Lactobacillus acidophilus AS A DIETARY ADJUNCT IN *Dahi* AND YOGURT

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Department of Dairy Science
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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1995

Dedicated to
my Dad and Mom

DECLARATION

I hereby declare that the thesis entitled **"Lactobacillus acidophilus as a dietary adjunct in Dahi and Yogurt"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Lactobacillus acidophilus as a dietary adjunct in Dahi and Yogurt"** is a record of research work done independently by **Sri. S. Appalo Eleven**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

INTRODUCTION

Fermented milk products have long been important components of diet in the countries of Europe, Asia and Africa. They were prepared at home by traditional methods of each country, using a natural starter. Such products include teatta (Scandinavia), Leben (Egypt), Kefir (Caucasus), Mart (Iran), Dahi (India), etc.

When fermentation process is carried out in a modern dairy, it can be characterised as a controlled chemical and biological preservation process. As a result of fermentation, conditions are provided for an incomplete metabolism of the components of milk and the production of intermediates, the most important of these being lactic acid and other organic acids.

The consumption of fermented milk products with selected lactobacilli is suggested as a remedy for gastro-intestinal and other infections, lactose intolerance symptoms, side effects of long term use of drugs, liver and bile malfunctions. It was also suggested to control serum cholesterol levels.

Lactose intolerance is a condition in which lactose, (milk sugar) is not hydrolysed due to the deficiency of

intestinal enzyme lactase. Thus the lactose passes unchanged from small intestine to the colon, where the osmotic equilibrium is disturbed, water is drawn into the colon and diarrhoea results. Some of the lactose is fermented by the natural flora of intestine. This results in gas formation and consequently cramping and bloating occur. This discourages the consumption of milk by such individuals, thus eliminating a major source of calcium and high quality protein.

Several studies suggested that fermented milk products like yogurt, acidophilus milk etc., are better tolerated than milk by lactose intolerant individuals. The reason attributed was production of lactose hydrolysing enzyme β-galactosidase by these starter organisms during their growth. Though there are some scanty reports regarding the β-galactosidase specific activity in yogurt from abroad, no work was seen to be reported from India particularly with regards to dahi, so as to compare the advantage of these two products in alleviating lactose intolerance.

Nowadays there is an increased awareness about the correlation between high blood cholesterol levels and coronary heart diseases. People are going for non or low fat diet. The prime victim of this new trend is the milk fat, which has got a high proportion of saturated fatty acids. There are

convincing evidences that the 3-hydroxy 3-methyl glutaric acid and orotic acid present in milk actually lowers the blood cholesterol. Various studies conducted at different parts of the world revealed that fermented dairy products are capable of reducing the blood cholesterol levels significantly, presumably by the assimilation of cholesterol by the starter bacteria.

One reason the scientists attribute for the changes during aging or senility is due to liberation of noxious compounds and autointoxication of the system. Presence of large numbers of enteric pathogenic bacteria is the culprit. Fermented milk products especially yogurt and dahi possess strong antibacterial activity, thus inhibiting a wide variety of pathogenic bacteria.

Eventhough conventional starter organisms of yogurt and dahi possess all the above beneficial effects, they lack one important quality to prolong the beneficial effects in the intestine, even after continued consumption. That is, they are incapable of colonising in the intestine. The main reason is they lack the acid and bile resistant properties.

Thus the need arose to search for a bacterium which is a normal inhabitant of human intestine, to be incorporated in these products to enhance the beneficial effects.

Lactobacillus acidophilus (L. acidophilus) is one such bacteria with all the properties to be an ideal dietary adjunct in Dahi and yogurt. L. acidophilus is widely accepted for its ability to inhibit various pathogenic organisms, improved lactose digestion, control serum cholesterol levels and anticarcinogenic activity (Gilliland, 1989). Dahi and yogurt could be a suitable vehicle to dessipate this useful bacteria to provide potential benefit to the consumer.

The present study is aimed at incorporation of L. acidophilus in yogurt and dahi and evaluate its beneficial effects in terms of

(i) β-galactosidase specific activity

(ii) Antibacterial activity

(iii) Hypocholesteremic effect and

(iv) Bile tolerance study of L. acidophilus, yogurt and dahi cultures used in the experiment

An attempt is also made to study the growth rate of rats fed with dahi and yogurt containing L. acidophilus.

Review of Literature

REVIEW OF LITERATURE

2.1.1 Lactose intolerance - incidence

Lactose is the main carbohydrate in the milk. The average content in cow's milk is little lower than five per cent (weight/volume). During digestion lactose is hydrolysed into glucose and galactose (Luyken, 1972; Lontie, 1973 and Porter, 1978).

Dalqvist (1977) reported that low lactose activity is a relatively common abnormality of small bowel in man. Andersen and Barefoed (1978) conducted a detailed study on the incidence of lactose intolerance and found that 64 per cent of adult Indian population are suffering from this condition.

The incidence is relatively high (66 per cent) in southern regions of India, when compared to the northern population (27.4 per cent) (Tandon et al., 1981). The reason attributed was that the north Indians, who were of Aryan descent, were known for traditional dairy farming. The south Indians had never raised dairy animals. Thus Savaiano and Levitt (1987) hypothesised, that lactose intolerance is a genetically linked trait. Primary lactose deficiency is common with a world wide occurrence of nearly 70 per cent.

2.1.2 Mechanism of lactose intolerance

Various studies reviewed the demographics and genetics of the loss of lactase enzyme in adults (Bayless et al., 1968 and Friedl, 1981).

Simoons (1978) reported that in most mammals, intestinal lactase concentrations were highest immediately after birth, decreases after weaning and drop to very low concentrations in adults. The genetically programmed loss of intestinal lactase activity occurs during the first or second decade of life in approximately 70 per cent of the world population. Only in the regions of the world, where there was a long tradition of dairying, do the native inhabitants retain the infantile amounts of intestinal lactase.

Levitt et al. (1981) reported that lactose is split into glucose and galactose by the lactase on the brush border of the small intestine. The monosaccharides are rapidly absorbed, resulting in the elevated blood glucose. Lactase deficient persons cannot digest significant quantities of lactose. This results in the fermentation of the disaccharide in the large intestine, by the microflora, producing hydrogen and carbon-di-oxide gases.

Lactose malabsorption may or may not result in lactose intolerance, the symptomatic response to the consumption of

lactose. Symptoms may range from borborygmus (Sounds of flatus in the intestine) distention, excessive flatulence, and intestinal pain to severe, acute diarrhoea (Kim and Gilliland, 1983; Gilliland and Kim, 1984; Saviano and Levitt, 1987; Shah and Jeelen, 1991).

Savaiano and Levitt (1987) reported that lactose deficiency was a condition of low intestinal lactase activity and it could result from either genetically programmed loss or a disease process that involved the small intestinal bowel mucosa. The loss rather than the retention of high amounts of intestinal lactase is the overwhelmingly predominant situation.

They classified lactase deficiency due to congenital and acquired causes. Acquired causes, were further divided into primary cause and secondary cause. Primary cause is the genetically programmed loss of lactase following weaning. Secondary deficiency results from a disease process that involves the small bowel mucosa and reduced lactose level or that causes insufficient exposure of mucosa to ingested lactose as in infectious diarrhoea, short gut, cetiac sprue, Crohn's disease, Tropical sprue, Malnutrition, Blind loop syndrome, Giardia, subintestinal gastrectomy and immunological deficiency syndrome.

2.1.3 Effect of lactic acid bacteria on lactose intolerance

Citti et al. (1965) studied the β-galactosidase specific activities of S. lactis 7962, and reported that the β-galactosidase specific activity differed, depending upon the assay buffer solution. The highest specific activity of 0.75 units was found when 0.05 M sodium phosphate buffer was used.

Kilara and Shahani (1974; 1976) conducted research on yogurt and acidophilus milk, and claimed that the micro-organisms partly would survive passage through the alimentary tract. This could also mean that hydrolysis of lactose through the gastro intestinal tract could be continued.

Various studies revealed that lactose malabsorbing humans digested lactose from yogurt much more efficiently than lactose from any other dairy product (Gallagher et al., 1974; Kilara and Shahani, 1976; Savaiano et al., 1984; Savaiano and Levitt, 1987 and Onwulata et al., 1989).

L. acidophilus added to milk as a dietary, adjunct is beneficial in that it improves lactose digestion in humans. The possible mechanism by which L. acidophilus could reduce breath hydrogen associated with lactose malabsorptions were explained by Buchanan and Gibbons (1974); Gilliland (1979) and Kim and Gilliland (1983).

(i) L. acidophilus could supply a source of lactose in the intestinal-tract, reducing the amount of lactose reaching the large intestine, thus reducing the formation of gases including hydrogen.

(ii) L. acidophilus exerts antagonistic effects on bacteria that could produce gases in the intestine.

(iii) L. acidophilus is a homofermentative bacterium and do not produce gas.

(iv) When the ratio of L. acidophilus to other bacteria in human intestine increases the total gas production from the action of heterofermentative bacteria may be decreased.

Several studies conducted on lactose intolerant individuals suggest that Lactose ingested in yogurt was more efficiently digested than lactose in milk (Kilara and Shahani, 1976; Savaiano et al., 1984; Gilliland, 1985 and McDonough et al., 1987).

Nonfermented dairy products containing L. acidophilus strains were less effective in reducing lactose intolerant symptoms when compared to the products containing yogurt strains (Kilara and Shahani, 1976; Savaiano and Levitt, 1987; Shah and Jeelan, 1990 and Lin et al., 1991).

Studies on improved lactose digestion by the milk containing viable L. acidophilus cells without subsequent fermentation gave equivocal results (Payne et al., 1981 and Kim and Gilliland, 1983). They attributed the strain differences, the concentration of organisms, variability in β-galactosidase activity, and bile tolerance as the possible reasons.

Alm (1982) reported that microorganisms used for fermented milk products reduced the lactose content of milk considerably. The result had shown that 500 millilitre of low fat milk caused abdominal distress and diarrhoea in lactose intolerant individuals, whereas the same quantity of yogurt or acidophilus milk did not result in any palpable symptoms. It was also reported that acidophilus milk prepared from L. acidophilus was better tolerated than ropy fermented milk of Streptococcus lactis.

Kim and Gilliland (1983) reported that the beneficial effects of L. acidophilus in alleviating lactose intolerance was immediate and did not require that microbe containing milk be consumed daily.

Kolar et al. (1984) reported that yogurt was well tolerated by lactose intolerant individuals even when

unusually large quantities of lactose (in yogurt) were ingested.

Gilliland (1985) studied the viability of yogurt culture organisms in the intestine and found out that they did not survive or grow in the intestinal tract, therefore, they only serve as a source for β-galactosidase in alleviating lactose maldigestion.

