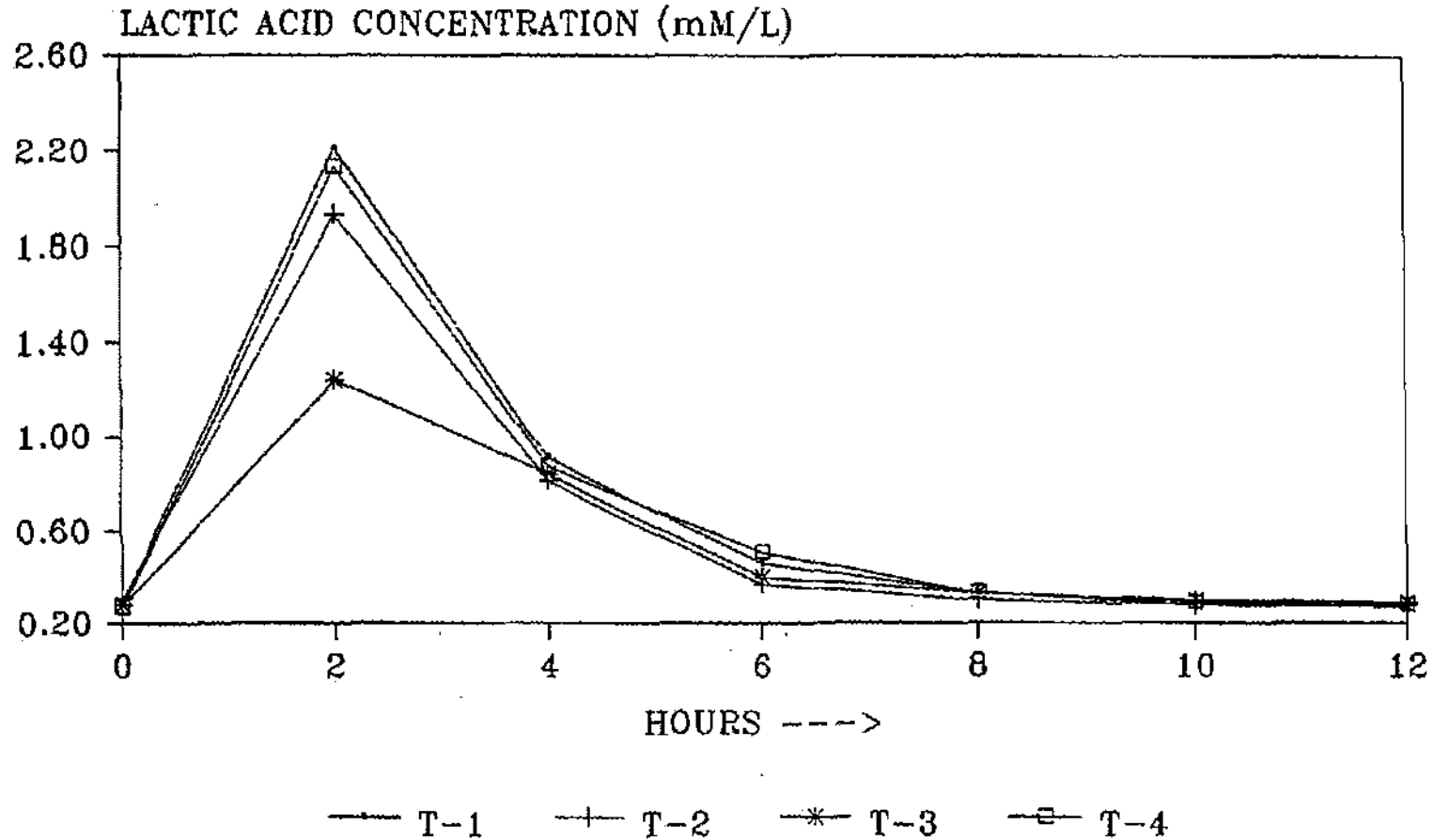
T₁, *L. acidophilus* (I); T₂, *S. cerevisiae* (B); T₃, *L. acidophilus* (I) + *S. cerevisiae* (B)
T₄, Control.

showed a higher microbial counts over control group (T_4) and (T_1) treatment groups. In treatment group T_2 microbial counts were 34.14, 23.25, 38.77, 59.64, 70.68, 83.82 and 73.07 percent higher at 2, 4, 6, 8, 10 and 12h post feeding respectively over control group. Similar but a higher response was observed in treatment group T_3 and the microbial counts were 9.75, 34.88, 57.02, 49.12, 105.17, 132.35 and 130.76 percent higher at 0, 2, 4, 6, 8, 10 and 12h post-feeding respectively compared to control group.

5.1.9 Lactic acid concentration in the rumen:

Lactic acid levels in the rumen liquor during different hours and treatment groups are presented in Table 5.6 (Fig. 5.11). Level of lactic acid reached to a maximum level after 2h of feeding, thereafter, it declined gradually. At 2h post feeding the level of lactic acid was considerably lower in treatment group T_3 (1.24 mM/l) which increased to 1.93 mM/l in treatment group T_2 , 2.13 mM/l in treatment group T_4 and 2.21 mM/l in treatment group T_1 . At 0h post feeding lactic acid concentrations were 7.40, 14.81 and 3.70 percent higher in treatment groups T_1 , T_2 and T_3 respectively over control group (T_4). Similarly treatment group T_1 also showed a higher concentration of lactic acid upto 4h post feeding. At 2 and 4h post feeding it was 3.75 and 4.59 percent higher respectively over control group (T_4). However, after 6, 8, 10 and 12h post feeding lactic

Fig. 5.11 : LACTIC ACID CONCENTRATION AT
DIFFERENT HOURS OF POST-FEEDING AND
PROBIOTIC SUPPLEMENTS



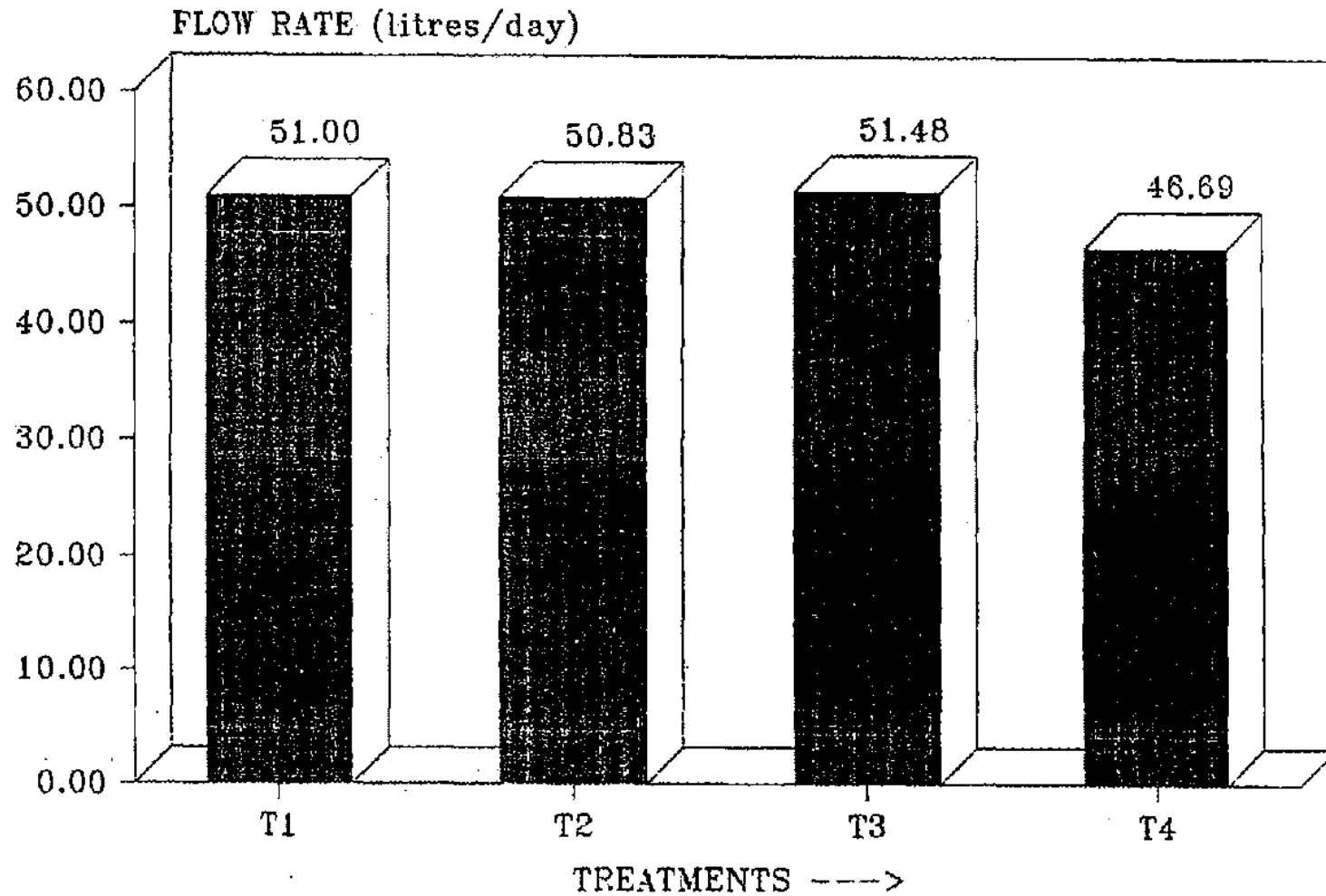
T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B)
T₄, Control.

acid concentration dropped by 9.80, 0.00 and 3.44 percent respectively over control group (T_4). Treatment group T_2 showed a lower concentration of lactic acid 9.38, 6.89, 27.45, 9.09 and 3.44 percent at 2, 4, 6, 8 and 10h of post feeding respectively. In treatment group T_3 lactic acid concentrations were 41.78, 3.44 and 21.56 percent lower at 2, 4, and 6h of post feeding respectively over control group (T_4). However, at 10 and 12h of post feeding it was 3.44 and 3.57 percent higher respectively compared to control group (T_4).

5.1.10 Flow rate of liquid digesta:

Ruminal digesta flow kinetics are presented in Table 5.6 (Fig. 5.12). It could be seen from table that the average values of rumen fluid volume (litres) were 30.25, 25.91, 26.67 and 25.16 in the animals of treatment groups T_1 , T_2 , T_3 and T_4 respectively. On further calculation it was found that rumen fluid volume presented 9.53, 8.75, 8.84 and 8.46 percent of the body weight in the animals of treatment groups T_1 , T_2 , T_3 and T_4 , respectively. Flow rates of liquid digesta (l/d) ranged between 46.69 (control group, T_4) to 51.48 (group, T_3). Flow rate of liquid digesta were 9.23, 8.86 and 10.25 percent higher in treatment groups T_1 , T_2 and T_3 over control group (T_4).

Fig. 5.12 : FLOW RATE OF LIQUID DIGESTA
ON DIFFERENT PROBIOTIC SUPPLEMENTS



T₁, L.acidophilus (I); T₂, S.cerevisiae (B); T₃, L.acidophilus (I) + S.cerevisiae (B)
T₄, Control.

5.2 DISCUSSION

5.2.1 DM disappearance in Nylon bags:

DM disappearance at 6h incubations were significantly higher in T_2 (by 10.23%) and T_3 (by 16.55%) treatment groups when compared to control group. The difference between treatment group T_1 (32.52%) and T_4 (32.14%) was non-significant. Similar response was obtained at 12h incubation. DM degradability (12h) were significantly higher by 7.21% in T_2 and by 11.66% in T_3 treatment groups over control group. At 24h incubations DM degradability was significantly higher by 4.97% in treatment group T_3 over control group. The difference between other treatment groups was non-significant. DM degradability (48h) was significantly higher by 3.40% in T_2 and by 5.24% in T_3 treatment groups over control group. However, the difference between T_2 and T_3 treatment groups was non-significant. It concluded that supplementation of S.cerevisiae (B) either alone or in combination with L.acidophilus (I) enhanced the DM degradability. As DM degradability in S.cerevisiae supplemented group (T_2) increased from 35.43, 45.48, 55.93 and 63.50 percent at 6, 12, 24 and 48h to 37.46, 47.37, 57.28 and 64.63 percent respectively when S.cerevisiae was supplemented with L.acidophilus (I) in treatment group T_3 . Similar results

have been reported by earlier workers (Gomez - Alarcon et al., 1987; Weidmeier et al., 1987; Arambel and Kent, 1990; Chademana and Offer, 1990). However, other workers obtained no such response (Harrison et al., 1988; Carro et al. (1992. Mutsvangwa et al., 1992). This could be due to difference of microbial strain supplemented in this experiment or differences of survivability of microorganisms at the time of feeding. In the present experiment DM degradability differences between different treatment groups were greater upto 12h of incubation and thereafter it declined. In the presence of yeast culture, the lag time for DM degradation reduced. Chademana and Offer (1990) confirmed the findings of present study and reported increased DM disappearance on rations varying in concentrate:forage proportion (10:90 to 60:40) and supplemented with yeast culture when incubated in the rumen upto 24h. However, no effect was visible at 48h incubation. Similar responses have been reported by Dawson et al. (1987), Williams and Newold (1990b), Williams et al. (1991). However, Ayala et al. (1992) found that supplementation of S. cerevisiae significantly improved the DM degradability upto 96h.