Cultured yogurt possessed considerable lactase activity mainly due to the presence of lactase as an endo enzyme in the yogurt organisms. Streptococcus thermophilus (S. thermophilus) contains approximately three times more lactase activity than did Lactobacillus bulgaricus (L. bulgaricus). The lactase activity was 1.5, 2.4 and 3.8 units/g for L. bulgaricus, S. thermophilus and combined culture respectively (Savaiano and Levitt, 1987).

Lin et al. (1989b) demonstrated a method for determining β-galactosidase activity of yogurt culture in skim milk. The specific activity was 4.5 units under optimal assay conditions.

Lin et al. (1991) estimated the β-galactosidase activities of various culture organisms. Mixed yogurt strain had a maximum activity (2.8 units) followed by single strains of L. bulgaricus (2.4 units) and S. thermophilus (1.8 units).

Of the three strains of L. acidophilus tested, all exerted lower activity than the yogurt organisms. Among the three strains also there was a considerable variation in their activity. L. acidophilus strain LA-2 showed the highest (1.2 units) and L. acidophilus strain NCFM the lowest (0.08 units) activity.

2.2.1 Importance of Antibacterial activity of Lactic acid bacteria

Metchnikoff (1908) proposed that the longevity of Bulgarians was due in part to their consumption of large quantities of fermented milk products. He postulated that the intestinal flora produced toxins which were detrimental to the host. The harmful effects of the intestinal bacteria were overcome by establishing a specific balance between the enteric flora and lactobacilli.

Vincent et al. (1959) reported that maintenance of gastrointestinal populations of L. acidophilus controlled potential pathogenic bacteria normally present in the gut.

Studies conducted by Niv et al. (1963) revealed that children suffering from infantile diarrhoea recovered more rapidly when fed yogurt as compared to neomycin-kaopectate.

Culture of Streptococcus lactis ssp diacetylactis (S. lactis ssp diacetylactis) extended the shelf life of cottage cheese, meat and numerous other products by preventing the growth of Pseudomonas sp. (Vedamuthu et al., 1966).

A decrease in the intestinal Escherichia coli (E. coli population is significant because, these organisms have been reported to produce Ethionine and Nitrosamines from nitrates and nitrites. These compounds are known to be potent carcinogens (Hill et al., 1971).

Gilliland (1979) while investigating lactose intolerance, reported that the abdominal cramps were mainly due to the gas production by the intestinal organisms. While fermenting lactose, Lactic acid bacteria produce acids, hydrogen peroxide and bacteriocins, which inhibited the gas producing organisms, thus reducing the severity of intolerance symptoms.

Probiotic lactic acid bacteria, which may include the traditional lactic acid bacterial species are said to improve the health of gastrointestinal tract, relieving constipation and preventing diarrhoea (Gorbach et al., 1988).

Gilliland and Walker (1990) reported that, L. acidophilus which occurs naturally in the intestine, could compete and grow well in the presence of similar bacteria, by

the virtue of bile tolerance and bacteriocin production, which provide them an advantage to establish and grow in the intestine.

2.2.2 Contaminants in yogurt and dahi

Bhat and Reporter (1949) studied the fate of some intestinal pathogenic bacteria in milk. They found that typhoid bacteria thrived in sour curd made from pasteurised milk, whereas cholera and dysentery bacteria showed a slow growth.

Tiwari and Singh (1964) reported the survival of E. coli, Salmonella paratyphi (S. paratyphi), Shigella dysenteriae (S. dysenteriae) and Staphylococcus aureus (S. aureus) in dahi.

No yeasts were detected in dahi prepared under laboratory conditions but developed in market samples to an average count of 8,85,000/ml after 72 hours of storage (Sreenivasan and Ranganathan, 1972).

Park and Marth (1972a, 1972b) determined the survival of Salmonella typhimurium (S. typhimurium) in skim milk during fermentation and in fermented milks during refrigerated storage. At low inoculum levels (0.25 per cent) of S. lactis and S. cremoris growth of S. typhimurium was inhibited but not

inactivated. Use of the same cultures at one per cent level resulted in inactivation of S. typhimurium during fermentation. S. thermophilus and L. bulgaricus showed much more inhibition to S. typhimurium and resulted in complete inactivation of the organisms.

Arnott et al. (1974) reported that some strains of S. aureus survived in commercial yogurt.

E. coli and Enterobactor aerogenous (E. aerogenous) incorporated in dahi before curdling of milk survived fairly longer periods during storage at room or refrigerated temperatures (Prasad et al., 1980).

Maciejska and Czarniakowa (1985) studied the ability of Micrococcus species to resist and grow in the presence of yogurt and cheese starter cultures. The organism survived and grew in the presence of L. bulgaricus, S. thermophilus and L. helveticus.

Mohanan et al. (1985) studied the growth of common contaminants of dahi, together with starter cultures. They found out that Enterobacteriacee died out with in 24 hours, aerobic spore formers were unable to multiply in the presence of starters and died out within 24 hours. E. coli was able to grow in the presence of S. thermophilus but not in the presence of L. bulgaricus, which suggested that L. bulgaricus

was mainly responsible for inhibiting growth of E. coli in dahi.

Ahmed et al. (1986) reported that Yersinia enterocolitica (Y.enterocolitica) not only survived during manufacture of yogurt, but also grew during the process, but the population steadily decreased during refrigerated storage of one week.

Walker (1988) reported that the most common spoilage microorganisms of milk and dairy products were those of gram negative rod shaped bacteria (Pseudomonas sp. and Coliforms), gram positive spore forming bacteria like bacillus sp. and Clostridium sp., lactic acid producing bacteria (Streptococcus sp.) yeasts and moulds.

While studying the ability of Listeria monocytogenes (L. monocytogenes) to grow and compete with mesophilic lactic acid bacteria (S. cremoris and S. lactis) Schaack and Marth (1988a) reported the survival of L. monocytogenes during fermentations. It was also observed that the organism also grew to some extent.

Khedkar et al. (1990a) studied the growth of S. aureus and E. coli in the presence of yogurt and acidophilus cultures. They reported that both the pathogens grew to 10^{5-7} cfu/ml and 10^{8} cfu/ml respectively before being inhibited.

Survival of pathogenic bacteria such as S. typhimurium, Y. enterocolitica and Campylobactor jejuni (C. jejuni) in yogurt and dahi stored at 5-7°C and 37°C was studied by Matta et al. (1991). None of the pathogens survived in dahi or yogurt at 37°C for more than 25 hours, but a few survived even after 48 hours of storage at 5-7°C.

2.2.3 In vitro antibacterial activity of lactic acid bacteria

Seneca et al. (1950) reported that yogurt was an active bactericidal and protozoidal milk product.

Antibacterial ability of the crude lactocidin a bacteriocin extracted from L. acidophilus was tested by Vincent et al. (1959) on several organisms. Proteus vulgaris (P. vulgaris), Salmonella enteritidis (S. enteritidis), Pasteurella multocida (P. multocida), E. coli, Pseudomonas aeruginosa (P. aeruginosa), S. aureus and Bacillus subtilis (B. subtilis) were some of the organisms which failed to survive in the presence of lactocidin.

Daly et al. (1970; 1972) conducted growth associated studies in which S. lactis ssp diacetylactis has significantly reduced the growth of Pseudomonas sp, S. aureus, E. coli, Clostridium perfringens (C. perfringens) and Alkaligenes sp.

Singh and Lakshminarayana (1973), reported the ability of L. acidophilus, L. bulgaricus, L. lactis and L. plantarum, to inhibit S. aureus, E. coli and B. subtilis.

Crude lactic cultures inhibited the growth of various psychrophilic bacteria (Gilliland and Speck, 1974; Reddy et al., 1975).

Gandhi and Nambudripad (1975) reported that the antibacterial activity of dahi collected from shops and homes against E. coli, Aerobacter aerogenous (A. aerogenous), Bacillus cereus (B. cereus) and S. aureus. Out of the 100 samples tested 62 showed inhibitory action towards one or more test organisms.

Shahani et al. (1976) reported that optimum temperature for L. acidophilus to produce antibacterial compounds was 37°C and milk medium had been indicated to be essential for production of these substances.

Babel (1976) reported that Nisin inhibited the streptococci of groups A, B, E, F, G, K and H, Pneumococci, Neisseria, Mycobacteria, Actinomyces, Staphylococci, Bacillus, Clostridia and Erysipelothrix.

Comparative studies on the inhibitory effect of S. lactis spp diacetylactis and S. thermophilus against

E. coli was done by Grinevich (1977). S. lactis ssp diacetylactis showed greater inhibition than S. thermophilus.

Pulusani et al. (1979) tested the antibacterial activities of various lactic acid bacteria against B. subtilis, B. pumulis, P. fluorescens, Flavobacterium capsulatum (F. capsulatum), S. typhimurium, E. coli, Shigella sp and S. lactis. Of the lactic acid bacteria tested, L. acidophilus, L. bulgaricus and S. thermophilus strongly inhibited the growth of test organisms.

In vitro studies conducted by Sandine (1979) revealed inhibition of bacilli, enteropathogenic E. coli., Klebsiella, Proteus, Pseudomonas, Salmonella, Shigellae, staphylococci, and Vibrio organisms by L. acidophilus.

Gandhi and Nambudripad (1979) reported that antibacterial activities exhibited by acidophilus milk foods varied with starter strain used. Those prepared using L. acidophilus-R showed greatest antibacterial activity.

Inhibitory activity of acidophilus sour milk against E. coli, B. subtilis, M. flavous and S. aureus was reported by Gandhi and Nambudripad (1980).

Dubois et al. (1982) reported that inhibitory activity of L.acidophilus against Pseudomonas sp was greater than that

exhibited by S. thermophilus. In vitro antibacterial activity of three strains of L. bulgaricus and one strain of L. acidophilus was tested by Reddy et al. (1984). Two of the three L. bulgaricus strains showed no inhibition against B. subtilis, E. coli, P. vulgaris, P. aeruginosa, P. fluorescens, Sarcina lutea (S. lutea), Serratia marcescens (S. marcescens), S. aureus and S. lactis. L. acidophilus showed moderate to strong inhibition against all the organisms tested. One strain of L. bulgaricus (DDS-14) exerted the strongest inhibition against all the organisms tested.

L. acidophilus was successfully used as a supplimentary culture in yogurt manufacture, to get a product with improved therapeutic properties. Such a product has got greater acceptability, as judged by sensory evaluations, than the conventional product (Sharma and Prasad, 1986 and Puhan, 1990).

Prasad and Gandhi (1987) reported that L. acidophilus-R exhibited strong inhibition against E. aerogenous, M. flavous, B. cereus, B. subtilis and S. typhosa. The yeast cultures were not inhibited. They also reported that the inhibition was maximum at pH 3.2 and became nil at pH 4.5.

The inhibitory effect of acidophilus yogurt, normal yogurt and acidified yogurt against S. aureus was tested by

Attaie *et al*. (1987). The acidophilus yogurt produced the highest inhibition against *S. aureus*.

Antibacterial activity of acidophilus milk was tested by Rao and Gandhi (1987) against common contaminants. *E. coli* was most susceptible followed by *S. aureus* and *B. subtilis*.

Cultured dairy products were antagonistic to growth and survival of pathogenic microorganisms as well as spoilage organisms. *S. typhimurium*, *E. coli*, *A. aerugenous*, *S. aureus* and *L. monocytogenes* were some of the sensitive pathogens (Schaack and Marth, 1988b).

Studies made by Khedkar *et al*. (1990b) with two strains of *L. acidophilus* of human origin showed variation in antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhosa* and *B. cereus*. Strain $LBKV_3$ exhibited higher inhibition than $LBKI_4$ against all the organisms tested.

Neutralised extracellular culture filtrate obtained from isolates of *L. acidophilus*, *L. bulgaricus*, and *S. lactis*, from dahi showed weak to moderate inhibition against *S. aureus*, *B. cereus*, *E. coli*., *B. brevis*, *B. circulans*, *B. coagulans*, *B. laterosporus*, *B. subtilis*, and *P. aeruginosa* (Varadaraj *et al*., 1990).

Geetha (1992) reported that the antibacterial activity of yogurt was highest against *E. coli*, *B. cereus* and *S. aureus*, when it was supplimented with *L. acidophilus*.

2.2.4 *In vivo* Antimicrobial activity

Smith (1924) reported that administration of *L. acidophilus* reduced the number of intestinal *E. coli*.

Acidophilus yeast milk produced with culture of acidophilus bacteria and a lactose fermenting yeast was antibiotically effective against *Mycobacterium tuberculosis* (*M. tuberculosis*) and several intestinal disorders (Skorodurnova, 1959).

A study conducted in patients suffering from Chronic colitis to see the effect of acidophilus milk showed its better tolerance by the patients, than fresh milk. It also had a beneficial effect on the general condition of most of the patients, bringing about complete disappearance of symptoms or a drastic reduction in their severity (Ekisennina and Tarnapolskaya, 1960).

Infants fed with milk fermented by *L. acidophilus* resulted in an increase in the numbers of these organisms and a decrease of gram negative bacilli in the intestinal flora. In 13 out of 17 cases of infantile diarrhoea, feeding 30

millilitres of fermented milk improved the condition (Aritaki and Ishikawa, 1962).

Patients suffering from serious urological infections by strains of E. coli, Proteus sp, S. aureus and S. haemolyticus, were fed with acidophilus milk resulted in 80 per cent recovery, 15 per cent improvement and unsuccessful only in five per cent of the patients (Tomic-Karovic and Krivec, 1964).

Yakult (1971) observed an increase in the acidophilus count and decrease in the coliforms, when specific pathogen free chickens were fed with L. acidophilus.

Similar observations were made in piglets by Muralidhara et al. (1977).

Sinha (1978) reported that feeding of unfermented milk containing L. acidophilus to rats increased the acidophilus count with a concomitant decrease in coliform count, in the intestinal flora.

Ayebo et al. (1980) reported that supplementing the regular diet of human subjects with low fat milk containing viable cultures of L. acidophilus increased the total lactobacilli count during the period of supplementation and remained at that level for atleast four weeks after culture

supplementation was discontinued. The total coliform counts during acidophilus supplementation decreased, but increased again to pre-trial level, once the acidophilus supplementation was discontinued.

Gilliland et al. (1980) compared two strains of L. acidophilus as a dietary adjunct in young calves. More lactobacilli and fewer coliforms were found in the intestine of calves which were given L. acidophilus than the control calves. The strain of L. acidophilus which originated from intestine of young calves produced greater changes than did a strain of human origin. They concluded that the strain of L. acidophilus of calf origin was more effective as a dietary adjunct in calf than a strain of human origin.

Kulpsh (1985) reported that L. acidophilus cells implanted and controlled the multiplication of putrifactive organisms which were responsible for intestinal disorders.

Teat dips containing L. acidophilus was effectively used to prevent bovine mastitis caused by S. aureus (Oliver and Mitchell, 1985).

Perdigon et al. (1987) found that L. acidophilus and S. thermophilus enhanced the enzymatic phagocytic activity of potential macrophages significantly, and also accelerated the phagocytic action of the reticulo endothelial system of mice.

The organisms activated lymphocyte and macrophages and produced an increase in the immune response.

Daily consumption of 50 millilitres of acidophilus milk shortened the Salmonella carrier state (Khedkar et al., 1990c).

Takahashi et al. (1991) reported that mucosal stimulation by orally administered L. acidophilus in mice induced a systemic immune response.

A cross over trial conducted by Hilton et al. (1992), indicated that daily consumption of yogurt containing L. acidophilus prevented vulvo vaginal candida infections. In treatment a three fold decrease in the infection was reported.

Effect of feeding milk fermented with L. acidophilus of human origin on faecal lactobacilli was studied by Patil et al. (1992). An increase in the lactobacilli count and decrease in coliform count was reported.

Mice challenged with L. monocytogenes and entero invasive E. coli, after giving L. acidophilus and L. casei cultured milk, obtained a 100 per cent protection while that of controls were 62-83 per cent. The level of antipathogenic sera and intestinal antibodies were two to four times higher in the treated mice, suggesting that cultured milk with

L. acidophilus and L. casei could be used as a prophylactic agent against selected pathogens (Nader de Macias et al., 1993).

2.2.5 Mode of inhibition by lactic cultures

Schaack and Marth (1988b) reported the possible conditions responsible for the inhibition of pathogenic bacteria by the lactic cultures. They include lactic, acetic, propionic and formic acids, diacetyl, hydrogen peroxide, low pH, anaerobic conditions and production of various bacteriocins.

2.2.5.1 Bacteriocins

Kodama (1952) reported the presence of an antibiotic substance called lactocin in lactic acid bacteria.

Bacteriocins are potent antibacterial substances which are produced by a large and diverse assortment of bacterial sp. Collectively bacteriocins form a heterogenous group with regard to producing bacteria, antibacterial spectrum, mode of action and chemical properties. S. lactis was reported to produce a low molecular weight peptide active against gram positive bacteria, namely nisin (Hawley, 1957 and Daeschel, 1989).

Vincent et al. (1959) tested the strains of L. acidophilus obtained from mice, rats, rabbits, hamsters and man. They were found to produce lactocidin, a bacteriocin, which exerted broad spectrum antibacterial activity against common enteric pathogens.

Branen et al. (1975) isolated some extracellular water soluble, low molicular weight compounds from the actively growing cultures of S. lactis ssp. diacetylactis and Leuconostoc citrovorum which had antimicrobial activity against pseudomonas sp, A. aerogenes, M. flavous, S. aureus and enteropathogenic E. coli.

Mikolajcik and Hamdan (1975a, b) identified an antibacterial agent in cultures of L. acidophilus and named it as Acidolin.

Shahani et al. (1976, 1977) purified a low molecular weight nitrogenous compound called Acidophilin from the cultures of L. acidophilus. Acidophilin was shown to posses very potent antibacterial activity. It was also found that different strains of L. acidophilus and L. bulgaricus differed greatly in their ability to produce bacteriocins.

Tagg et al. (1976) defined bacteriocins as a protein containing macromolecules produced by lactic acid bacteria,

which exerted a bactericidal mode of action on susceptible bacteria.

Kozak et al. (1977) reported that the strains of S. lactis which were non nisin producing produced lactostreptins.

S. thermophilus was reported to produce strong antibacterial compounds. They were identified as heat stable amines of low molecular weight (700 daltons) (Pulusani et al., 1979).

Neutralised samples of commercial fruit yogurt produced inhibition zones of 14.5-19.5 millimetre diameter against B. subtilis when assayed by agar diffusion test. Inhibition was more at pH 6 than at pH 8 (Foissy and Wolfslenher, 1982).

Production of bacteriocins by lactic acid bacteria was described by Shahani (1982). S. lactis produced nisin, S. cremoris produced diplococcin, L. brevis produced lactobacillin and lactobrevin, L. plantarum produced lactolin, L. acidophilus produced acidolin, acidophilin, lactocidin, lactocin-B and L. bulgaricus produced bulgarican.

Increase in the pH of pooled fraction reduced bulgarican activity and stability. Reacidification with 2N

hydrochloric acid restored full activity of the bulgarican although hydrochloric acid itself possess no inherent activity. This suggested that protonated carboxyl groups may play a role in bulgrican's antibacterial activity. Acidophilin, acidolin and lactocidin isolated from L. acidophilus and bulgarican from L. bulgaricus exhibited significant inhibition towards a wide range of pathogens, both gram positive and gram negative bacteria. But it showed no appreciable antifungal activity (Reddy et al., 1984).

2.2.5.2 Acid and pH

Antibacterial properties of lactobacilli of yogurt and acidophilus milk were due to lactic acid and hydrogen peroxide (Vincent et al., 1959).

Park and Marth (1972a,b) reported that inhibition of S. typhimurium in yogurt was due to intracellular dissociated moity of lactic acid and some factors other than lactic acid.

Ganske and Branen (1973) reported that inhibitory activity of S. lactis ssp. diacetylactis was due to the production of organic acids and hydrogen peroxide.

Lactic acid percentage of the dahi samples did not correlate to the inhibitory activity of the product (Gandhi and Nambudripad, 1975).

Rubin (1977) reported that lactic acid was the main inhibitory factor in yogurt against S. typhimurium.

Gilliland and Speck (1977b) reported that the antagonistic activity exhibited by L. acidophilus upon S. aureus and E. coli was not directly related to the amount of acid produced by the lactobacilli.

Patkal et al. (1977) established a relationship between rate of acid production and degree of inhibition of coliforms by lactic acid bacteria.

Shahani (1982) reported that the antibacterial activity of lactic acid bacteria had been attributed in part to the production of lactic acid which decreased the pH of the medium, thus preventing the growth of acid sensitive bacteria.

Low pH of fermented dairy products contributed to the inactivation of pathogens during ripening and storage (Northolt, 1984).

Results of a series of experiments conducted by Prasad and Gandhi (1987) showed that the antibacterial activity of L. acidophilus cultures were maximum at pH 3.2 and decreased with an increase in the pH. This indicated low pH had also contributed to the inhibitory activities of lactic acid bacteria.

Low pH and organic acids were the major factors responsible for inhibitory action of lactic acid bacteria towards coliforms (Reinheimer et al., 1990).

2.2.5.3 Diacetyl

Sandine et al. (1972) studied the factors affecting the viability of pathogens in fermented milk products. He reported that factors like diacetyl production and lower fatty acids, contributed to the inhibition.

Kulshrestha and Marth (1974) studied 25 compounds associated with milk for their inhibitory properties towards E. coli. Diacetyl was found to be the most inhibitory, ketone and formaldehyde were more detrimintal than acetaldehyde.

Maximum accumulation of flavour substances including diacetyl, in fermented milk products containing cultures of S. lactis ssp. diacetylactis occurred during first few hours of fermentation (Erzinkyan et al., 1981).