5.2.2 Fibre degradability:

At 6h incubation treatment group T_2 (23.10%) and T_3 (23.88%) have significantly higher fiber degradability (by 14.98 and 18.87 percent) over control group (20.09%). At 12h of incubation treatment group T_3 (25.65%) had significantly (by 8.87%) higher fiber degradability over

control group (23.56%). Fiber degradability (12h) in treatment groups T_2 (24.23%), T_1 (23.90%) and control group (T_4) (23.56%) were non-significantly different. Similarly at 24 and 48h of incubation, there was a non-significant difference among the treatments. The improvement in the fiber degradability during first 12h of incubation supports the data of earlier studies (Wallace and Raleigh, 1960; Weidmeier et al., 1987; Williams, 1989b; Chandemana and Offer, 1990 and Offer, 1990). The ability of S.cerevisiae to stimulate fiber digestion in the hind gut of horses is also reported (Glade, 1988; Glade and sist, 1988 and Pagan, 1990). Weidmeier et al. (1987) observed that addition of S.cerevisiae in the ration of ruminants increased the number and proportion of cellulolytic bacteria in the rumen, thereby resulting into improved digestibility of structural carbohydrate. The precise mechanism by which S.cerevisiae modifies the degradation of forage not known. It seems likely that the S.cerevisiae stimulate the initial colonisation of the plant fragment in the rumen allowing more rapid commencement of fiber breakdown.

5.2.3 Ruminal total volatile fatty acids (TVFA) concentration :

A non-significant difference of TVFA concentration existed before feeding among all the treatment groups. The TVFA concentration in treatment groups T_1 did not differ significantly from 0 to 12h post feeding when compared to

control group. In treatment group T_3 TVFA concentration was significantly higher by 6.64, 12.50, 11.13, 9.81, 11.80, 17.49 and 7.19 percent at 0, 2, 4, 6, 8, 10 and 12h post feeding respectively. The concentration was significantly higher by 6.29 and 7.19 percent in treatment group T_2 at 2h and 4h post feeding. It appears from present data that supplementation of L.acidophilus (I) possessed no effect on TVFA production. Whereas supplementation of S.cerevisiae increased the production. Again supplementation of S.cerevisiae (B) with L.acidophilus had a prominent effect on TVFA production. The data of Mutsvangwa et al. (1992) supported the present observations that total concentration of VFA were significantly higher in bulls receiving S.cerevisiae as compared with that of control. Several other workers (Newman and Dawson, 1987; Dawson and Newman, 1988; Harrison et al., 1988; Williams et al., 1988; Martin and Nisbet, 1989 and Carro et al., 1992) have reported a higher concentration of ruminal TVFA. However, other workers (Phillips and Von Tungelin, 1985; Chademan and Offer, 1990 and Frikins et al., 1990) found that total concentration of VFA was not affected by yeast culture supplement at any time. Harrison et al. (1988) reported that molar proportion of propionate and valerate was higher. A higher proportion of these VFA may account for a higher VFA level occurred in present experiment. Although the primary factor for a higher TVFA with addition of yeast culture is unclear.

5.2.4 Concentration of individual volatile fatty acids in the rumen:

The concentration of acetate in all treatment groups was statistically similar at different hours except at 4h post feeding in T_2 treatment group, where acetate concentration was higher compared to control group (62.9 Vs 49.9 mM/100 M). Similarly the concentration of propionate in T_1 treatment group was also statistically similar at different hours. However, the concentration of propionate improved in treatment groups T_2 and T_3 . In the treatment group T_2 the propionate concentration was significantly higher at 2, 4, and 6h post-feeding (by 9.91, 9.55 and 10.42 percent respectively) compared to control group (T_4). Even propionate concentration at 0, 8, 10 and 12h post feeding was also improved in T_2 treatment group but did not reach to statistical significance. Treatment group T_3 showed a significantly improved propionate concentration (by 13.06, 15.45, 17.54 and 10.89 percent at 2, 4, 6 and 12h post feeding respectively) compared to control group (T_4). The concentration of propionate at 8h (23.5 mM/100 M) and 10h (22.3 mM/100 M) post feeding was also non-significantly higher in treatment group T_3 , compared to control group (T_4). The concentration of butyrate between different groups and hours was almost similar except in treatment group T_3 at 0h (11.8 mM/100M) and in control group (T_4) at

6h (10.8 mM/100 M), where exceptionally higher butyrate concentration was observed.

The results of the present study are at par with the results of Hession et al. (1992). They found that yeast culture supplement had no effect on acetate concentration. Harrison et al. (1987) found that yeast culture supplementation in fistulated Holstein cows showed increased concentration of propionic acid (27.4 Vs 30.0 M%) and did not affect the concentration of butyric acid (14.7 Vs 14.5 M%). Similarly Adams et al. (1981) reported that yeast culture supplement in the diet of lambs had no significant effect on acetic acid concentration (at 7h 63.2 Vs 61.2 and at 11h 62.2 Vs 60.9 mol/100 mol in yeast culture supplemented group). In the same experiment propionate concentration was increased (at 7h 22.5 Vs 24.9 and at 11h 24.1 Vs 25.6 mol/100 mol in yeast supplemented group) and concentration of butyric acid was not affected by yeast culture supplement (at 7h 11.7 Vs 11.0 and at 11h 11.3 Vs 10.9 mol/100 mol in yeast culture supplemented group). As in the present experiment a higher concentration of propionate with yeast culture was also obtained in steers by Malcolm and Kiesling (1990) (15.8 Vs 16.2 mol/100 mol), in sheep by Newbold et al. (1990) (132 Vs 165 m mol/mol) and in bulls by Mutasvangwa et al. (1992) (at 8h 31.74 Vs 33.87 and at 15h 33.71 Vs 36.79 mM/l).

However, the results of the present study differ from other studies. many workers reported that inclusion of yeast culture in the diet resulted in a higher acetic acid concentration (Harrison et al, 1987, 1988, Weidmeier et al, 1987, Williams, 1989, Malcolm and Kieslin, 1990, Nutsvangwa et al, 1992). This difference in VFA proportion might be due to level of yeast culture in the diet of animals. Williams (1989) compared two levels of yeast culture and found that concentration of acetate increased with increasing the level of yeast culture supplement. This also might be due to differences in the proportion of forage to concentrate in the diet. Carro et al. (1992) found a lower acetate and higher butyrate when yeast culture was included in the medium and low concentrate diets. However, yeast culture had the opposite effect in high concentrate diets. The data of the present study indicated that the variations depend on the feeding pattern and time of sampling. The microbial cultures influence fermentation pattern and alter fermentation stoichiometry.

5.2.5 Acetate propionate ratio in the rumen

Acetate:propionate ratio was non-significantly differed between all treatment groups except T_3 before the start of feeding. At all time intervals group T_1 showed a non-significantly higher acetate to propionate ratio, due to a higher tendency in acetate production. In contrast, group T_2 showed non-significantly lower acetate to propionate ratio at all time intervals of sampling.

Similarly in treatment group T_3 , acetate to propionate ratio was 9.90, 15.08, 16.17, 8.42 and 9.49 percent lower at 0, 2, 4, 8 and 10h of post feeding respectively over control group (T_4). The reduction in acetate to propionate ratio was due to a higher proportion of propionate at the expense of acetate. It appears from the present data that S.cerevisiae caused a shift in more propionate production at the cost of acetate. In the present trial the reduction in the acetate to propionate ratio confirm earlier results (Harrison et al., 1988; Newbold et al., 1990; Carro et al., 1992) indicating that S.cerevisiae (B) with L.acidophilus (I) had a major effect on fermentation stoichiometry in the rumen. Several authors (Adams et al., 1981; Teh et al., 1987; Dawson and Newman, 1988; Martin and Nisbet, 1989; Chademana and Offer, 1990; Mutsvangwa et al., 1992) have reported either small or no change in acetate to propionate ratio when S.cerevisiae was supplemented to a high forage diet. In the present study, ruminal VFA patterns were altered by addition of S.cerevisiae (B) with L.acidophilus (I). Molar proportion of acetate was lower and molar proportion of propionate was greater in animals receiving mixed cultures. The shift in molar proportion of acetate and propionate resulted in a lower acetate to propionate ratio in rumen fluid of animal receiving mixed culture.

5.2.6 Levels of ruminal ammonia-N

Ammonia-N concentration was significantly lower by 5.39, 2.99 and 6.68 percent before feeding in treatment group T_1 , T_2 and T_3 respectively as against control group. In treatment group T_3 , ammonia-N was significantly lower by 6.68, 13.91, 15.04, 10.40, 6.72, 6.03 and 6.83 percent at 0, 2, 4, 6, 8, 10 and 12h post feeding respectively over control group (T_4). Similarly treatment group T_2 also showed a lower ammonia-N at all time intervals after feeding. Level of ammonia-N was significantly lower (by 3.77 and 4.61 percent) at 2 to 4h of post feeding. But in treatment group T_1 , ammonia-N concentration was similar as compared to control group (T_4). The data revealed that supplementation of S.cerevisiae (B) lowered the level of ammonia-N but combination L.acidophilus (I) had added effect. However, L.acidophilus (I) alone did not show any response over control. Greater concentration of total anaerobic bacteria due to increased protein synthesis (Table 5.5) supported the contention of lower level of ammonia concentration in animals fed S.cerevisiae. Ammonia is the preferential source of N for a large proportions of the ruminal microbial population (Bryant and Robinson, 1961) and incorporation of ammonia into ruminal bacteria has been demonstrated (Mathison and Milligan, 1971). Lower

concentration of ammonia in the rumen of animals fed S.cerevisiae (B) may reflect increased transportation of ammonia into microbial protein. A high proportion of microbial-N derived from ammonia-N with the yeast supplemented vesels as compared to the control one, support this suggestion (Carro et al., 1992). Similar results have been reported in vivo by Harrison et al. (1988), Newbold et al. (1990) and Chademana and Offer (1990) and in vitro by Dawson and Newman (1987) suggested an improved microbial capture of ammonia (Chademana and Offer, 1990). However, Adams et al. (1981), Frikins. et al. (1990) and Mutsvangwa et al. (1992) found that yeast culture had no significant effect on ruminal ammonia but bulls given yeast culture tended to have lower ruminal ammonia levels (Mutsvangwa et al., 1992).

5.2.7 Levels of microbial protein in the rumen:

Microbial protein level in rumen liquor drawn before feeding was non-significantly different among the treatment groups. The level in treatment group T₃ was significantly higher by 15.85, 13.68, 12.25, 12.03, 12.66 and 10.74 percent at 2, 4, 6, 8, 10 and 12h post feeding and in treatment group T₂ it was higher by 12.19, 14.21, 5.67 and 5.37 percent at 2, 4, 10 and 12h post feeding respectively over control group (T₄). The observations revealed that supplementation of S.cerevisiae (B) improved the microbial

protein synthesis in the rumen but L.acidophilus alone did not influence the microbial synthesis to any extent though in combination with S.cerevisiae exhibited a stimulatory effect. Similar responses were recorded by Harrison et al. (1988) and Weidmeier et al. (1987). Williams et al. (1991) found that presence of yeast culture tended to increase non-ammonia nitrogen at the duodenum in sheep. Similarly Weidmeier et al. (1987) indicated that supplementation of yeast culture enhanced microbial protein synthesis for which ammonia-N available during fermentation was effectively utilized. A lower level of ammonia-N and a higher microbial population support this contention (Cairo et al., 1992).

5.2.8 Microbial population in the rumen content:

The microbial population was non-significantly higher in 11 groups supplemented with S.cerevisiae (B) upto 48h post feeding compared to control group. However, after 6h post feeding the microbial population was significantly higher by 59.64, 70.68, 83.82 and 73.07 percent at 6, 8, 10 and 12h of post feeding in treatment group T_2 over control group (T_4). Similarly after 8 to 12h post feeding microbial population was significantly higher by 105.17, 132.35 and 130.76 percent at 8, 10 and 12h post feeding in treatment group T_3 over control group (T_4). Even microbial

population was significantly higher in treatment group T_3 as compared to treatment group T_2 after 10 to 12h post feeding. The present data clearly indicated that supplementation of S.cerevisiae (B) with or without L.acidophilus significantly increased the ruminal microbial population. The result suggested that S.cerevisiae increased the population of fiber degrading bacteria and their activity (Carro et al., 1992). S.cerevisiae may provide factors stimulatory to rumen cellulolytic bacteria (Bryant, 1973; Dawson et al., 1990) and proteolytic bacteria (Weidmeier et al., 1987; Williams, 1990a). Feeding yeast culture has increased number of cellulolytic rumen bacteria (Hoyos et al., 1987; Weidmeier et al., 1987; Harrison et al., 1988; Dawson, 1990; Firkins et al., 1990) and this has been confirmed by present experiment. Specific growth factors (B vitamins or branched chain fatty acids) exhibited with yeast culture have been reported to stimulate the growth of certain types of rumen bacteria (Dawson 1990; Chedemana and Offer, 1990). This mechanism could also explain the increased fiber degradability observed in our experiment with S.cerevisiae treatment.

5.2.9 Lactic acid concentration in the rumen:

Lactic acid concentration reached to a maximum at 2h Post-feeding, thereafter it declined gradually. The concentration of lactic acid before feeding was non-significantly different among all treatment groups. At

2h post feeding the lactic acid concentration was significantly lower (by 41.78%) in the treatment group T_3 over control group (T_4). Similarly treatment group T_2 also had a non-significantly lower (by 9.38%) concentration of lactic acid. At 4h post feeding a non-significantly lower concentration level of lactic acid was maintained in treatment groups T_2 and T_3 , except at 10 and 12h of post feeding in treatment group T_3 where lactic acid concentration was slightly higher over control group (T_4). In treatment group T_1 , a highest concentration of lactic acid was observed at 2h post feeding compared to treatment groups T_2 and T_3 . However, it was not significantly higher over control group (T_4). It appears that *S.cerevisiae* (B) with or without *L.acidophilus* (I) significantly lowered the concentration of lactic acid at peak hour (2h post feeding). Lactic acid is not a substrate used by *S.cerevisiae* for growth (Panchal, 1984a), therefore, the major reduction in lactate concentration may result from the use of lactate precursor or stimulation of lactate used by other microorganisms. A similar peak in lactic acid concentration was reported in cows fed starch based concentrate (Malestein *et al.*, 1981). Their peak concentration (6.5 mM/l) occurred 1h post feeding, followed by a rapid decline to a base line levels. Recent studies on sheep fed a mixed diet, have shown a similar significant reduction in ruminal lactate concentrations when yeast culture was supplemented to the

diet (Newbold et al., 1990). Similar responses were also recorded by other workers with a low ruminal pH (Williams, 1990b and Williams et al., 1990).

5.2.10 Flow rate of liquid digesta:

A non-significant difference was observed among the different treatment groups with respect to flow rate of liquid digesta. However, flow rate was higher by 10.25, 8.86 and 9.23 percnt in treatment groups T_3 , T_2 and T_1 , respectively, compared to control group (T_4). It seems that supplementation of S.cerevisiae (B) and L.acidophilus (I) had a tendency to increase the flow of liquid digesta but not significantly. The increased liquid dilution rate may improve the efficiency of bacterial growth and flow of α -linked glucose polymer, total amino acids and total microbial amino-acids to small intestine (Stouthamer and Bettenhausen, 1973; Harrison et al. 1975). Similar observations were made by Galyean et al. (1979), Rogers et al. (1979), Adams et al. (1981), Weidmier et al. (1987), Harrison et al. (1988) and Malcolm and Kiesling, (1990). Harrison et al. (1988) reported that liquid dilution rate was 10% higher in cows fed yeast culture as compared to control although difference was non-significant.

Table 5.1 Percent dry matter disappearance of substrate at different hours of incubation periods and probiotic supplements.

Incubation Period (h)	TREATMENT GROUPS							
	T ₁		T ₂		T ₃		T ₄	
	Measured	Fitted	Measured	Fitted	Measured	Fitted	Measured	Fitted
6	32.52 ^a ±0.80	32.39 ±0.84	35.43 ^b ±0.50	35.54 ±0.48	37.46 ^c ±0.51	37.51 ±0.55	32.14 ^a ±0.79	32.02 ±0.75
12	42.93 ^a ±0.95	43.27 ±0.91	45.48 ^b ±0.39	45.22 ±0.48	47.37 ^c ±0.53	47.26 ±0.42	42.42 ^a ±0.87	42.72 ±0.82
24	54.71 ^a ±0.64	54.44 ±0.65	55.93 ^{ab} ±0.46	55.99 ±0.16	57.28 ^b ±0.27	57.92 ±0.27	54.57 ^a ±0.56	54.31 ±0.60
48	60.86 ^a ±0.68	60.97 ±0.64	63.50 ^b ±0.61	63.43 ±0.53	64.63 ^b ±0.29	64.60 ±0.32	61.41 ^a ±0.13	61.50 ±0.11

a,b,c, figures with different superscripts in the same row differ significantly (P<0.01)

T₁, L.acidophilus (I); T₂, S.cerevisiae (B); T₃, L.acidophilus (I) + S.cerevisiae (B);

T₄, Without supplement. (Control).

Table 5.2 Percent crude fibre disappearance of substrate at different hours of incubation periods and probiotic supplements.

Incubation Period (h)	TREATMENT GROUPS							
	T ₁		T ₂		T ₃		T ₄	
	Measured	Fitted	Measured	Fitted	Measured	Fitted	Measured	Fitted
6	20.55 ^a ±1.31	20.53 ±1.21	23.10 ^b ±1.01	22.90 ±1.02	23.88 ^b ±0.52	23.85 ±0.57	20.09 ^a ±0.93	20.13 ±0.90
12	23.90 ^a ±1.29	23.86 ±1.30	24.23 ^{ab} ±1.03	24.79 ±0.98	25.65 ^b ±1.26	25.68 ±1.05	23.56 ^a ±1.21	23.84 ±1.28
24	26.88 ^a ±1.60	27.06 ±1.51	27.54 ^a ±1.00	27.17 ±1.06	27.97 ^a ±1.29	27.97 ±1.46	26.65 ^a ±1.40	26.74 ±1.34
48	28.92 ^a ±1.78	28.82 ±1.82	28.99 ^a ±1.35	29.11 ±1.32	29.94 ^a ±1.47	29.93 ±1.41	28.60 ^a ±1.06	28.56 ±1.08

a, b, c, figures with different superscripts in the same row differ significantly (P/0.01)

T₁, L.acidophilus (I); T₂, S.cerevisiae (B); T₃, L.acidophilus (I)+S. cerevisiae (B);

T₄, Without supplement (Control).

Table 5.3 Parameters in the equation of Orskov and Mc Donald (1979)¹ to describe the rate of degradation of DM and fibre in diets supplemented with different probiotic supplements.

Treatment groups	Dry Matter				Crude Fibre			
	a	b	c	d	a	b	c	d
T ₁	15.39 ±1.36	47.00 ±0.41	0.0752 ±0.0069	43.50 ±0.67	14.43 ±1.95	14.45 ±1.44	0.0837 ±0.0171	23.80 ±1.42
T ₂	21.15 ±1.09	44.31 ±00.89	0.0657 ±0.0052	46.20 ±0.29	20.28 ±1.17	9.66 ±0.47	0.0535 ±0.0064	25.20 ±1.10
T ₃	22.74 ±0.73	43.43 ±0.72	0.0693 ±0.0013	47.97 ±0.38	21.29 ±0.47	9.33 ±1.39	0.0484 ±0.0127	26.13 ±0.92
T ₄	15.65 ±1.30	47.58 ±1.14	0.0704 ±0.0056	43.40 ±0.46	14.53 ±0.50	14.48 ±0.84	0.0814 ±0.0159	23.43 ±0.99

¹ $P = a + b(1 - e^{-ct})$, where P=Percent of DM/Fibre degraded after time t (h);

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component of DM/fibre which will in time be degraded;

c = the rate constant for the degradation of the component with potential degradability;

d = effective degradability at a flow rate of 0.05 %/h.

T₁, *L.acidophilus* (I); T₂, *S.cerevisiae* (B); T₃, *L.acidophilus* (I) + *S.cerevisiae* (B);

T₄, Without supplement (Control).

Table 5.4 TVFA, individual VFA and acetate:propionate ratio at different hours post-feeding and probiotic supplements.

Treat- ment groups	h	TVFA (mM/100ml)	Acetate	Propionate		Butyrate	Acetate Propionate ratio
				(mM/100M)			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
T ₁	0	8.77±0.32 ^a	65.1 ^a	20.4 ^a	7.3 ^a	3.19 ^{ab}	
	2	11.33±0.30 ^a	64.5 ^a	22.1 ^a	6.6 ^a	2.92 ^b	
	4	12.32±0.29 ^a	66.8 ^a	21.1 ^a	8.8 ^a	2.69 ^b	
	6	14.37±0.34 ^a	62.1 ^a	21.7 ^{ab}	8.5 ^a	2.86 ^a	
	8	11.20±0.45 ^a	63.0 ^a	20.8 ^a	9.7 ^a	3.03 ^b	
	10	8.98±0.16 ^a	65.5 ^a	20.1 ^a	7.5 ^a	3.26 ^b	
	12	8.14±0.20 ^a	61.3 ^a	19.1 ^a	8.1 ^a	3.21 ^a	
Mean ±S.E.		10.73±0.84	64.1 ±0.75	20.8 ±0.38	8.1 ±0.39	3.02 ±0.08	
T ₂	0	9.51±0.28 ^a	65.4 ^a	21.1 ^a	5.7 ^a	3.10 ^{ab}	
	2	12.67±0.32 ^{bc}	66.4 ^a	24.4 ^b	6.0 ^a	2.72 ^b	
	4	13.86±0.33 ^b	62.9 ^b	24.1 ^b	6.5 ^a	2.61 ^b	
	6	15.64±0.35 ^a	63.8 ^a	23.3 ^{bc}	6.0 ^a	2.74 ^a	
	8	11.90±0.56 ^a	63.3 ^a	22.7 ^{ab}	5.2 ^a	2.79 ^{ab}	
	10	9.88±0.29 ^a	67.5 ^a	22.5 ^b	6.4 ^a	3.00 ^{ab}	
	12	8.63±0.11 ^a	66.0 ^a	22.0 ^{bc}	6.6 ^a	3.00 ^a	
Mean ±S.E.		11.73±0.96	65.1 ±0.66	22.9 ±0.44	6.1 ±0.19	2.85 ±0.07	
T ₃	0	9.47±0.33 ^a	59.1 ^a	20.3 ^a	11.8 ^b	2.91 ^a	
	2	13.41±0.39 ^c	60.7 ^a	25.1 ^b	6.6 ^a	2.42 ^a	
	4	14.37±0.41 ^b	57.9 ^a	25.4 ^b	9.3 ^a	2.28 ^a	
	6	16.56±0.35 ^b	64.5 ^a	24.8 ^c	8.5 ^a	2.60 ^a	
	8	13.16±0.30 ^b	61.3 ^a	23.5 ^b	8.3 ^a	2.61 ^a	
	10	11.89±0.50 ^c	63.8 ^a	22.3 ^b	6.3 ^a	2.86 ^a	
	12	9.39±0.07 ^b	67.9 ^a	22.4 ^c	5.8 ^a	3.03 ^a	
Mean ±S.E.		12.61±0.98	62.1 ±1.30	23.4 ±0.70	8.1 ±0.79	2.67 ±0.10	

...contd.

Table 5.5 Ammonia-N, Microbial protein, microbial counts and lactic acid concentration at different hours post-feeding and probiotic supplements.

Treat- ment group	Hours	Ammonia-N (mg/100ml)	Microbial protein (mg/100ml)	Microbial counts ^B ($\times 10^8$)	Lactic acid (mM/L)
(1)	(2)	(3)	(4)	(5)	(6)
T ₁	0	11.04±0.22 ^a	62.0±3.0 ^a	7.6±0.92 ^a	0.29±0.010 ^a
	2	16.49±0.50 ^c	75.5±3.5 ^a	8.4±1.03 ^a	0.21±0.010 ^c
	4	19.44±0.30 ^c	95.0±4.0 ^a	11.0±1.09 ^a	0.91±0.005 ^a
	6	14.31±0.37 ^c	102.0±3.0 ^a	11.8±1.18 ^a	0.46±0.010 ^a
	8	12.79±0.30 ^b	108.0±3.0 ^a	12.2±0.44 ^a	0.33±0.010 ^a
	10	11.74±0.23 ^{ac}	110.0±5.0 ^a	15.0±1.41 ^a	0.28±0.005 ^a
	12	11.24±0.12 ^{ab}	114.5±3.5 ^a	16.4±1.02 ^a	0.27±0.005 ^a
Mean±S.E.		13.86±1.18	95.29±7.4	11.8±1.21	0.68±0.027
T ₂	0	11.32±0.30 ^a	82.0±3.0 ^a	11.0±0.77 ^a	0.31±0.005 ^a
	2	15.56±0.38 ^b	92.0±7.0 ^{bc}	10.6±0.87 ^a	1.93±0.002 ^b
	4	18.39±0.26 ^b	108.5±9.5 ^b	13.6±1.44 ^a	0.81±0.070 ^a
	6	13.69±0.27 ^b	108.0±3.0 ^{ab}	18.2±1.16 ^b	0.37±0.020 ^a
	8	12.64±0.15 ^b	114.5±3.5 ^{ab}	19.8±1.16 ^b	0.30±0.005 ^a
	10	11.74±0.08 ^{ac}	121.0±3.0 ^{bc}	25.0±1.48 ^b	0.28±0.010 ^a
	12	11.16±0.20 ^{ab}	127.5±3.5 ^{bc}	27.0±0.94 ^b	0.28±0.005 ^a
Mean±S.E.		13.50±1.00	104.79±8.3	17.9±2.47	0.61±0.033
T ₃	0	10.89±0.33 ^a	68.5±3.5 ^a	9.0±1.00 ^a	0.28±0.005 ^a
	2	13.92±0.27 ^a	95.0±4.0 ^c	11.6±0.92 ^a	1.24±0.030 ^a
	4	16.38±0.42 ^a	108.0±3.0 ^b	14.8±1.07 ^a	0.84±0.005 ^a
	6	12.75±0.25 ^a	114.5±3.5 ^b	17.0±0.89 ^a	0.40±0.005 ^a
	8	11.93±0.21 ^a	121.0±3.0 ^b	23.8±2.48 ^b	0.33±0.000 ^a
	10	11.36±0.22 ^a	129.0±5.0 ^c	31.6±1.69 ^c	0.30±0.005 ^a
	12	10.74±0.15 ^a	134.0±3.0 ^c	36.0±4.03 ^c	0.29±0.000 ^a
Mean±S.E.		12.57±0.76	110.0±8.5	20.5±3.87	0.52±0.014

... Contd.