Jay (1982) while reporting about the antimicrobial properties of diacetyl stated that it was most efficient against gram negative bacteria and least effective against lactobacilli.

S. lactis ssp. diacetylactis is added to milk products primarily for flavour production, however, some strains

produce a considerable amount of acid. This bacterium differ from S. lactis in the ability to ferment citrate to diacetyl (Schleifer, 1987).

2.2.5.4 Hydrogen peroxide

Dahiya and Speck (1968) reported that hydrogen peroxide present in the culture filtrate of L. bulgaricus was responsible for its antibacterial activity against S. aureus.

Price and Lee (1970) reported that inhibition of Bacillus, Pseudomonas and Proteus sp. was due to the presence of hydrogen peroxide in the lactobacilli.

The antagonistic activity of L. acidophilus towards enteric pathogens and psychrophilic spoilage organisms has also been attributed to the production of hydrogen peroxide by L. acidophilus (Gilliland and Speck, 1977a and Collins and Aramaki, 1980).

Accumulation of hydrogen peroxide in growth media of lactobacilli occurs because they do not possess catalase enzyme. Hydrogen peroxide can react with other compounds to produce inhibitory substances. In raw milk, hydrogen peroxide generated by lactic acid bacteria can react with endogenous thiocyanate to produce compounds which are inhibitory to

microorganisms (Reiter and Harnulv, 1984; and Kandler and Weiss, 1986).

Matalon and Sandine (1986) also reported that hydrogen peroxide produced by some lactobacillus cultures had been associated with their inhibitory effect against a variety of bacteria.

However, Dominguez et al. (1987) reported that hydrogen peroxide, selectively enrich the product for L. monocytogenes thus giving an adverse effect.

2.3.1 Importance of bile tolerance

L. acidophilus possess several characters that enable it to survive and grow in the intestine. Among this is the ability to grow in the presence of bile. This character had been identified as an important one in preparation and storage of concentrated cultures for use as dietary adjunct (Gilliland, 1979).

In a feeding trial by Gilliland et al. (1984) in new born dairy calves, L. acidophilus was supplemented with feed. The strain with more bile resistance caused greater increase in the number of facultative lactobacilli in the upper small intestine than did the strain with lower bile resistance.

Gilliland et al. (1985) reported that L. acidophilus when grown anaerobically in the presence of bile assimilated cholesterol. As the concentration of bile was increased from 0-0.5 per cent, the amount of cholesterol assimilated was also increased.

Gilliland and Walker (1990) suggested that a culture of L. acidophilus of human origin which assimilates cholesterol, grow well in presence of bile and produces bacteriocins, should be selected for use as dietary adjunct in humans. A culture of L. acidophilus possessing all these characters will have an advantage over one, that does not, in establishing and functioning in the intestine. If the dietary culture is to function as a result of surviving and growing in the intestinal tract, it is important that it be able to grow in presence of bile.

Overdahl and Zottola (1991) reported that bile tolerance is necessary for survival of lactobacilli in the intestinal tract. An outer polysaccharide layer might be responsible for adherence to human intestinal tract. They suggested that these two factors may be the basis for use of L. acidophilus as a dietary adjunct.

Noh and Gilliland (1993) studied five strains of L. acidophilus. They found that β-galactosidase activity of

all the five strains were significantly high in the presence of 0.3 per cent oxgall than in its absence. They explained that in the presence of bile the cellular permeability of L. acidophilus increased, permitting more substrate to enter into the cell, thus increasing the β-galactosidase activity of whole cells.

2.3.2 Bile tolerance of L. acidophilus

In vitro studies made by Pattersson et al. (1983a) indicated that L. acidophilus survived better than L. bulgaricus in gastric juice.

Pattersson et al. (1983b) reported the presence of viable L. acidophilus in human ileum upto 4.5 hours after ingestion of acidophilus milk.

Lindwall and Fonden (1984) studied the viability of various lactic acid bacteria in presence of bile and gastric juice. Both in vitro and in vivo experiments showed a better survival of L. acidophilus as compared to L. bulgaricus.

While studying the ability of seven strains of L. acidophilus isolated from calf intestine, Gilliland et al. (1984) reported a marked difference among the strains to tolerate bile. In presence of 0.3 per cent oxgall strains 27SC, 25SB and 36 SB showed higher resistance and attained

optical density 0.3 in 3.22 hours while three strains had not reached optical density 0.3 even after six hours.

Khattab and Abour-Donia (1987) reported that out of the six strains of lactic acid bacteria tested for their ability to grow in the presence of 0.3 per cent of bile salt, S. thermophilus and S. lactis failed to grow in the presence of bile salt at any concentration (0.15, 0.2 and 0.3 per cent) L. bulgaricus eventhough grew in low concentration, failed to show any growth at 0.3 per cent. L. acidophilus grew well in all the concentrations tested.

Conway et al. (1987) reported that L. acidophilus-ADH and L. acidophilus-N2 survived better than L. bulgaricus and S. thermophilus, in normal saline at low pH, and gastric juice. The survival rate of all these organisms in gastric juice was extended by the addition of milk.

Hood and Zottola (1988) reported that L. acidophilus was able to survive and adhere to human intestinal cells in vitro at a low pH values of 3-4.

L. acidophilus and Bifidobacterium bifidum (B. bifidum) were compared for their viability in acid pH (Holcomb, 1991). Only L. acidophilus survived and grew after exposure to 0.01 N hydrochloric acid for two hours at 37°C.

Dierdahl and Zottola (1991) tested 17 strains of L. acidophilus for bile tolerance. Fourteen strains tolerated one per cent of bile and one strain tolerated upto 0.6 per cent of bile. Of the strains tested only two strains were unable to tolerate any of the bile concentrations used in the study.

L. acidophilus and B. bifidum were compared for bile tolerance by Hoier (1992). Both of them were not inhibited except at high concentrations, which was unlikely to be found in normal intestinal condition.

Walker and Gilliland (1993) reported that there was a considerable variation among 19 strains of L. acidophilus tested for bile tolerance. All the strains of L. acidophilus exhibited some degree of bile tolerance. Some of the differences in the bile tolerance was attributed to the natural difference in growth of the individual strain.

Five strains of L. acidophilus were compared for their growth in PMN broth, with or without 0.3 per cent oxgall. All the strains grew more slowly in the presence of oxgall. However, only the growth of two strains were significantly lower in presence of oxgall than in its absence ($P>0.05$) (Noh and Gilliland, 1993).

2.3.3 Implantation of L. acidophilus in the intestine

Savage (1972) reported that the mechanism of attachment of L. acidophilus to the intestinal wall was mediated by acidic mucopolysaccharides occurring on the surface of the bacteria.

Moore and Holdeman (1972) isolated the common lactobacilli from human intestine. They were identified as L. acidophilus, L. bifidus, L. plantarum, L. casei and L. fermentum. Of these only L. acidophilus and L. bifidus were present in sufficient numbers.

Electron microscopic studies by Brooker and Fuller (1975) showed the adhesion of lactobacilli to gastrointestinal tract by means of a carbohydrate rich layer on the bacterial cell.

Members of lactobacilli especially L. acidophilus, which are indigenous to the intestinal tract of man and animals. Their presence in the intestine helps to establish and stabilize the microflora of healthy individuals (Sandine, 1979; and Menon, 1991).

Eventhough many lactobacilli survive selective pressures of the gastro intestinal environment, flow rates of digesta through the small intestine washes out any organism

which is unable to multiply rapidly enough to avoid dilution or to maintain their residence by physical attachment to the intestinal epithelium (Robins-Browne and Levine, 1981).

Kleeman and Klaenhammer (1982) established a screening system to test L. acidophilus for adhering ability. They reported a considerable variation among the strains in their ability to adhere to the intestinal epithelium. Eventhough all the 27 strains tested, adhered well in presence of calcium, only four of them retained the ability to adhere without calcium.

Elevated L. acidophilus counts in the intestine of human subjects was reported even after the cesstion of ingestion of L. acidophilus, one week prior the estimation (Lindwall and Fonden, 1984).

Conway et al. (1987) compared the ability of two of L. acidophilus strains (LA-ADH and LA-N2), L. bulgaricus and S. thermophilus to adhere to human and pig ileal, caecum or colon cells, in vitro. Both of the L. acidophilus strains survived and adhered better than L. bulgaricus and S. thermophilus. Among the two strains of L. acidophilus, LA-ADH showed better survival and adhesion.

Hood and Zottola (1988) reported that L. acidophilus

was able to adhere to human intestinal cells even after subjected to low pH treatment for five hours.

Overdahl and Zottola (1991) reported that an outer polysaccharide layer present in L. acidophilus was responsible for adhesion to the human intestinal tissue. Of the 17 strains they tested, ten exhibited ruthenium red stained outer polysaccharide layers, which indicated a stronger adherence property.

2.4.1 Hypercholesteremia

Cholesterol is the prime suspect in Coronary Heart Disease (CHD), because the formation of atherosclerotic lesion, is an inflammatory response to this substance. Spain (1966) conducted studies on 6000 men and found that cholesterol levels in blood and blood pressure had a positive correlation with atherosclerosis.

Kurski and Narayana (1976) reported that the chickens fed with feed containing cholesterol showed an increase in the HDL level from 25.6 $\pm$ 6.7 to 152.3 $\pm$ 41.3.

The lipids in plasma circulate with lipoproteins, namely chylomicrones, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). About 70 per cent of total plasma cholesterol in

normal human beings is contained in LDL, the lipoprotein most strongly correlated with atherosclerosis. LDL carries cholesterol into the blood vessels, whereas HDL takes plasma cholesterol to the liver for metabolism. There is an inverse relationship between levels of HDL and the development of atherosclerosis (Robbins and Cotran, 1981).

Brown and Goldstein (1984) Nobel lauretes in medicine, shed more light on the cholesterol issue. Cholesterol being hydrophobic in nature does not circulate freely in the blood but only in association with lipoproteins, LDL and HDL. High levels of LDL in blood causes atherosclerosis to develop. The level of LDL particles in blood is affected by specialized proteins called LDL receptors. These receptors bind LDL particles and extract them from the fluid that bathes the cells. The LDL is broken down in the cells and cholesterol is used for biological functions. When the need is low the cell makes fewer LDL-receptors, thus LDL levels in blood raises, accumulating excess cholesterol in arteries, which accelerates the atherosclerosis. The inadequacy in LDL receptors has been attributed to both genetic and environmental factors.

Kansal (1990) reported that almost one million people die annually from cardiovascular disorders. Elevated serum cholesterol levels clearly increase the risk of cardiovascular disease. It was predicted that a two per cent reduction in

cardiovascular diseases for every one per cent reduction in serum cholesterol.

2.4.2 Milk and coronary heart disease

Cholesterol and triglyceride contents of milk and meat foods in a pastoral area were studied by Wang et al. (1982). They reported that triglyceride content of milk products were much higher than that of meat.