.....Contd... Table 5.5

(1)	(2)	(3)	(4)	(5)	(6)
	0	11.67±0.16 ^b	68.5±3.5 ^a	8.2±1.16 ^a	0.27±0.010 ^a
	2	16.17±0.46 ^c	82.0±8.0 ^{ab}	8.6±0.67 ^a	2.13±0.030 ^{bc}
	4	19.28±0.42 ^c	95.0±4.0 ^a	9.8±1.32 ^a	0.87±0.020 ^a
T ₄	6	14.23±0.46 ^{bc}	102.0±3.0 ^a	11.4±1.60 ^a	0.51±0.010 ^a
	8	12.79±0.23 ^b	108.0±3.0 ^a	11.6±1.81 ^a	0.33±0.020 ^a
	10	12.09±0.20 ^{bc}	114.5±3.5 ^{ab}	13.6±1.83 ^a	0.29±0.005 ^a
	12	11.53±0.22 ^b	121.0±3.0 ^{ab}	15.6±1.60 ^a	0.28±0.005 ^a
Mean±S.E.		13.97±1.08	98.71±7.0	11.3±1.01	0.67±0.026

a, b, c, figures with different superscripts at similar hours in a column differ significantly (P < 0.01).

T₁, L. acidophilus (I); T₂, S. cerevisiae (B);

T₃, L. acidophilus (I) + S. cerevisiae (B);

T₄, Without supplement (Control).

Table 5.6 Rumen fluid volume and flow rate of liquid digesta on different probiotic supplements.

Treatment groups	<u>PARAMETERS</u>			
	Body weights of animals (Kg)	Rumen fluid volume (Lit.)	Rumen fluid volume as percentage of body weight (%)	Flow rate of liquid digesta (Lit./day)
T ₁	316.67±6.39 ^a	30.25±2.39 ^a	9.53±0.57 ^a	51.00±3.79 ^a
T ₂	296.00±3.46 ^a	25.91±0.73 ^a	8.75±0.17 ^a	50.83±7.28 ^a
T ₃	301.67±5.04 ^a	26.67±0.41 ^a	8.84±0.04 ^a	51.48±7.76 ^a
T ₄	297.33±5.21 ^a	25.16±0.85 ^a	8.46±0.16 ^a	46.69±3.85 ^a

a,b,c, figures with different superscripts in the same row differ significantly (P<0.01)

T₁, L.acidophilus (I); T₂, S.cerevisiae (B); T₃, L.acidophilus (I) + S.cerevisiae (B);

T₄, Without supplement (Control).

Table 5.7 Statistical analysis of different parameters of *in vivo* rumen fermentation.

	d.f.	MSS		d.f.	MSS	
		DM	OM		TVFA	NH ₃ -N
Between hours	3	1854.38	121.34	6	212.61	254.24
Between treatments	3	48.86*	11.06*	3	31.69*	25.10*
Error	41	1.02	3.98	242	0.93	11.30

	d.f.	MSS				d.f.	MSS	
		Acetate	Prop.	Buty.	Acetate:Prop		M. Protein	Lactic Acid
Between hours	6	17.41	10.93	8.29	0.14	6	3351.41	2.79
Between treatments	3	33.43	4.78*	1.50	0.23*	3	594.92*	0.07*
Error	18	8.46	0.52	2.18	0.01	46	28.69	0.02

	d.f.	MSS		d.f.	MSS
		Rumen fluid Vol.	Flow rate		Microbial counts
Between hours	2	5.35	370.49	6	603.21
Between Treatments	3	15.23	14.8	3	682.71*
Error	6	5.38	18.9	130	21.29

* Significant at 5% level.

CHAPTER - VI

PHASE - III

GROWTH RATE NUTRIENT UTILISATION AND FEED

CONVERSION EFFICIENCY ON VARIOUS PROBIOTIC SUPPLEMENTS

PHASE - III

6. Growth rate nutrient utilisation and feed conversion efficiency on various probiotic supplements

6.1 RESULTS

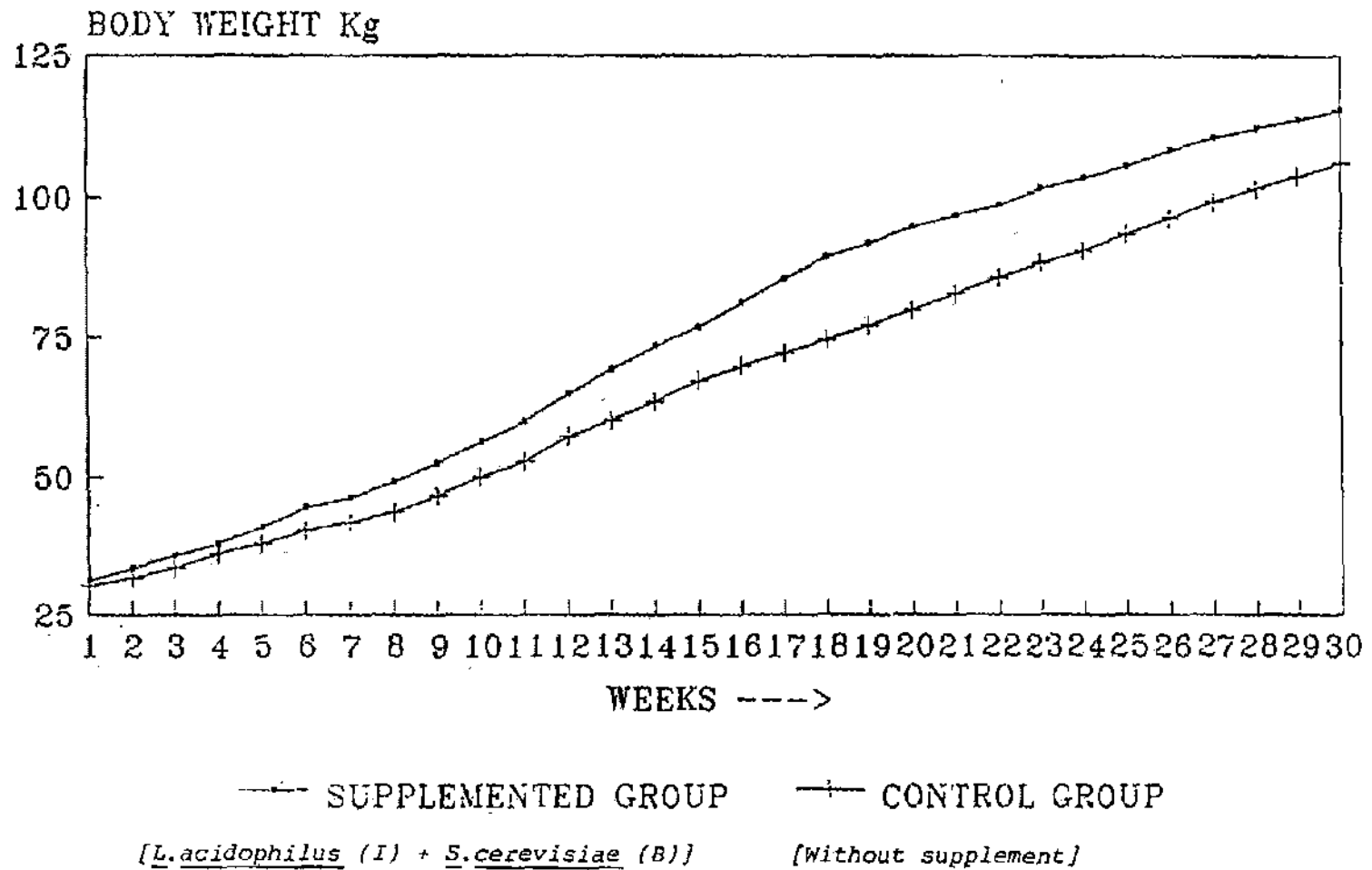
Earlier observations on in vitro and in vivo experiments revealed that supplementation of a mixed culture of S.cerevisiae (B) with L.acidophilus (I) proved better compared to other treatments. Therefore two growth trials were conducted, one on pre-ruminant and the other on post-ruminant calves supplementing the above mixed culture. With

pre-ruminant calves (1 week old) a growth trial of 98 days duration was conducted on 20 animals, divided into two groups of ten animals in each group. Another growth trial was conducted on other 20 post-ruminant calves, divided into two groups of ten animals in each group, for a period of 238 days. A metabolic trial was conducted on all 20 animals in the post-ruminant group towards the end of growth trials. The results of this study are presented in ensuing paragraphs.

6.1.1 Effect of supplementation on live weight gain in pre-ruminant calves :

The data on live weight gain in pre-ruminant growing calves is presented in Table 6.1 (Fig 6.1). While the initial body weights were similar between groups, the body weight after 98 days of experimental feeding were 67.1 and 76.7 kg in control and supplemented groups respectively. The total body weight gain in 98 days was lower in control group (36.9 kg) than the supplemented group (45.6 kg). The average daily body weight gain (g) was 376.53 and 466.21 in control and supplemented groups respectively. After discontinuation of culture supplement average final body weight (after 98 days) also appeared higher in supplemented group (115.3 kg) in comparison to control group (105.9 kg). However, the total gain in body weight during

Fig. 6.1 : BODY WEIGHT GAIN IN PRE-RUMINANT CALVES WITH AND WITHOUT PROBIOTIC SUPPLEMENT



post-supplementation looked more or less similar in both groups. The average daily body weight gain was comparatively higher in supplemented group (395.92 g/d) over control group (390.82 g/d). From these results it appeared that during culture supplementation the calves grew at a faster rate. However, on withdrawal of supplementation the rate of growth slowed down.

6.1.2 Effect of supplementation on incidence of diarrhoea in the pre-ruminant calves

In general the occurrence of diarrhoea, expected to be higher in pre-ruminant calves. Therefore, the calves were inspected daily and cases of diarrhoea were recorded in both groups. It was observed that calves remained in diarrhoea for 5.9 days in control and 3.8 days in supplemented group (Table 6.1). Incidence of diarrhoea seems to be less pronounced in culture supplemented group compared to control group. However, supplementation do not have a complete check on diarrhoea.

6.1.3 Feed Conversion efficiency and feed cost per unit gain in pre-ruminant calves

The total DM intake through feed during 98 days was 68.26 kg in control and 74.41 kg in culture supplemented group (Table 6.2). The average DM consumption per kg of live weight gain appears to be lower in culture supplemented group (1.72 Vs 2.08 kg) as compared to control group.

Similarly, feed cost per kg of live weight gain was considerably less in group supplemented with culture (Rs. 49.60 Vs 61.00) in comparison to control group.

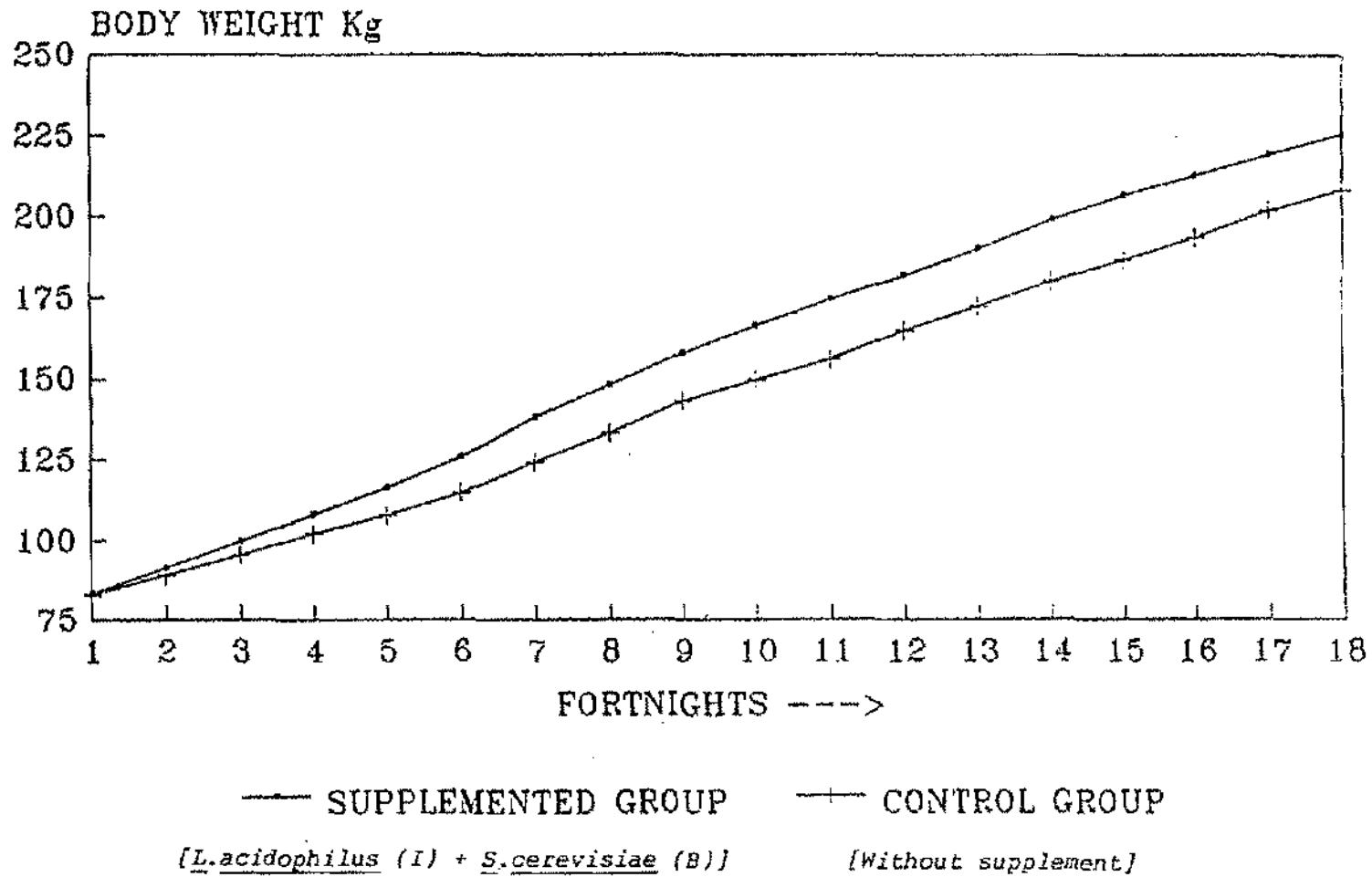
6.1.4 Effect of supplementation on live weight gain in post-ruminant calves

Average live body weight of post-ruminant calves taken fortnightly presented in Table 6.3 (Fig. 6.2). The mean initial body weight of calves on control group was 82.9 kg and on culture supplemented group 83.1 kg. All the calves gained body weight during the experimental period and attained the average body weight of 208.0 and 225.0 kg on respective treatment at the end of growth trial (238 days). Total body weight gain during the 238 days of feeding trial was higher in supplemented group (141.9 kg) over control group (125.1 kg) representing 596.19 g daily weight gain in supplemented group compared to 525.63 g in control group.

6.1.5 Effect of supplementation on feed conversion efficiency and feed cost per unit gain in post-ruminant calves

Table 6.4 presents the efficiencies of feed conversion towards live weight gain in post-ruminant calves fed with and without supplementation of culture. The average total DM intake during the 238 days of feeding trial were

Fig. 6.2 : BODY WEIGHT GAIN IN POST-RUMINANT CALVES WITH AND WITHOUT PROBIOTIC SUPPLEMENT



809.16 and 860.43 kg in control and supplemented groups respectively. However, feed consumed per kg of live body weight gain was lower in culture supplemented group (6.11 kg) Vs control group (6.55 kg). Total feed cost during feeding trial was high in the culture supplemented group (Rs. 4173.15) over control group (Rs 3912.94). Whereas feed cost per kg of live body weight gain was Rs 31.48 in control and Rs 29.60 in the culture supplemented group.

6.1.6 Chemical composition of feeds and residues:

Table 6.5 provides information on the percent chemical constituents of green fodder (Berseem) and concentrate offered and residue left during metabolic trial.

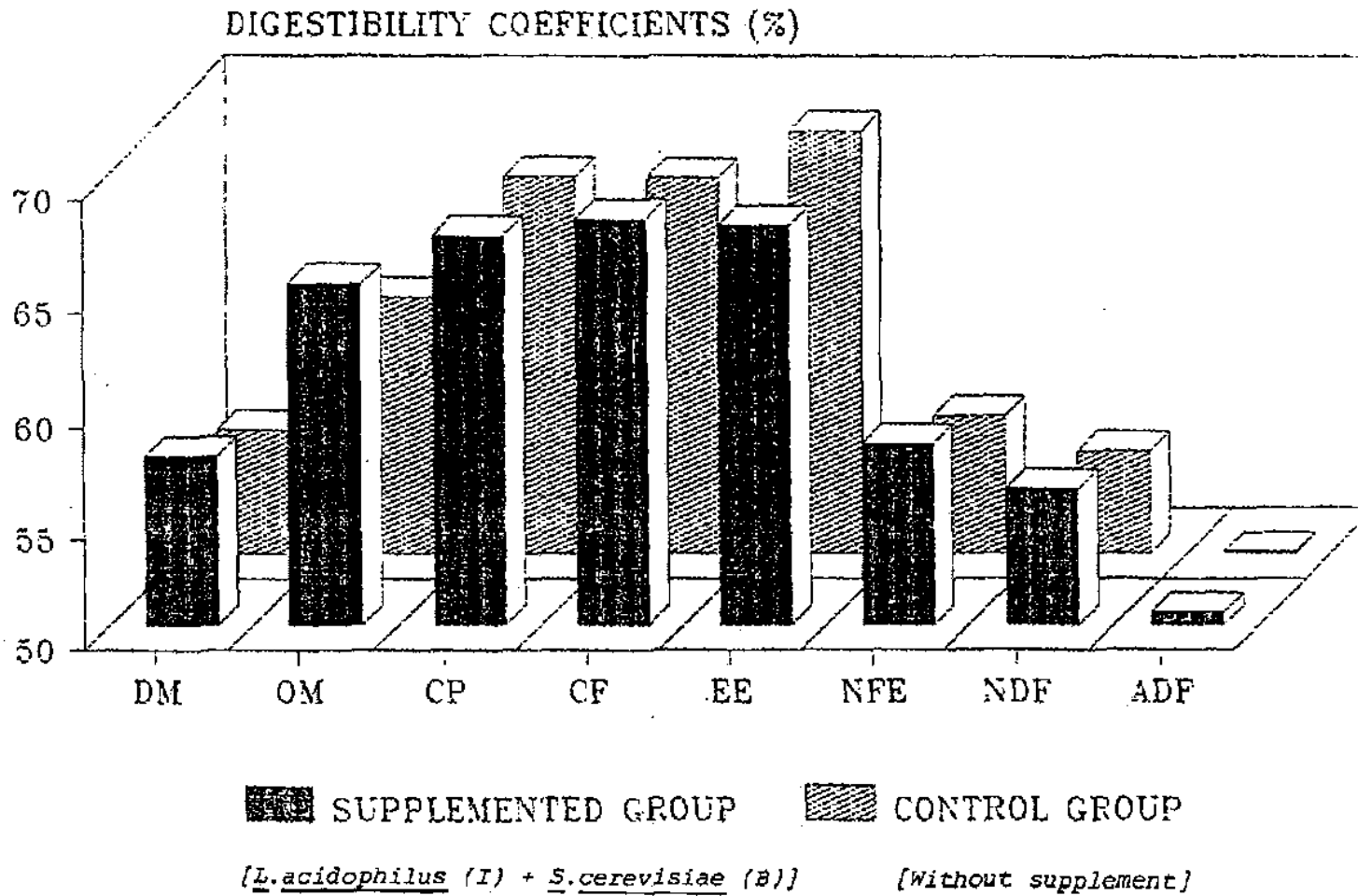
6.1.7 Effect of supplementation on feed intake by post-ruminant calves during metabolic trial

The data showing average body weights, and DM intake from concentrate and fodder has been displayed in Table 6.6. The total DM intake was higher in group supplemented with culture (5.42 kg/d) as compared to control group (4.90 kg/d). The DM intake per 100 kg body weight was incidently similar in both groups. However, DM intake per kg $W^{0.75}$ was lower for control group (105.26 kg) over supplemented group.

6.1.8 Effect of supplementation on digestibility coefficients of feed constituents

perusal of Table 6.7 (Fig 6.3) indicated that digestibility of DM, OM, CP, CF, NFE, NDF & ADF appeared to

Fig. 6.3 : DIGESTIBILITY COEFFICIENTS OF NUTRIENTS IN POST-RUMINANT CALVES WITH AND WITHOUT PROBIOTIC SUPPLEMENT



be higher in the group of animals supplemented with culture than those of control group. However, digestibility of EE was slightly lower in culture supplemented group (66.73%, over control (67.73%). Digestibility coefficient of ADF was almost similar in group supplemented with culture (50.57%) and control group (50.06%).

6.1.9 Effect of supplementation on nutrient intake in post-ruminant calves during metabolic trial

Table 6.8 shows the data on intake of different nutrients of $g/kg W^{0.75}$ and $kg/day/100$ kg body weight in both groups. Total intake of CP (g/day) was 649.54 and 688.88 in control and culture supplemented groups respectively. The CP intake per 100 kg body weight was similar in control group (369.71 g/day) and supplemented group (372.59 g/day). The CP intake per kg of metabolic body size was also not different in the supplemented group compared to control group (13.73 Vs 13.83 g/day). DCP intake was higher in group supplemented with culture over control group (463.31 Vs 432.45 g/day). However, DCP intake per 100 kg body weight was 259.03 and 255.79 g/day in control and supplemented groups respectively. Whereas DCP intake per kg of metabolic body size was 9.23g/d in supplemented group and 9.31 g/day in control group. TDN intake was 2.71 and 2.90 kg/day in control and supplemented groups, respectively. However, TDN intake per 100 kg body weight was 1.54 Vs 1.62 kg for supplemented and control group.

Similarly TDN intake per kg metabolic body size was slightly lower in supplemented group (57.45 g/day) than those of control group (58.18 g/day).

6.1.10 Effect of supplementation on nitrogen balance:

The average nitrogen intake (g/day) was 103.78 and 110.22 in control and supplemented groups, respectively (Table 6.9). Nitrogen excreted in dung appears to be higher in supplemented group over control (36.09 Vs 34.39 g/day). However, N excreted in dung as percentage of nitrogen intake was higher in control (33.06%) compared to supplemented group (32.71%). N excreted in urine was 43.70 and 43.51 g per day in control and supplemented group, respectively. However, N excreted in urine as percent of nitrogen intake was 41.91 and 39.39 in control and supplemented group, respectively. The efficiency of N-retained as per cent of N intake was higher in supplemented group (27.89%) over control group (25.49%). Similarly N-retained as percent of N-absorbed was higher in supplemented group (46.01%) over control (43.34%). From the data it appears that efficiency of nitrogen retention from feed and absorbed nitrogen was higher in calves fed culture supplemented feed.

6.2 DISCUSSION

6.2.1 Effect of supplementation on live body weight gain in pre-ruminant calves :

Initial body weight in both groups were statistically similar ($P < 0.05$). However, at 98 day the body weight of

calves was significantly higher (by 14.31 percent) in treatment group supplemented with mixed culture (76.7 kg) compared to control group (67.1 kg). The total body weight gain during 98 days of supplementation in mixed culture supplemented group (45.6 kg) was significantly higher by 23.58 per cent over control group (36.9 kg). Similarly average daily body weight gain in culture supplemented group (466.21g) was significantly higher by 23.81 percent compared to control group (376.53 g). After discontinuation of supplementation final body weight (115.3 Vs 105.9 kg) and total body weight gain (38.8 Vs 38.3 kg) were similar in both the groups. It clearly indicates that supplement significantly increased growth rate of calves. At the same time when supplement is withdrawn growth slowed down to a normal level comparable to control. Similar results have been observed in young growing calves (Fallon and Harte, 1987 and Hughes, 1988). In both series of experiments yeast culture was included in the calf starter ration at 2 kg/tonne which significantly increased the live weight gain as a result of proportional increase in feed intake and digestibility. Similarly in the present experiment also a higher DM intake (Table 6.2) was noticed in culture supplemented group (74.41 Vs 68.20 kg). This indicates that additional nutrient came from improved intake and might also from improved digestibility of feed, as the DM intake

(Table 6.2) in supplemented group was lower (1.72 Vs 2.02 kg/kg gain in body weight).

6.2.2 Effect of supplementation on incidence of diarrhoea in pre-ruminant calves

The average days of diarrhoea per animal during 98 days was significantly lower by 35.59 per cent in supplemented group (3.8 days) compared to control group (5.9 days). Further in culture supplemented group only 6 animals suffered from diarrhoea, however, in control group all the 10 animals suffered. The result of the present study are consistent with the findings of Guo et al. (1990). They used Chinese, Russian and Japanese strains of lactic acid bacteria and found symptoms of diarrhoea disappeared with a cure rate of 95.81 per cent. Similar findings were also observed by Kimura (1984), Bonaldi et al. (1986), Kopenoy (1989) and Steven and Johnston (1988). They found that when selected strains of lactic acid bacteria included in the diet, the calves had no digestive disturbance with regard to diarrhoea. As earlier reported by Bechman et al. (1977) a low incidence of diarrhoea was due to attachment of lactic acid bacteria on intestinal epithelial with a removal of pathogenic microorganism. Gilliland et al. (1979) showed that feeding humans unfermented milk containing cells of L.acidophilus caused significant increase in the numbers of lactobacilli in their faeces. In a similar type of study in which unfermented whole milk containing cells of

L.acidophilus was fed to young calves, Ellinger et al. (1978) observed decrease in number of coliform bacteria in their faeces. It concluded from the present study that lactic acid bacteria with S.cerevisiae had a significant check on diarrhoea. However, further research need to carry out to select the strains to completely check the diarrhoea.