Raheja (1987) has emphasized that poor dietary habbits, altered lifestyle and the use of fried fatty foods in the diet which provide higher levels of peroxides, with the predisposition to diabetes, may contribute to the development of coronary heart disease.

Dairy products may made an appreciable contribution to saturated fat and cholesterol intake. For this reason restricted use of full cream milk is usually included in the physicians dietary recommendations to hyper cholesterolemic patients (Kansal, 1990).

In past years milk has received some adverse publicity in terms of its effect on the blood cholesterol level of consumer. Indeed, milk fat when consumed as butter or cream does exert a hypercholesterolemic response in humans. Milk fat contains a moderate amount of cholesterol. The fatty acid

profile of milk fat is unique. It is primarily a saturated fat, containing 50 mol/100 mol long chain saturated fatty acids and about 15 mol/100 mol short and medium chain fatty acids (Boudreau and Arul, 1993).

2.4.3 Milk and hypocholesteremia

Orotic acid a pyrimidine intermediate in the nucleic acid synthesis generally exist in milk at a concentration ranging from 72-122 mg/litre (Hallanger et al., 1953).

Windmuller (1963) observed that plasma lipids, particularly triglycerides, were depressed in the experimental rats as early as 16 hours, after orotic acid was introduced in the diet. He also found that LDL fraction almost disappeared from the plasma and a reduction of 60 per cent in HDL fraction in the orotic acid fed rats.

Similar reports by Roheim et al. (1965) stated that orotic acid depresses the formation or release of VLDL.

About one third of the fatty acids of milk are monounsaturated, which neither raises nor lowers the blood cholesterol levels. Poly unsaturated fatty acids account for approximately four per cent and have been reported to lower the blood cholesterol levels. Of the remaining (approximately 63 per cent) saturated fatty acids, nearly 13 per cent are

short chain fatty acids which are metabolised in a manner that has no effect on blood cholesterol and are not deposited in the adipose tissue. Stearic acid which constitutes about 13-14 per cent probably is not involved in increasing the plasma cholesterol content. Therefore only about one third of the milk fatty acids are of the kind suspected of elevating the blood cholesterol (Kahn, 1970; Hurt, 1972; Gurr, 1984 and Hornstra, 1989).

Pottenger and Getz (1971) confirmed that rats fed a diet one per cent in orotic acid for seven days developed fatty livers and that there was an apparent inhibition of the secretion from the liver of LDL without altering general liver protein synthesis.

Boguslawski and Wrobel (1974) demonstrated that *in vitro* addition of cow milk to rat liver homogenate completely abolished sterol synthesis from its precursors acetate and mevalonate.

Bernstein *et al*. (1976, 1977) reported that orotic acid of milk may be involved in its hypocholesteremic effect. They also found out that choline and myo-inositol commonly found in milk inhibits acetate incorporation into cholesterol, but their inhibitory effect was considerably lower than orotic acid.

Howard (1977) proposed that calcium is one of the factors in the milk responsible for the hypocholesteremic effect. This was predicted merely on observations that calcium lowered serum cholesterol levels by forming insoluble salts with glycoconjugated bile acids, thus increasing their fecal excretion.

Howard and Marks (1977) demonstrated a hypocholesteremic response of unfermented whole milk and skim milk in a large group of subjects. There was a significant reduction in serum cholesterol in both groups on the milk supplement. After two weeks the fall in serum cholesterol was five per cent for the whole milk group and 15 per cent for those on skim milk. They postulated that lactose may be a contributing factor in milk towards hypocholesteremic effect.

Mann (1977a,b) reported that biosynthesis of cholesterol from acetate. The acetate was activated by enzyme Acetyl CoA synthetase and suggested that inhibition of this enzyme by milk probably be the reason for cholesterol biosynthesis. He also postulated that a hypocholesteremic milk factor 3-Hydroxy 3-methyl glutaric acid (HMG) which inhibits the rate limiting enzyme HMG CoA reductase in cholesterol biosynthesis, present in the fermented milks.

Nair and Mann (1977) included 0.1 per cent of HMG into diets with or without cholesterol, the hypocholesteremic effect was similar to those observed for skim milk. These workers therefore postulated that HMG is a hypocholesteremic factor (MF) in milk.

Studies conducted by Kritchevsky et al. (1979) showed a reduction of 40 per cent of the activity of HMG CoA reductase, a rate limiting enzyme in cholesterol biosynthesis in the liver fractions of rat given whole or skim milk.

Ahmed et al. (1979) demonstrated the presence of two compounds in bovine milk, active in reducing cholesterol biosynthesis. One of them, orotic acid is present in the dialysate of the whey, which inhibited the synthesis of cholesterol before formation of mevalonie. The other component was retentate and exerted the effect beyond the formation of mevalonate. The author suggested that the effect of orotic acid was mediated through uracil. They observed that orotate 6-14 C administered to rat was converted into uracil in the liver, and the uracil inhibited hepatic biosynthesis in the same manner as orotic acid did.

Stahelin et al. (1981) in their experiments found out that skim milk, yogurt, whey and fermented whey produced a hypocholesteremic effect in swine. However lactose and casein

failed to decrease the cholesterol levels of swine used in the experimentation. They suggested that the cholesterol lowering principle of milk was neither casein nor lactose.

Studies conducted by Connolly et al. (1982) showed no conclusive result on the contribution of milk products on coronary heart disease (CHD). They studied the effect of high and low levels of dairy products on Irish subjects and concluded that dairy products had no adverse effect on CHD.

Chawla and Kansal (1984) conducted experiments with rats and reported that milk feeding in rats reduced the deposition of cholesterol in liver and blood vessels.

The casein has got a higher ability to increase the HDL-content to high levels when compared to soy protein (Lefevre and Schneemann, 1984).

Subbiah and Yenker (1985) added milk to rat liver homogenates and found a 50 per cent increase in the activity of cholesterol 7C hydroxylase, a rate limiting enzyme in cholesterol breakdown by bile acids.

Research works on buffalo milk was conducted by Srinivasan and Kansal (1986) and reported that buffalo milk despite its high saturated fatty acid content, induced

hypocholesteremia in rats. The treated rats had an increased HDL and reduced LDL significantly.

The same authors (1988) suggested that the hypocholesteremic effect of milk was due partly to enhance excretion of bile acids in faeces in rats fed cow whole milk.

A recent epidemiological study had shown that people who regularly consumed milk were much less likely to suffer a heart attack than those who consumed skimmed milk (Medical Research Council, 1991). Of the people included in the survey, 9.9 per cent of them experienced major Ischemic Heart Disease (IHD) in non milk drinking group. The incidence was 6.3, 5.8 and 1.2 per cent in half pint, half to one pint and more than one pint milk drinking groups respectively.

2.4.4 Hypocholesteremic effect of fermented milks

Role of microorganisms in the cholesterol destruction or degradation in the rat had been reported by Danielsson and Gustafsson (1959).

Wostmann et al. (1966) found an accelerating effect of normal intestinal microflora on systemic cholesterol catabolism and elimination of solubilization and absorption of lipids.

Mann and Spoerry (1974) studied a group of Maasai tribesmen in Africa. In spite of very high intake of milk and meat, they maintained low levels of serum cholesterol and low incidence of clinical coronary heart disease. The researchers accidentally discovered that consumption of large quantities of fermented milk by the Maasai, actually lowered their serum cholesterol levels and cardiac risk factor. This inverse relationship between serum cholesterol level and consumption of milk fermented with a wild strain of Lactobacilli, suggested that there must be a factor in the fermented milk that somehow inhibits the biosynthesis of cholesterol.

Serum cholesterol levels of laying hens were studied by Tortuero et al. (1975) and found that implantation of L. acidophilus on the caeca considerably reduced the cholesterol levels in serum.

Harrison and Peat (1975) reported that the orotic acid content of milk did not decrease during the manufacture of fermented milk products.

Mann (1977a) conducted experiment on American volunteers. The subjects were fed whole milk yogurt, skim milk yogurt or fresh milk daily for 12 days. In general there was a reduction in serum cholesterol during the feeding period, with a slow return towards normality upon cessation of

the yogurt diet. Both whole and skim milk yogurt produced a statistically significant reduction of cholesteremia. Fresh milk at an intake of two litres daily did not statistically affect cholesteremia and he seemed to think that the factors affecting serum cholesterol were produced or enhanced in milk by the microbial action. In the same experiment the author administered radioactive acetate to human volunteers and observed that incorporation of the acetate into cholesterol was inhibited during the consumption of yogurt resulting in decreased cholesterol biosynthesis. He postulated that 3-hydroxy 3-methyl glutaric acid (HMG) in the fermented milk inhibited the rate limiting enzyme in cholesterol biosynthesis, HMG CoA reductase. Mann also suggested that in the biosynthesis of cholesterol from acetate, the acetate would be activated by acetyl CoA synthetase and a decrease in the cholesterol biosynthesis occurred by the inhibition of this enzyme.

Mann (1977b) claimed that the milk factor (MF) which was responsible for hypocholesteremia was slightly more in fermented milk. He also stated that the MF is non protein, dialyzable, heat and acid stable and polar.

Gilliland and Speck (1977a) demonstrated that L. acidophilus could deconjugate both taurocholic and glycholic acids under anaerobic conditions. They suggested

that an increased excretion of bile acid might lead to a faster rate of catabolism of cholesterol by bile acids.

Hepner et al. (1979) conducted experiments on human volunteers and confirmed the ability of yogurt in reducing serum cholesterol levels.

Thakur and Jha (1981) conducted a research on rabbits, in which, they were fed with stock diet, milk, yogurt or calcium carbonate. The milk reduced the effect of cholesterol, but the yogurt and calcium carbonate were similar and had more marked effects. Atheroscleretic lesions and aortic sudanophilia was maximum in the control group. The groups receiving yogurt, and calcium carbonate showed an intermediate degree of sudanophilia. It is suggested that the calcium was responsible for the cholesterol lowering effect of yogurt, but that other hypocholesteremic agents might also be present.

Stahelin et al. (1981) reported that the lipid lowering principle of yogurt was not lactose or casein.

Rats fed with milk fermented by S. thermophilus exhibited a reduction in plasma cholesterol levels (Rao et al., 1981).

Grunewald (1982) tested the effects of reconstituted skim milk, and the same fermented by L. acidophilus in rats. At the end of the feeding trial it was found that fermented milk had lower serum cholesterol levels (65 mg/dl) than did the water fed (78 mg/dl) or milk fed (79 mg/dl). The data indicated that the factors influencing serum cholesterol levels were produced during fermentation of the milk.