6.2.3. Effect of supplementation on feed conversion efficiency and feed cost per unit gain in pre-ruminant calves:

The statistical analysis of data ($P \leq 0.05$) on DM intake during 98 days culture supplementation significantly improved the DM intake by 9.11 per cent in supplemented group (74.41 kg) compared to control (68.20 kg). However, the DM intake per kg of live weight gain was significantly lower by 17.31 per cent in supplemented group (1.72 Vs 2.06 kg). This indicated that culture supplement in the pre-ruminant calf increased the efficiency of feed utilization. Similarly total feed cost and feed cost per kg gain in body weight were significantly lowered by 9.43 and 33.54 per cent respectively compared to control treatment. In several studies it has been found that yeast culture supplement significantly increased the feed intake in calves (Bonaldi et al., 1986; Fallon and Harte, 1987; Hughes, 1988), Steers (Adams et al., 1981), lactating cow (Malcolm and Kiesling, 1986; Quinonez et al., 1988, 1989; Williams 1990a; Williams et al., 1991; Wohlt et al., 1991)

and in bulls (Drennan, 1990). This additional DM intake by calves provided extra energy for a higher growth rate as observed in the present experiment.

Bhoi (1992) reported that supplementation of strains of yeast and lactic acid bacteria significantly increased the feed conversion efficiency in the kids. Similarly, Steven and Johnston (1988) also recorded 9 percent advantage in daily weight gain and 8 percent advantage in feed efficiency when only lactic acid bacterial culture was added in the diet of calves.

6.2.4 Effect of supplementation on live body weight

gain in post-ruminant calves :

The initial body weight in the both groups being similar, the body weight after 238 days of growth period were significantly higher by 8.17 percent (225 Vs 208 kg) in group supplemented with mixed culture of S.cerevisiae (B) + L.acidophilus (I) as compared to control group. Similarly total live body weight gain in supplemented group was significantly higher by 13.43 percent (141.90 Vs 125.10Kg) over control group. The average daily live body weight gain in supplemented group of calves was significantly higher by 13.42 per cent over control group calves.

The results of the present study corroborated to reports obtained with supplementation of S.cerevisiae and

lactic acid bacteria by Bonaldi et al. (1986), Kopecny et al. (1989), Vuetic et al. (1989) and Liberse (1991) in calves. However, Phillips and Vontungeln (1985), Quinonez et al. (1989), and Mutsvangwa et al. (1992) did not find positive response with supplementation of S.cerevisiae and lactic acid bacteria in the young and adult cattle. This lack of responses might be due to the differences of strains and quality of culture used in the present experiment. The supplementation of same strains [S.cerevisiae (B) + L.acidophilus (I)] as used in the present study has also resulted to improve the body weight gain significantly in kids (Bhoi, 1992).

Growth is influenced by different factors such as nutritional, hormonal, biochemical or genetical. Since there is no reason to suggest that culture supplementation stimulates hormone profiles in the body (Williams, 1990a), the influence of culture on rumen functioning might have affected the growth rate. The additional nutrients available in the diet due to higher intake might have also a supportive factor for increased growth rate. There may be an increase in the metabolisability of digesta absorbed from the rumen or change in rate of passage of the digesta.

6.2.5 Effect of supplementation on feed conversion efficiency and feed cost per unit gain in post-ruminant calves

Feed gain ratio is one of the important nutritional parameter to evaluate the effect of culture supplement in the diet of growing calves. The efficiency of feed supplement for growth in calves is discussed hereunder.

Feed conversion efficiency on live body weight gain in both groups is given in Table 6.4. Animals supplemented with mixed culture of S.cerevisiae (B) and L.acidophilus (I) consumed significantly higher DM by 6.34 percent (860.43 Vs 809.16 kg). However, the consumption of DM per kg of live body weight was significantly lower by 6.72 percent (6.11 Vs 6.55 kg/kg body weight gain). During trial total feed cost was significantly higher by 6.65 per cent (Rs 4173.15 Vs 3912.94). Feed cost per kg body weight gain was lowered by 5.97 percent (Rs 29.60 Vs 31.48) in group supplemented with mixed culture.

An improved DM intake was observed in post-ruminant on culture supplement in the present study. Similarly Adams et al (1981), Phillips and Vontungeln (1985); Bonaldi et al. (1986), Malcolm and Kiesling (1986), Fallon and Harte (1987), Hughes (1988) Williams et al. (1991), Wohlt et al. (1991) and Mutsvangwa et al (1992) found a higher DM intake with supplementation of yeast culture.

In accordance to the present study Bonaldi et al. (1986) reported that when DM intake calculated in terms of DM consumed per kg of live body weight gain, it was lower in group supplemented with S.cerevisiae and L.acidophilus.

More recently Drennon (1990) reported improvements in live weight gain, feed conversion efficiency and DM intake when yeast culture containing S.cerevisiae was included in the diet of bulls over a 205 day trial period. Similarly, Bhoi (1992) reported a higher feed conversion efficiency in kids, where same strains of S.cerevisiae and L.acidophilus were supplemented in the diet. However, Quinonez et al., (1989) did not find any response in DM intake in HF calves. But they also did not find any effect on body weight gain in the calves. This may be the effect of supplement of different strains of microbial culture used in his study.

Based on the over all treatment means in Table 6.4 after accounting for maintenance requirement of calves given culture, required an extra energy for a higher live body weight gain. The additional DM intake provided extra energy for calves given culture. No other physiological effects need to be proposed to explain the effects of culture.

The increase in voluntary feed intake by calves getting culture supplement in their feed may be related to pH modulation via reduction in lactic acid concentration

(Williams, 1990a). Williams *et al.* (1991) reported that low ruminal pH from diets high in rapidly fermentable starch fed to young calves was a factor limiting appetite and that low pH was related to high levels of lactic acid in the developing rumen (Frost and Nevison, 1989). But in present study the level of concentrate was not high since calves were fed forage and concentrate in a ratio of 60:40. *In vivo* results also supported that lactic acid concentration was within a normal range.

It is clear from the results that the addition of mixed culture of *S.cerevisiae* (B) and *L.acidophilus* (I) in the ration of calves stimulated higher DM intake and rumen fermentation with a higher concentration of propionate and TVFA, along with enhancing the feed conversion efficiency.

6.2.6 Chemical composition of feeds and residues:

In the present study level of nutrition was not changed in both groups, even with regard to source of feed stuffs. During metabolism trial the calves of both the groups were offered green fodder (Berseem) and concentrate. CF content of berseem was comparatively higher due to the late cut. The proximate constituents of berseem were well within the range reported by Sen and Ray (1971). The concentrate mixture was adequate in protein and energy contents. Residue collected in the morning was mainly from fodder.

6.2.7 Effect of supplementation on feed intake by post-ruminant calves during metabolic trial:

The average body weights of calves at the start of metabolic trial were significantly higher in culture supplemented group since the calves in this group grew significantly due to treatment effect. During the period of metabolic trial the calves on culture supplement group again exhibited the higher gain in body weight. During metabolic trial DM intake through concentrate was similar in both groups. However, DM intake through green fodder (Berseem) was significantly higher in culture supplemented group, thus total DM intake was also significantly higher in the same treatment group. DM intakes per 100 kg body weight and per Kg metabolic body size were not statistically different in both groups. The calves in supplemented group were heavier therefore, physiologically DM intake was also higher.

The performance response can be explained by the increased nutrient supply resulting from increased intake. Yeast culture has been shown to improve nutrient supply to the animal at a fixed intake (Williams et al., 1990b), but in farm trials its effects on intake appears to be the most important cause of improved performance. The stimulatory effects of culture on fiber digestion provide an immediate explanation of increased intake.

6.2.8 Effect of supplementation on digestibility

coefficients of feed constituents:

Results of digestibility of feed constituents are presented in Table 6.6.

6.2.8.1 DM digestibility :

DM digestibility in control and culture supplemented groups were 55.54 and 57.58 percent, respectively. The DM digestibility was significantly higher (3.67%) in calves of group supplemented with mixed culture in the diet. DM digestibility results of the present study is in accordance with response observed by Bhoi (1992) with supplementation of mixed culture of strains of S.cerevisiae and L.acidophilus in the kids. Other workers also found a higher DM digestibility with supplementation of yeast culture in steers (Williams et al., 1991) and in bulls (Mutsvangwa et al., 1992). Supplementation of probiotic in the form of mixed culture of S.cerevisiae (B) and L.acidophilus (I), seems to improve rumen environment which enhanced DM digestibility. The stimulatory effects of culture on DM digestion provide an explanation of increased intake noticed in present experiment.

6.2.8.2 OM digestibility :

Digestibility of OM in control and supplemented group were 61.35 and 65.19 percent, respectively. OM digestibility was significantly enhanced (6.26%) by the supplementation of culture.

6.2.8.3 CP Digestibility :

Digestibility of CP in control and supplemented groups were 66.73 and 67.29 per cent, respectively. CP digestibility was slightly higher in supplemented group but variations observed were non-significant. Similar results have been reported by Le Gendre et al. (1957); Adams et al., (1981); Arambel and Kent (1988) and Mutsvangwa et al., (1992). However, Weideier et al., (1987) and Wohlt et al. (1991) found that supplementation of yeast culture improved the CP digestibility when included in the diet of ruminant. They reported yeast culture provided some factors stimulatory towards proteolytic bacteria. But in the present experiments presence of any stimulatory factors were not noticed.

6.2.8.4 CF Digestibility:

Digestibility of CF in control and supplemented groups were 66.64 and 68.03 per cent respectively. CF digestibility tended to be higher in supplemented group but without showing any significant effect. However, Bhoi (1992) found in kids that supplementation of mixed culture containing same strains of S.cerevisiae (B) and L.acidophilus (I) significantly improved the CF digestibility. Whereas supplementation of alone S.cerevisiae (B) did not improve the CF digestibility. Contrary to the results of present experiment, supplementation of yeast culture also reported to improve digestibility of cellulose (Wohlt et al., 1991) and hemicellulose (Weidmeier et al., 1987).

Jung and Varel (1987) noted that increase in the number of cellulolytic bacteria did not correspond to increase in digestion of cell wall, cellulose or hemicellulose. Similarly, Firkins *et al.* (1990) also did not find any response in hemicellulose digestibility by supplementation of yeast culture. These factors might be responsible for not showing improvement in fiber digestibility in the present experiment.

6.2.8.5 EE Digestibility :

Digestibility of EE was similar in both groups. The results of the present study with regard to digestibility of EE are at par with results obtained by Bhoi (1992) in the kids. Culture supplement did not show any effect on performance/or proliferation of lipolytic bacteria and no such report is available in earlier literature.

6.2.8.6 NFE Digestibility

Digestibility of NFE in control and supplemented groups were 56.17 and 58.14 percent respectively which are statistically non-significant. Bhoi (1992) also reported that inclusion of S.cerevisiae (B) and L.acidophilus (I) either in combination or individually not produced any significant response when included in the diet of kids. The digestion of NFE is neither checked nor enhanced in the present study.

6.2.8.7 NDF Digestibility:

Digestibility of NDF in control and supplemented groups were 54.59 and 56.12 percent respectively. However, the difference between both groups was statistically non-significant. Harrison (1987); Arambel and Kent (1988); Firkins *et al.* (1990); Wohlt *et al.* (1991) and Mutsvangwa *et al.* (1992) also reported that supplementation of yeast culture had no effect on digestibility of NDF. This statement confirms the present findings under this project. However, in contrary to present results Bhoi (1992) found that supplementation of *S.cerevisiae* (B) with or without *L.acidophilus* (I) improved the digestibility of NDF in the kids. This might be due to differences in the species of animals and their feeding regimes. The results of the present experiments concluded that a mixed culture of *S.cerevisiae* (B) with *L.acidophilus* (I) had no effect on the digestibility of calves fed under 60:40 forage concentrate ratio.

6.2.8.8 ADF Digestibility

Digestibility of ADF in the control and supplemented groups were 50.06 and 50.57 percent respectively. The difference between both groups was non-significant. Earlier workers (Adams *et al.*, 1981; Harrison, 1987b; Weidmeier *et al.*, 1987; Arambel and Kent, 1988; Firkins *et al.*, 1990; and Wohlt *et al.* 1991) also did not find any response of

yeast culture to enhance ADF digestibility which confirms the results of the present study. However, Bhoi (1992) reported that in goats supplementation of S.cerevisiae (B) alone or in combination with L.acidophilus (I) increased the ADF digestibility due to improved buffering action or altered rumen environment. But such type of response is not encountered in the present investigation with calves.

In the present experiment, the presence of yeast culture in the rumen had effects on rumen stoichiometry and increased rate of DM degradation which resulted in increased forage intake and growth of animals. Williams et al. (1990) found that presence of yeast culture tended to increase the DM flow in duodenum and absorption from small intestine in sheep. It is particularly important with diets where physical limitations on rumen fill may restrict the amount of material entering the rumen. The accepted conceptual model for the effect on intake assumes a maximum capacity of the reticulo-rumen for digesta volume. The animal eats forage until this is achieved, after which further intake depends on the creation of space in the rumen as a result of the outflow. This in turn depends on rate of reduction of forage particle size and hence rate of digestion.

significantly higher by 16.95 percent (30.63 Vs 26.19) in culture supplemented group over control. Nitrogen retained as percent of intake and absorbed were calculated and found statistically similar in both groups. But again a higher tendency of nitrogen retention was observed in the culture supplemented group. Earlier studies also indicated that supplementation of cultures increased the nitrogen retentions in the kids (Bhoi, 1992) and in buffalo calves (Bakshi and Langer (1990). This might be due to increased absorption of protein from the small intestine, which was slightly higher in the present study but did not reach to a statistically significant level. William *et al.* (1990) also found that addition of yeast culture in sheep significantly increased the absorption of non-ammonia nitrogen from small intestine. Erasmus (1991) conducted his experiment on lactating HF cows with yeast culture supplementation. He reported that cows given yeast culture showed non-ammonia-N and flow rate higher over control mainly due to increased microbial-N flow. Microbial-N was 56 and 47 percent of N-intake on yeast culture and control. Amino acid concentration of duodenal digesta in cows on yeast culture was significantly higher for threonine, serine cystine and methionine, and lower for glutamic acid compared to control group. These contentions support present finding where nitrogen retention was found high on mixed culture [*S.cerevisiae* (B) and *L.acidophilus* (I) supplementation.]

However, other workers reported faecal and urinary nitrogen losses and nitrogen retention were unaffected by the addition of yeast culture (Le Greidre et al., 1957; Adams et al., 1981; Wohlt et al., 1991 and Mutsvangwa et al., 1992. This might be due to differences in quality of protein present in the diet.

Table 6.1 Body weight gain and incidence of diarrhoea in pre-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Initial body weight (Kg)	30.2±1.80	31.1±1.53	NS
Final body weight (Kg) (98 day trial) during supplementation	67.1±4.82	76.7±3.76	S
Total gain in body weight (Kg) during supplementation	36.9±4.43	45.6±3.65	S
Average daily body weight gain (g/d) during supplementation	376.53±45.21	466.21±37.01	S
Average days in diarrhoea	5.9±1.66	3.8±0.93	S
Final body weight (Kg) at 98 day post supplementation	105.9±4.74	115.3±4.12	NS
Total gain in body weight (Kg) at 98 day post supplementation	38.3±1.20	38.8±1.04	NS
Average daily body weight gain (g/d) during post supplementation	390.82±12.27	395.92±10.63	NS

Each value is the average of 10 observations ($P < 0.05$).

Table 6.2 Feed conversion efficiency and feed cost per unit gain in pre-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Total DM intake (kg) (98 days trial) during supplementation	68.20 ±2.75	74.41 ±2.18	S
Total gain in body weight (kg)	36.90 ±4.43	45.60 ±3.65	S
DM intake per kg gain in body weight (Kg)	2.08 ±0.26	1.72 ±0.13	S
Total feed cost (Rs)	1942.03 ±96.18	2144.19 ±74.67	S
Feed cost (Rs) per Kg gain in body weight	61.00 ±8.33	49.60 ±3.84	S

Each value is the average of 10 observations ($P/0.05$).

Table 6.3 Body weight gain in post-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Initial body weight (Kg)	82.90 ±1.38	83.10 ±3.03	NS
Final body weight (Kg) (238 days trial)	208.00 ±3.49	225.00 ±3.68	S
Total gain in body weight (Kg)	125.10 ±3.69	141.90 ±4.50	S
Average daily body weight gain (g/d)	525.63 ±15.50	596.19 ±18.92	S

Each value is the average of 10 observations ($P/0.05$).

Table 6.4 Feed conversion efficiency and feed cost per unit gain in post-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Total DM intake (Kg) (238 days trial)	809.16 ±8.97	860.43 ±14.10	S
Total gain in body weight (Kg)	125.10 ±3.69	141.90 ±4.50	S
DM consumed (Kg) per kg body weight gain	6.55 ±0.19	6.11 ±0.20	S
Total feed cost (Rs)	3912.94 ±44.88	4173.15 ±48.58	S
Feed cost (Rs) per kg body weight gain	31.48 ±0.86	29.60 ±0.95	S

Each value is the average of 10 observations ($P/0.05$).

Table 6.5 Promixate and Van Soest composition of feeds and residue of post-ruminant experimental calves (% , on DM basis).

Parameters	Greed fodder	Concentrate	Residue
Organic matter	87.60±0.50	92.27±0.34	87.58±0.54
Crude protein	15.90±0.71	19.30±0.59	16.94±0.87
Crude fibre	21.11±1.00	10.19±0.28	17.20±0.91
Ether extract	1.45±0.10	3.80±0.18	1.72±0.11
Nitrogen free Extract	49.15±1.31	58.99±0.48	51.73±2.21
Ash	12.41±0.50	7.73±0.34	12.42±0.54
Neutral detergent fibre	51.37±0.79	49.37±1.11	51.04±0.23
Acid detergent fibre	40.29±0.88	8.39±0.48	37.14±1.57

Each value is the average of four observations.

Table 6.6 Average daily feed intake during metabolic trial in post-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Average body weight (Kg) at the beginning of metabolic trial	167.3±4.42	184.3±3.99	S
Average body weight (Kg) at the end of Metabolic trials	169.9±4.43	187.6±3.91	S
Concentrate DM Intake (Kg/d)	1.99±0.07	2.13±0.06	NS
Green fodder DM intake (Kg/d)	2.95±0.07	3.29±0.07	S
Total DM intake (kg/d)	4.94±0.10	5.42±0.10	S
DM intake per 100 kg body weight (Kg/d)	2.93±0.11	2.93±0.09	NS
DM intake per kg W ^{0.75} (g/d)	105.26±3.44	107.93±2.82	NS

Each value is the average of 10 observations (P/0.05).

Table 6.7 Digestibility coefficients (%) of nutrients in post-ruminant calves kept on with or without probiotic supplement.

Parameters	Control group	Probiotic supplemented group	Statistical observations
Dry matter	55.54 ± 0.49	57.58 ± 0.81	S
Organic matter	61.35 ± 0.64	65.19 ± 0.90	S
Crude Protein	66.73 ± 0.72	67.29 ± 0.69	NS
Crude Fibre	66.64 ± 0.57	68.03 ± 0.63	NS
Ether Extract	68.73 ± 0.80	67.73 ± 0.63	NS
Nitrogen Free Extract	56.17 ± 0.59	58.14 ± 1.04	NS
Neutral detergent fibre	54.59 ± 0.64	56.12 ± 0.90	NS
Acid detergent fibre	50.06 ± 0.71	50.57 ± 0.89	NS

Each value is the average of 10 observations (P/0.05).

Table 6.8 Nutrient intake in post-ruminant calves kept on with or without probiotic supplement

Parameters	Control group	Probiotic supplemented group	Statistical observations
CP intake (g/d)	649.54 ± 18.96	688.88 ± 14.36	NS
CP intake per 100 kg body weight (g/d)	369.71 ± 23.19	372.59 ± 13.52	NS
CP intake per kg W ^{0.75} (g/d)	13.81 ± 0.60	13.73 ± 0.43	NS
DCP intake (g/d)	432.45 ± 11.51	463.31 ± 9.82	S
DCP intake per 100 kg body weight (g/d)	259.03 ± 12.13	255.79 ± 7.75	NS
DCP intake per kg W ^{0.75} (g/d)	9.31 ± 1.61	9.23 ± 0.26	NS
TDN intake (kg/d)	2.71 ± 0.08	2.90 ± 0.08	NS
TDN intake per 100 kg body weight (kg/d)	1.62 ± 0.06	1.54 ± 0.08	NS
TDN intake per kg W ^{0.75} (g/d)	58.18 ± 2.20	57.45 ± 2.28	NS

Each value is the average of 10 observations ($P/0.05$).

Table 6.9 Nitrogen balance in post-ruminant calves kept on with or without probiotic supplement

Parameters	Control group	Probiotic supplemented group	Statistical observations
N-intake (g/d)	103.78 ± 2.98	110.22 ± 2.30	S
N-excreted in dung (g/d)	34.39 ± 1.41	36.09 ± 1.20	NS
N-excreted in dung as % of intake	33.06 ± 0.68	32.71 ± 0.69	NS
N-excreted in urine (g/d)	43.70 ± 2.19	43.51 ± 1.46	NS
N-excreted in urine as % of intake	41.91 ± 1.16	39.39 ± 0.71	NS
N-balance (g/d)	26.19 ± 1.02	30.63 ± 0.54	S
N-retained as % of N-intake	25.49 ± 1.37	27.89 ± 0.78	NS
N-retained as % of N-absorbed	43.34 ± 1.67	46.01 ± 1.04	NS

Each value is the average of 10 observations ($P < 0.05$).

Table 6.10 Statistical analysis of body weight gain and feed conversion efficiency in pre- and post- ruminant calves.

Parameters	Calculated 't' value	
	Pre-ruminant	Post-ruminant
Initial body weight	0.86	1.10
Final body weight	2.44*	3.46**
Total gain in body weight	2.56**	3.51**
Daily body weight gain	2.11*	3.63**
Days in diarrhoea	2.38*	-
Final body weight (Post-supplementation)	1.02	-
Total gain in body weight (Post-supplementation)	1.96	-
Daily body weight gain (Post-supplementation)	1.74	-
Total DMI	2.37*	2.40*
DM intake/kg gain	2.24*	2.19*
Total feed cost	2.29*	3.14**
Feed cost/kg gain	2.46*	3.53**

For each parameter number of paired observations were 10.

* Significant at 5% level.

** Significant at 1% level.

Table 6.11 Statistical analysis of different parameters recorded during metabolic trial in post-ruminant animals.

Parameters	Calculated 't' value	Parameters	Calculated 't' value
Initial body wt.	3.21**	Final body wt.	3.34**
Concentrate DMI	1.26	Green Fodder DMI	2.33*
Total DMI	2.26*	DMI/100 kg Body wt.	0.93
DMI/kg W ^{0.75}	0.84	DM Dig.	2.84**
OM Dig.	2.46*	CP Dig.	0.69
CF Dig.	1.03	EE Dig.	0.81
NFE Dig.	0.44	NDF Dig.	0.64
ADF Dig.	0.91	CP intake/day	1.53
CP intake/100 kg body wt.	1.45	CP intake/kg W ^{0.75}	2.32*
DCP intake :		TDN intake per :	
Per day	1.37	day	1.96
per 100 kg body wt.	1.48	100 kg body weight	1.51
per kg W ^{0.75}	1.87	Kg W ^{0.75}	1.64
N intake/day	3.61**	N balance	3.94**
N excreted in dung as:		N excreted in urine as:	
g/day	1.53	g/day	1.74
% of intake	1.16	% of intake	1.08
N retained as:			
Percent of N intake	1.01		
Percent of N absorbed	1.68		

For each parameter number of paired observations were 10.

* Significant at 5% level

** Significant at 1% level

CHAPTER - VII

SUMMARY AND CONCLUSIONS

7. SUMMARY AND CONCLUSIONS

SUMMARY:

Manipulation of intestinal micro-flora in both ruminants and non-ruminants has a significant effect on feed efficiency and growth rate. Manipulation for optimum rumen fermentation and digestion can be achieved by supplementation of live microbes as dietary adjunct. The commonly used dietary cultures (Yeast or/and lactic acid producing bacteria) also called probiotic have been reported to enhance

ruminal production of total volatile fatty acid, anaerobic and cellulolytic bacteria along with reduction of ammonia, methane and lactic acid concentration. The achievement of optimal ruminal condition is known to improve feed intake and efficiency of feed conversion towards growth and milk production. However, different strains of lactic acid bacteria reduce pathogenic microorganisms in the gastro-intestinal tract and increase immunity towards diseases and anti-tumor activities. These supplements are safer to use since they have no ill effect on the health of animals or consumers like that of antibiotics. The use of probiotics as growth promoter is therefore an answer to enhance animal productivity.

The information on the influence of different strains of microflora on rumen fermentation and feed utilization for growth is lacking in India. The present investigations were therefore, carried out with two strains of each L.acidophilus, S.thermophilus (New name Streptococcus salivarius subsp. thermophilus) and S.cerevisiae. Six strains were evaluated through ruminal studies (in vitro and in vivo), metabolic trials and growth experiments. The results obtained in different experiments have been summarized in the ensuing paragraphs.

7.1 In vitro rumen fermentation influenced by the supplementation of individual microorganisms

In the first phase six strains of microorganisms were evaluated in vitro using wheat straw and concentrate mixture (60:40) as substrate. In vitro system was supplemented with each strain of microorganissms (@ 10^9 cfu/vessel) and data collected at 24 and 48 h incubations. The different treatments were as follows:

<u>Treatments</u>	<u>Microorganisms supplemented</u>
T ₁	<u>Lactobacillus acidophilus</u> (R)
T ₂	<u>L.acidophilus</u> (I)
T ₃	<u>Streptococcus thermophilus</u> (HST)
T ₄	<u>S. thermophilus</u> (CH)
T ₅	<u>Sachromyces cerevisiae</u> (522)
T ₆	<u>S.cervisiae</u> (B)
T ₇	Without supplement (Control)

7.1.1 Effect of supplmentation on DM and OM degradability:

DM degradability at 24 h and 48 h incubation periods improved in treatment groups T₁ (50.48 and 52.34%); T₂ (51.02 and 53.81%), T₅ (52.52 and 54.19%) and T₆ (55.57 and 56.86%) respectively over control group (T₇) (49.30 and 51.40%). OM degradability also showed the similar trend at both incubation periods. The degradability increased by

supplementation of both strains of S. cerevisiae followed by L. acidophilus but S. cerevisiae strain B exhibited better performance. However, the strain of S. thermophilus did not perform significantly.