In another experiment, Pulusani and Rao (1983) compared the effect of water, skim milk and skim milk fermented by S. thermophilus, L. bulgaricus or L. acidophilus for hypocholesteremia. After the feeding trials the plasma cholesterol levels (mg/dl) and whole body lipids (mg/g dry matter) for the treatments one to five were 61.3, 54.7, 56.0, 57.1 58.1 and 3.68, 3.58, 3.27, 3.18, 3.00 respectively. They postulated that the hypocholesteremia of fermented milks might be due to an increased excretion of cholesterol or its metabolites, and inhibition of cholesterol biosynthesis by metabolites produced by lactic cultures.

Jaspers et al. (1984) fed adult human volunteers with yogurt and found that there was a significant reduction in total serum cholesterol of 10-12 per cent in the initial period, but returned towards control values with continued yogurt consumption. Differences in concentration of uric acid, orotic acid and HMG in yogurt were insufficient to

account for the differences in the temporary hypocholesteremia seen between trials.

Similar experiments were conducted by Chawla and Kansal (1984). They reported that the rats fed with dahi and acidophilus milk had considerably lower serum cholesterol levels than the rats fed with control unfermented milk.

Nelson and Gilliland (1984) in their research, evaluated the ability of L. acidophilus strain of human origin for cholesterol assimilation in vitro. The strain removed both cholesterol and bile salts when incubated in growth medium at 37°C. They also reported that the strains exhibiting low bile tolerance were less active in removing cholesterol from the growth medium while strains exhibiting high bile tolerance varied in their ability to remove cholesterol.

Gilliland et al. (1985) made extensive in vitro and in vivo studies on the cholesterol reduction by L. acidophilus. Tests using high cholesterol diet as model, cholesterol in serum was lower when the diet was supplemented with L. acidophilus. During the same study, they observed deconjugation of bile acids by L. acidophilus, in vitro and suggested that an increased bile acid might lead to a faster rate of catabolism of cholesterol by bile acids. It was also found that L. acidophilus could assimilate cholesterol in

presence of oxgall (<0.05 per cent) during anaerobic growth in MRS broth. The authors suggested that L. acidophilus being part of the normal intestinal flora, was expected to affect the cholesterol metabolism in the intestine.

Germ-free rats were inoculated with the human intestinal bacteria by Chikai et al. (1987). They found that bile acid excretion in faeces was significantly higher in rats inoculated with intestinal microorganisms than in gnotobiotic rats and most of these bile acids were deconjugated. They suggested that free bile acids adhered to bacteria or dietary fibres, thus enhancing excretion of bile acids. This action might trigger the feedback mechanism that regulates the hepatic cholesterol synthesis and subsequent transformation into bile acids, which might reduce cholesterol concentrations.

In an experiment conducted by Danelsson et al. (1989), the hypercholesteremic pigs when fed with acidophilus yogurt showed a significant reduction in total cholesterol and low density lipoproteins. A comparable reduction in triglycerides, was also noticed. The HDL-cholesterol level increased in all the acidophilus yogurt fed pigs.

Lin et al. (1989a) explored the effect of tablets containing L. acidophilus and L. bulgaricus on cholesterol.

In vitro tests revealed that the organisms significantly reduced the cholesterol in the growth medium. The bacteria assimilated cholesterol only when they were alive and at numbers above 10^8 cfu/millilitre. Oxgall inhibited the growth the bacteria especially *L. bulgaricus* thus reducing its ability to assimilate cholesterol. During *in vivo* trials, human subjects were either fed normal diet as control or normal diet with lactobacillus tablet. In all the subjects treated with lactobacillus tablet, mean concentrations of total cholesterol (TC) and low density lipoproteins (LDL) reduced to statistically significant level. High density lipoproteins (HDL) increased 1.8 to three mg/dl. The cardiac risk factor TC/HDL was unchanged in the control group (4.45-4.43) but decreased with time in lactobacillus treated group significantly.

In the same experiment a commercial hypolipidemic tablet placebo and lactobacillus tablet were tested for their hypocholesteremic effect on human subjects. Both treatments reduced TC significantly and HDL increased significantly. The cardiac risk factor (TC/HDL) also had not changed significantly for either treatment. In placebo group it reduced from 4.39 to 3.68 while in Lactobacillus treated group the reduction was from 4.22 to 3.45.

Gilliland and Walker (1989) reported that out of 12 strains of L. acidophilus of human origin compared for characteristics desirable for use as a hypocholesteremic factor, there were significant variation in the ability to grow in presence of bile and to assimilate cholesterol in vitro. The more bile resistant cultures did not necessarily assimilate the greatest amount of cholesterol. They suggested that bile resistance was not directly related to the ability to assimilate cholesterol. Another important character in this regard was the ability to compete well with the other cultures of lactobacilli or lactic acid bacteria.

Khedkar et al. (1990d) studied the efficiency of L. acidophilus-LBKV-3 fermented buffalo skim milk on human volunteers of 50-60 years of age for hypocholesteremia. The feeding significantly reduced the serum cholesterol levels (LDL and VLDL). Eventhough serum cholesterol level (SCL) increased after 15 days of stopping the test feed, it was still significantly lower than the SCL before feeding test diet. This indicates that the human strain of L. acidophilus was either able to uptake the cholesterol from gastro intestinal tract or inhibit cholesterol biosynthesis, thus, it would be helpful in reducing the chances of atherosclerosis.

Gilliland and Walker (1990) tested many strains of L. acidophilus for bile tolerance and cholesterol

assimilation. There were significant differences in the ability to grow in the presence of 0.3 per cent oxgall. The time taken to increase the optical density of broth by 0.3 varied from 2.93 hours to more than seven hours. Cholesterol assimilation also showed significant variation among the strains. The amount of cholesterol assimilated ranged from 103.9 to 27.5 ug/ml. The cultures also showed a considerable variation in bacteriocin production. The author suggested that the most bile resistant culture of L. acidophilus having other desirable characteristics should be selected for use as a dietary adjunct.

Serum cholesterol concentrations of rats fed high cholesterol diets containing skim milk or cultured milk were estimated by Suzuki et al. (1991). Various strains of L. acidophilus were tested and it was observed that strain SBT 2056 had the highest hypocholesteremic effect. The organism had an inhibitory effect against cholesterol absorption in the intestine.

Nakajima et al. (1992) studied the effect of ropy fermented milk on serum cholesterol in rats. Basal diets containing slime forming L. lactis ssp. cremoris-SBT 0495, its non slime forming variant SBT 1275, and acidified reconstituted skim milk with 0.5 per cent cholesterol added,

were fed to rats for seven days. Serum cholesterol levels of rats fed with the ropy fermented milk were lowest among the treatment group. It was 102.6 mg/dl, 115 mg/dl and 126.2 mg/dl for the ropy milk, non ropy milk and control respectively. The serum HDL cholesterol/total cholesterol were highest for rats fed with ropy fermented milk. They postulated that the slime materials produced by S. lactis ssp. cremoris SBT 0495 had a beneficial effect on rat cholesterol metabolism.

Rasic et al. (1992) reported that S. thermophilus assimilated less cholesterol than that of L. bulgaricus. A significant difference was found between strains of L. acidophilus which assimilted significantly more cholesterol than S. thermophilus in a commercial yogurt culture studied.

Walker and Gilliland (1993) reported a considerable variation among the 19 strains of L. acidophilus in their ability to grow in presence of bile, to deconjugate sodium traurocholate and to assimilate cholesterol. All the strains of L. acidophilus tested exhibited some degree of bile tolerance. Cholesterol removal was highest in strain ATCC (50 mg/dl). They found a positive correlation between cholesterol assimilation and bile salt deconjugation. But bile tolerance, bile salt deconjugation and assimilation of cholesterol put together revealed no significant correlation.

2.4.5 Physiological values of laboratory rat

Harkness and Wagner (1989) reported the physiological values of laboratory rat serum proteins 5.6-7.6 g/dl, serum lipis 70-415 mg/dl, phospholipids 36-130 mg/dl, triglycerides 26-145 mg/dl, and total cholesterol 40-130 mg/dl.

2.5.1 Need for L. acidophilus to be incorporated in yogurt and dahi

In India, eventhough dahi is consumed widely, products based on L. acidophilus are not popular. Acidophilus milk, being a new product to Indian palates, had not received ready acceptance due to its high acidity, harsh taste and lack of buttery flavour (Gandhi and Nambudripad, 1975; 1980).

In spite of beneficial roles ascribed to the ingestion of L. acidophilus, little have been done to include this bacterium as a dietary compound. The unappetizing flavour of regular acidophilus milk, discourages its consumption for purposes other than, an urgent need to correct intestinal malady; Consequently it is essentially unavailable to public. There are a number of products containing L. acidophilus that are dispensed as pharmaceuticals. These often are dried preparations. Also there are a number of products available in health food stores. But viable lactobacilli in these products vary considerably and the cost is relatively high.

Efforts are being made to include L. acidophilus in the culture used to make yogurt. The merits of ingesting L. acidophilus by this means are not well documented (Speck, 1976).

Starter culture bacteria L. bulgaricus and S. thermophilus used for the manufacture of yogurt did not survive and grow in the intestinal tract. Therefore the benefits received from yogurt were derived from the contents of the culture rather than the viability of the culture in the intestinal tract (Speck, 1977 and Gilliland and Kim, 1984).

Hargrove and Alford (1978) reported that eventhough L. bulgaricus was frequently isolated in the intestinal tract during feeding trials, it disappeared three days after the discontinuation of yogurt feeding. S. thermophilus never was isolated below the upper small intestine. L. acidophilus was usually present and persisted even when it was no longer ingested in the diet.

Deeth and Tamime (1981) reported that neither L. bulgaricus nor S. thermophilus used in modern yogurt making are native to gastero intestinal tract and stated that prolonged beneficial effects could only be obtained if adherent strains of starter organisms like L. acidophilus were used.

Klaenhammer (1982) reported that primary objective of supplying L. acidophilus through dietary adjuncts was to establish this bacteria in the intestinal tract.

Consumption of acidophilus milk resulted in implantation of L. acidophilus in the intestine whereas the organisms of dahi could not be implanted in the intestinal tract, to control gastero intestinal disorders (Gandhi and Rao, 1989).

Strains of L. acidophilus and L. bulgaricus were compared for their ability to implant in the intestine by Tomar and Prasad (1989). L. bulgaricus had poor ability to implant when compared to L. acidophilus.

Gilliland and Walker (1990) reported that bacteriocins produced by L. acidophilus provide them an advantage over similar bacteria, to establish and grow in the intestinal tract.

Materials and Methods

MATERIALS AND METHODS

3.1.1 Starter cultures used

The following pure freeze dried cultures were procured from National Dairy Research Institute (NDRI), Karnal.

(i) Lactobacillus acidophilus AB-1

(ii) Streptococcus salvarius ssp. thermophilus-YH-S

(iii) Lactobacillus delbrueckii ssp. bulgaricus

(iv) Lactococcus lactis

(v) Lactococcus lactis ssp. diacetylactis

3.1.2 Maintenance of starter cultures

The cultures were activated by three consecutive transfers in sterile skim milk, subcultured and maintained by transfers at fortnightly intervals. Culture combinations used in each treatment were paired just before the experiment to see the inhibitory effect, if any, by one organism over the other.