7.1.2 Effect on in vitro total volatile fatty acid (TVFA) Concentration

TVFA concentration (meq/100 ml) was higher in all culture supplemented groups at 24h and 48h incubation (Treatments T₁, 6.64 and 8.61; T₂, 7.65 and 9.45; T₃, 6.56 and 7.79; T₄, 6.53 and 7.68; T₅, 7.71 and 9.78; T₆, 8.96 and 10.89; T₇, 6.03 and 7.59). Strain S. cerevisiae (B) exhibited higher TVFA production over other strains.

7.1.3 Effect on in vitro microbial population:

Microbial counts ($\times 10^7$) were significantly higher in all culture supplemented groups at 24h and 48h of incubation (Treatment T₁, 26.35 and 35.45; T₂, 32.30 and 34.10; T₃, 18.25 and 25.60; T₄, 17.05 and 24.55; T₅, 36.70 and 49.25; T₆, 49.05 and 77.00; T₇, 12.75 and 15.98, respectively), but strains of S. cerevisiae excelled the microbial population over the strains of lactic acid producing bacteria.

7.1.4 Effect on in vitro ammonia-N concentration:

Ammonia-N concentration (mg/100 ml) was significantly lower in treatment groups T_5 (17.68 and 19.08) by 13.88 and 17.58% and T_6 (15.93 and 16.45) by 22.41 and 28.94%, respectively at 24 h and 48 h incubation periods compared to Control group (20.53 and 23.15). The reduction in ammonia-N was more pronounced on supplementing strains of S.cerevisiae.

7.1.5 Effect on in vitro microbial protein-N:

Microbial protein-N (g/kg OM digested) was significantly higher in all culture treatment groups. The respective values at 24 h incubation were 42.35, 45.22, 40.11, 39.50, 46.67, 48.75 and 38.91 and at 48 h were 50.64, 56.09, 49.81, 52.49, 55.47, 61.03 and 45.15 for treatment groups T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7 .

7.1.6 Effect on in vitro total gas production:

Supplementation of L.acidophilus (I) and both strains of S.cerevisiae significantly reduced the gas production. The total gas production (ml/g substrate) was 75.0, 71.4, 68.4 and 83.2 at 24 h and 100.1, 97.4, 93.1 and 110.8 at 48 h incubation period for respective treatment groups T_2 , T_5 , T_6 and T_7 .

7.2 Supplementation of live microorganisms on in vivo rumen fermentation:

Two microbes L.acidophilus (I) and S.cerevisiae (B) were selected on the basis of earlier experiment and evaluated in vivo either individually or in combination.

The treatments were

T ₁ ,	<u>L.acidophilus</u> (I)
T ₂ ,	<u>S.cerevisiae</u> (B),
T ₃ ,	<u>L.acidophilus</u> (I) + <u>S.cerevisiae</u> (B)
T ₄ ,	Without supplement (Control)

7.2.1 DM disappearance in nylon bag:

DM degradability was significantly higher in group T₂ by 10.28 and 7.21% at 6h (35.43 Vs 32.14%) and 12h (45.48 Vs 42.42%) respectively. Similarly DM degradability was higher in mixed culture by 16.55, 11.66 and 4.97% at 6, 12 and 24 h.

7.2.2 Fiber degradability:

The fiber degradability on treatment group T₂ (23.10%) was significantly higher by 14.98% at 6 h compared to control group (20.09%) and treatment group T₃ compared to control by 18.87 and 8.87 % at 6 h (23.88 vs 20.09%) and at 12 h (25.65 vs 23.56%), respectively. Supplementation of S.cerevisiae alone had stimulatory effect upto 6 h but in

combination with L.acidophilus (I), the degradability was higher upto 24 h.

7.2.3 Ruminal total volatile fatty acid (TVFA) concentration:

The TVFA concentration (meq/100 ml) was significantly higher on mixed culture (T_3) by 6.64, 12.50, 11.13, 9.51, 11.80, 17.49 and 7.19% at 0h (9.47 vs 8.85), 2h (23.41 vs 11.92), 4h (14.37 vs 12.93), 6h (16.56 vs 15.08), 8h (12.16 vs 11.77), 10h (11.89 vs 10.12) and at 12 h (9.39 vs 8.76) compared to control group. However, on S.cerevisiae alone (T_2) showed a higher TVFA concentration by 6.29 and 7.19% at 2h (12.69 vs 11.92) and 4h (13.86 vs 12.93) compared to control group.

7.2.4 Concentration of individual volatile fatty acid in the rumen:

Group T_2 (S.cerevisiae alone) showed a higher concentration of propionate by 9.91, 9.55 and 10.42% at 2, 4 and 6 h and group T_3 (mixed culture) by 13.06, 15.45, 17.54 and 10.89 % at 2, 4, 6 and 12 h compared to control treatment. However, concentration of acetate and butyrate were not significantly affected by the treatments.

7.2.5 Acetate:Propionate ratio in the rumen:

In treatment group T_3 (mixed culture acetate

propionate ratio was lowered by 9.90, 15.08, 16.17, 8.42 and 9.49 % at 0, 2, 4, 8 and 10 h., respectively compared to control group. However, other groups did not differ significantly.

7.2.6 Levels of ruminal ammonia-N:

Ruminal ammonia-N (mg/100 ml) in group T_2 was lowered by 2.99, 3.77 and 4.61% at 0h (11.32 vs 11.67), 2h(15.56 vs 16.17) and at 4h (18.39 vs 19.24), respectively compared to control group. Similarly, group T_3 showed a lower ruminal ammonia-N by 6.68, 13.91, 15.04, 10.40, 6.72, 6.03 and 6.83 % at 0, 2, 4, 6, 8, 10 and 12h respectively compared to control. However, supplementation of L. acidophilus alone did not affect significantly.

7.2.7 Levels of microbial protein in the rumen:

In group T_3 , microbial protein (mg/100 ml) level was higher by 15.85, 13.68, 12.25, 12.03, 12.66 and 10.74 % at 2, 4, 6, 8, 10 and 12 h and in group T_2 it was higher by 12.19, 14.21, 5.67 and 5.37% at 2, 4, 10 and 12 h respectively compared to control group.

7.2.8 Microbial population in the rumen:

In treatment group T_2 microbial population ($\times 10^8$) was similar upto 4 h, however, after 4 h, it increased by 59.64, 70.68, 83.82 and 73.07% at 6, 8, 10 and 12 h, respectively, compared to control group. However, group T_3 showed a higher concentration of microbial cells by

105.17, 132.35 and 130.76% at 8, 10 and 12 h, compared to control.

7.2.9 Lactic acid concentration in the rumen:

Except at 2 h (peak period) the concentration of lactic acid (mM/l) was similar between all groups. At 2 h, lower concentration was observed on treatments T_2 and T_3 compared to control.

7.2.10 Flow rate of liquid digesta:

Flow rate of liquid digesta (l/d) was non-significantly higher in treatment groups T_3 (51.48), T_2 (50.83) and T_1 (51.00) respectively as compared to control (46.69).

7.3 Growth rate, nutrient utilisation and feed conversion efficiency on various probiotic supplements.

Two growth trials on pre-ruminant and post-ruminant calves were conducted to study the effects of mixed culture [*S.cerevisiae* (B) and *L. acidophilus* (I)] on feed conversion efficiency towards gain in body weight. The treatment group was compared to the non-supplemented group. In each trial 20 calves were separately grouped into two and metabolic trial was conducted on post ruminant calves.

7.3.1 Effect of supplementation on live weight gain in pre-ruminant calves:

The initial body weight of pre-ruminant calves was

31.1 kg on treatment group and 30.2 kg on control group. However, after 98 days trial the respective live weights were 76.7 kg and 67.1 kg. Group supplemented with mixed culture showed significantly higher body weight gain (466.2 vs 376.53 g/d). After discontinuation of treatment there was no difference in body weight gain in both the groups.

7.3.2 Effect of supplementation on incidence of diarrhoea in pre-ruminant calves:

Incidence of diarrhoea was lowered by 35.59 percent in supplemented group (3.8 days/animal), where only six animals were suffered from diarrhoea as compared to control group (5.9 days/animals) where all the ten animals were suffered.

7.3.3 Feed conversion efficiency and feed cost per unit gain in pre-ruminant calves:

Supplemented group showed a higher DM intake (74.41 Vs 68.20 kg), a lower DM intake per kg of body weight gain (1.72 Vs 2.08 kg) and a lower feed cost per kg body weight gain (Rs. 49.60 vs 61.00)

7.3.4 Effect of supplementation on live weight gain in post-ruminant calves:

The initial body weight of post-ruminant calves was 83.10 kg on treatment group and 82.90 kg on control group. However, after 238 days trial the respective live weights

were 225.00 and 208.00 kg. The group supplemented with mixed culture showed significantly higher body weight gain (596.19 vs 525.63 g/d).

7.3.5 Effect of supplementation on feed conversion efficiency and feed cost per unit gain in Post-ruminant calves:

In supplemented group total feed intake was higher (860.43 Vs 809.16 Kg), consumption of DM (kg)/kg live body weight gain was lower (6.11 vs 6.55 kg), and feed cost per kg body weight gain was lower (Rs 29.60 Vs 31.48).

7.3.6 Chemical composition of feeds:

The CP content in Berseem and concentrate was 15.91% and 19.30%. The feed was adequate to supply essential nutrients to the animals.

7.3.7 Effect of supplementation on feed intake by post-ruminant calves during metabolic trial:

Total DM intake during metabolic trial was higher in supplemented group (5.42 vs 4.94 kg/d) compared to control. However, DM intake per 100 kg body weight and per kg $W^{0.75}$ were similar in both groups.

7.3.8 Effect of supplementation on digestibility coefficients of feed constituents:

In supplemented group, digestibility coefficients of DM (57.58 vs 55.54%) and OM (65.19 vs 61.35%) were higher by 3.67 and 6.26 % respectively compared to control group.

However, digestibility coefficients of other constituents were not significantly affected by treatment.

7.3.9 Effect of supplementation on nutrient intake in post-ruminant calves during metabolic trial:

CP and TDN intake were similar in both groups. However, DCP intake (g/d) increased by 7.14 percent over control group.

7.3.10 Effect of supplementation on nitrogen balance:

In the culture supplemented group nitrogen intake (g/d) was higher by 6.21 % and nitrogen balance (g/d) was higher by 16.95% (30.63 vs 26.19) compared to control group. However, nitrogen excretion remained similar in both groups.

CONCLUSIONS

1. Supplementation of L.acidophilus (I), S.cerevisiae (522) and S.cerevisiae (B) enhanced in vitro DM and OM degradability. The strains of yeast [S.cerevisiae (522) and S.cerevisiae (B)] enhanced microbial-nitrogen, microbial counts, TVFA and reduced ammonia-N and total gas production. These effects further improved when L.acidophilus culture was added along with S.cerevisiae.
2. Supplementation of S.cerevisiae (B) alone or along with L.acidophilus (I) increased the in vivo DM and CP degradability. The concentration of ammonia-N and lactic acid reduced on supplementation of

S.cerevisiae (B) alone or with combination of L.acidophilus (I). The propionate concentration increased with supplementation of S.cerevisiae (B). However, combination with L.acidophilus (I) caused lower acetate:propionate ration.

3. Pre-ruminant calves significantly gained body weight at reduced feed cost on supplementation of S.cerevisiae (B) + L.acidophilus (I) and experienced low incidence of diarrhoea.

Post-ruminant calves kept on supplementation of S.cerevisiae (B) and L.acidophilus (I) together showed a higher body gain, lower DM intake/kg body weight gain and lower feed cost/kg body weight gain. DM digestibility and N-balance improved on mixed culture.

* * *

EFFECT OF LIVE MICROBES AS DIETARY ADJUNCT ON
RUMINAL FERMENTATION, NUTRIENT UTILIZATION
AND GROWTH OF CALVES

RAMAN MALIK

ABSTRACT

Supplementation of probiotic have been reported to improve the ruminal fermentation, feed intake and feed efficiency towards productive traits in ruminants. Therefore, two strains of each L.acidophilus, S.thermophilus and S.cerevisiae were evaluated through ruminal studies (in vitro and in vivo), metabolic trial and growth experiments. In vitro system (24 and 48h) supplemented with individual strains of L.acidophilus and S.cerevisiae (@ 10^9 cfu/vessel) showed improved degradability of DM and OM with increased concentration of TVFA, microbial protein, microbial counts along with reduced ammonia-N and total gas production. In vivo experiments showed that S.cerevisiae (B) supplementation (@ 10^9 cfu/kg DMI/d) increased degradability of DM and crude fibre with increased concentration of TVFA, propionate, microbial protein, microbial counts along with reduced concentration of lactic acid, ammonia-N and reduced ratio of acetate:propionate. Supplementation of S.cerevisiae (B) along with L.acidophilus (I) showed a stimulatory effect. However, supplement do not have significant effect on liquid digesta flow rate. Data from growth experiment exhibited a significantly higher body weight gain and lower feed cost/kg body weight gain on supplementing mixed culture [S.cerevisiae (B) and L.acidophilus (I)] in pre-ruminant and post-ruminant calves. Incidence of diarrhoea significantly reduced on culture supplemented pre-ruminant calves. Digestibility of proximate principles did not differ but N balances increased on supplementing mixed culture.

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