3.1.3 Preparation of Dahi

Fresh good quality cow milk was procured from University Dairy Plant, Mannuthy. It was pre heated to 35-40°C, and filtered. The filtered milk was standardized to

3.5 per cent fat and 8.5 per cent solid non-fat, heat treated at 85°C for 30 minutes, and then cooled to room temperature.

The milk was then divided into two equal parts of 500 millilitres each. These two parts were used to prepare. Control Dahi (DC) and treatment Dahi (DT).

Control dahi (DC) was prepared with a mixed starter of Lac. lactis and Lac. lactis ssp. diacetylactis, each at one per cent level.

Treatment dahi (DT) was prepared with a mixed starter at two per cent level. Starter culture consisted of Lac. lactis, Lac. lactis ssp diacetylactis and L. acidophilus at 1:1:2 ratio.

(The ratio was arrived after a preliminary trial in which different levels of L. acidophilus was incorporated in the normal starter culture, so that the total L. acidophilus, cells in the finished product would be 10^8 cells/millilitre. At 1:1:2 ratio of Lac. lactis, Lac. lactis ssp diacetylactis and L. acidophilus, in the inoculum, the desired count of L. acidophilus was obtained in the finished product).

The DC and DT thus inoculated, were incubated at room temperature for 16 hours, till a pH of 4.6 was attained. The

DC and DT thus prepared were stored at refrigeration temperature until analysis.

3.1.4 Preparation of yogurt

Fresh, good quality cow milk was received from University Dairy Plant, Mannuthy. After prewarming, the milk was subjected to filtration. Then it was standardized to 3.5 per cent fat and 16 per cent solid non-fat, by the addition of sufficient amount of skim milk powder. The fortified milk was heated to 60°C and homoginised at 2000-2500 psi. The milk was then heat treated at 85°C for 30 minutes and cooled to 42°C.

The yogurt mix thus prepared was divided into two equal parts of 500 millilitre each, and used for the preparation of Control Yogurt (YC) and Treatment Yogurt (YT).

Control Yogurt (YC) was prepared with a mixed starter of *S. salivarius* ssp *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* each at one per cent level.

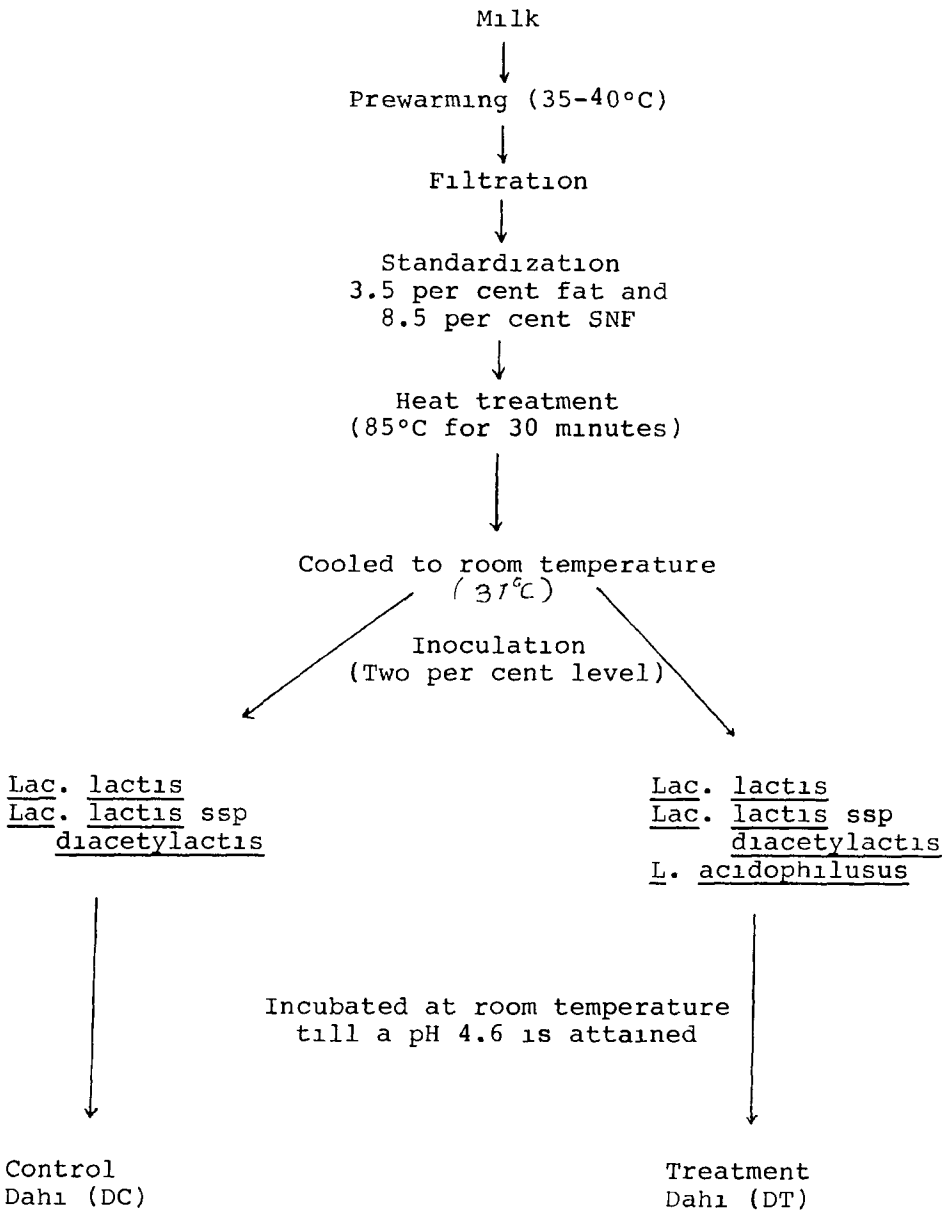
Treatment Yogurt (YT) was prepared with a mixed starter at two per cent level. Starter culture mix consisted of *S. salivarius* ssp *thermophilus*, *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* at 1:1:2 ratio. (A preliminary trial was conducted to arrive at this ratio *L. acidophilus* was added with the normal yogurt starters at different levels and

the level at which the viable cell count of L. acidophilus was 10^8/ml in the end product, was determined.

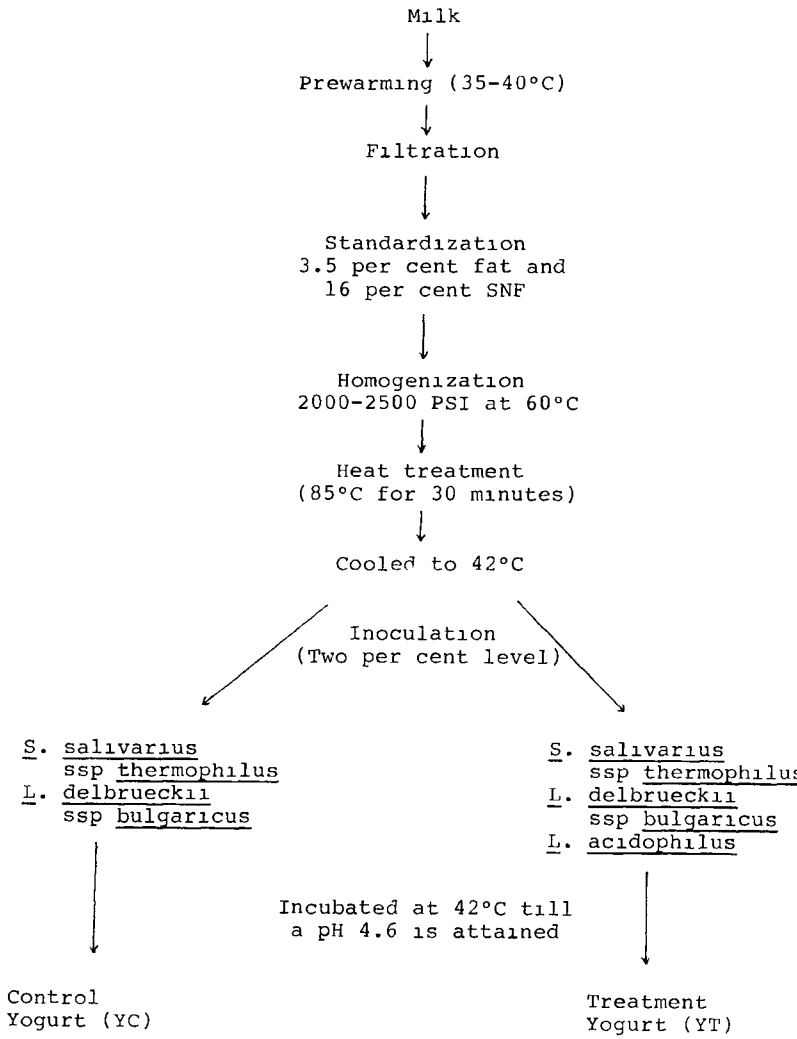
The inoculated mix of YC and YT were incubated at 42°C for four hours, till a pH of 4.6 was attained. The Yogurt YC and YT thus prepared were stored at refrigeration temperature until further analysis.

The flow diagram for the products under different treatments groups is given below.

Flow diagram for manufacture of control and treatment dahi



Flow diagram for manufacture of control and treatment yogurt



The samples of products under DC, DT, YC and YT were then subjected to the following tests.

(i) β-Galactosidase specific activity

(ii) Antibacterial activity

(iii) Bile tolerance

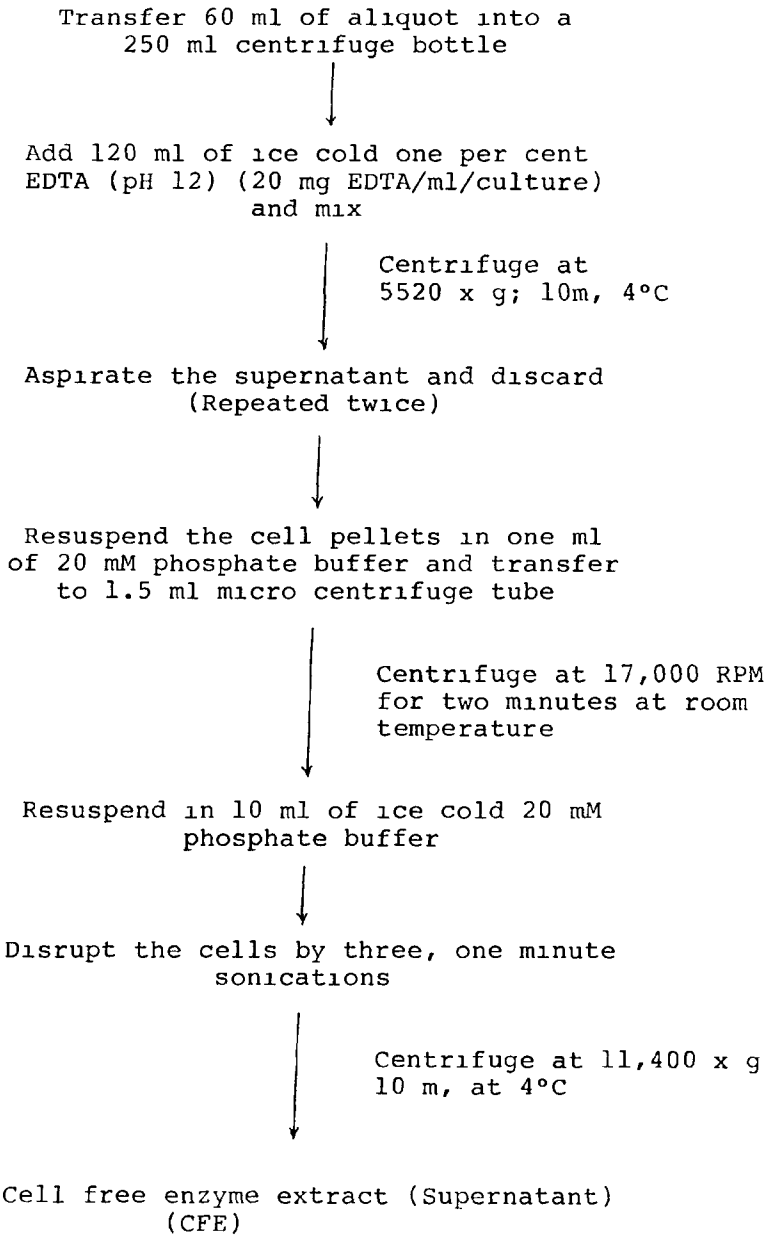
(iv) Hypocholesteremic effect and

(v) Growth rate in rats

3.2.1 β-Galactosidase specific activity

β-Galactosidase specific activity was measured in DC, DT, YC and YT as per the procedure developed by Lin et al. (1989a) the detailed procedure is given below.

Flow diagram



One millilitre aliquot
(CFE)
↓
β-Galactosidase assay
↓
μMol ONP released
per minute per millilitre

One millilitre aliquot
(CFE)
↓
Add one millilitre of
10 per cent TCA
↓
Centrifuge at 900 x g, 10 m
↓
Wash the precipitate with
5 per cent TCA
↓
Resuspend in one
millilitre of 0.5 N NaOH
↓
Lowry protein assay
↓
mg protein/ml

$$\beta\text{-Galactosidase specific activity} = \frac{\mu\text{Mol ONP released}}{\text{mg protein}}$$

3.2.2 Preparation of cell free enzyme extract

Sixty millilitres of the sample (DC, DT, YC or YT) was dissolved in 120 ml of ice cold one per cent Ethylene Diamine Tetrachloro Aceticacid (EDTA) (pH 12) to solubilize milk protein and centrifuged at 5520 x g for 10 minutes. The supernatant was discarded. The procedure was repeated twice to get clear cell pellets. The cell pellets were washed twice with one millilitre of potassium phosphate buffer (20 mM, containing 5 mM $MgSO_4$ - pH 7). The cell pellets were

resuspended in 10 millilitres of phosphate buffer and sonicated for three times, one minute each, with sufficient interval using Imeco ultrasonic sonifier.

Samples were maintained on ice throughout the procedure, to prevent enzyme denaturation by the heat generated during sonication. Then the cell suspension was centrifuged at 11,400 g for 10 minutes to remove the cell debris and whole cells. The supernatant cell free, enzyme extract was immediately assayed for β-Galactosidase specific activity and protein content.

3.2.3 β-Galactosidase assay

β-Galactosidase activity was measured using a chromogenic substance O-nitrophenyl-β-D-Galactopyrinocide (ONPG). Reaction mixtures were prepared by mixing four millilitres of 32 μM of ONPG solution and one millilitre of cell free enzyme extract as above. The reaction mix was incubated at 37°C in a waterbath for 30 minutes. Colour development at the end of incubation was measured at 420 nm, using a Spectronic-20 colorimeter. Total ONP released was calculated by interpretation with a standard curve.

(Standard curve was prepared by dissolving O-Nitrophenol in minimum quantity of alcohol and making up the volume by phosphate buffer to give concentrations two to 48

µmol/ml. The optical density of each concentration was measured in a Spectronic-20 colorimeter at 420 nm. The readings were plotted in a graph).

3.2.4 Protein assay

The protein content of the cell free enzyme extract was estimated using the procedure described by Lowry et al. (1951).

Reagents

(i) Four per cent Sodium carbonate in distilled water (Reagent-I).

(ii) 0.5 per cent Copper sulphate in one per cent potassium sodium tartrate in distilled water (Reagent-II).

(iii) Alkaline copper reagent was prepared by mixing 50 millilliters of Reagent I with two millliliters of Reagent-II.

(iv) 0.1 N Sodium hydroxide

(v) Diluted folin reagent (Folin reagent was diluted with equal volume of 0.1 N Sodium hydroxide)

(vi) Standard protein solution (Bovine serum albumin 100 g/ml).

One millilitre of cell free enzyme extract (CFEE) was added with one millilitre of 10 per cent Trichloro aceticacid (TCA) and centrifuged at 900 x g for 10 minutes at 4°C. Supernatant was discarded and the protein precipitate was washed twice with five per cent TCA and resuspended in 0.5 N Sodium hydroxide solution (one millilitre). To this 1.5 millilitre of alkaline copper reagent was added. It was shaken well and allowed to stand for 10 minutes, after which exactly 0.15 millilitre of diluted Folin reagent was added with continuous shaking. The tubes were allowed to stand for 30 minutes. Then the color development was read at wave length 720 nm.

The protein was measured by interpreting the value with a standard curve.

(Standard curve was prepared by dissolving bovine serum albumin in distilled water to get concentrations from 25 g/millilitre to 400 g/millilitre. Each concentration was used for color development and the optical density was measured at 720 nm. The values were plotted in a graph)

3.3.1 Antibacterial activity

In order to know the antibacterial activity of Lactic acid bacteria used in the present study, selected pathogenic organisms were used as test organisms. The procedure

described by Khedkar *et al*. (1990b) was followed. The following test organisms were used.

(i) *Enterobacter aerogenous*

(ii) *Micrococcus flavous*

(iii) *Escherichia coli*

(iv) *Staphylococcus aureus* and

(v) *Bacillus cereus*

The above mentioned test organisms were obtained from National Chemical Laboratories (NCL) Pune, in the form of slant cultures. The cultures were maintained by propagation in nutrient agar once in fifteen days. The composition of Nutrient agar is given below.

Beef extract	-	3 g
Peptone	-	5 g
Agar agar	-	15 G
Distilled water	-	1000 ml

3.3.2 Preparation of inoculum

The individual cultures were grown in nutrient broth (Beef extract-3 g, Peptone-5 g, Distilled water to make up 1000 millilitres) for overnight. Cells were harvested by centrifuging at 3000 RPM for 30 minutes in sterile tubes. Supernatant was discarded and the cell pellets were

resuspended in sterile normal saline. This suspension was used to adjust the optical density of sterile normal saline to 0.6 at 550 nm. This will give approximately 10^7 cells/ millilitre. Nutrient agar was melted and tempered to 45 + 2°C. One millilitre of readjusted cell suspension was poured in the petridishes and 15 millilitres of the media was mixed and allowed to solidify. Five wells each of seven millimeter diameter were made at equal distance with the use of sterile cylindrical hollow stainless steel gel cutter (7 mm diameter).

3.3.3 Preparation of cell free culture filtrate (CFC)

Twenty millilitres of each sample DC, DT, YC and YT were centrifuged at 3000 RPM for 15 minutes. The supernatant was aspirated and sterilized by passing through a Seitz filter. The CFC thus prepared were transferred in 0.1 ml aliquots in appropriate wells made on the agar surface (as described above, 3.3.2). The plates were pre-incubated at 4°C for 60 minutes to facilitate the diffusion of CFC into the media. Then the plates were incubated at 37°C for 24 hours without inversion. The diameter of inhibition zone was measured using a vernier caliper and the results were recorded.

3.4.1 Bile tolerance

In order to know the ability of the Lactic acid bacteria used in this study, to grow in the presence of bile, a test was done according to the method described by Gilliland et al. (1984).

3.4.2 Preparation of inoculum

The cultures of all the Lactic acid bacteria used in present study were propagated in ten millilitres of MRS broth for 24 hours at 37°C in a Carbon-di-Oxide atmosphere.

Composition of MRS broth

Peptone	- 10 g
Beef extract	- 10 g
Yeast extract	- 5 g
Glucose	- 20 g
Tween-80	- 1 ml
Di-potassium Hydrogen phosphate	- 2 g
Sodium acetate-$3H_2O$	- 5 g
Tri ammonium citrate	- 2 g
Magnesium sulphate-$7H_2O$	- 0.2 g
Mangnus sulphate $4H_2O$	- 0.05 g
Distilled water to make up	1000 ml

The propagated cultures were centrifuged at 3000 x g at 4°C for 10 minutes. The supernatant was discarded and cell pellets resuspended in two millilitre of fresh MRS broth. This suspension was used to adjust 10 millilitre of MRS broth to an Optical density of 0.62 - 0.64 at 650 nm.

3.4.3 Preparation of test broth

Lactobacilli MRS broth was prepared with and without 0.3 per cent oxgall. It was dispensed in 10 millilitre volumes and sterilized by autoclaving at 121°C for 15 minutes. For the culture to be tested, one tube of each media was inoculated with 0.1 millilitre of readjusted inoculum.

The inoculated media were incubated at 37°C in a waterbath. Growth was monitored by measuring the increase in optical density at 600 nm with a spectronic-20 Colorimeter. Growth to reach an optical density of 0.3 was determined.

3.5.1 Hypocholesteremic effect

The hypocholesteremic effect of the samples DC, DT, YC and YT were studied by a biological experiment using adult albino rats.

Forty two adult albino rats of uniform weight and age were selected from Small Animals Breeding Station (SABS) of the University. They were divided into seven groups of six

rats in each group. To eliminate sex variation each group was allotted with equal numbers of male and female rats. They were caged individually. The following feeding pattern was followed.

3.5.2 Feeding pattern

All the rats in each group were fed with a basal rat ration having the following composition.

Maize	-	24 per cent
Groundnut oil cake	-	12 per cent
Linseed oil cake	-	12 per cent
Wheat bran	-	50 per cent
Mineral mixture	-	one per cent
Multi vitamin mix	-	one per cent

In order to make the ration isoproteinic, basal feeds of Groups III to VI (DC, DT, YC and YT Groups) were fortified with casein. The following feeding pattern were followed.

Group I Basal rat ration

Group II Basal rat ration with 0.5 per cent cholesterol

Group III Basal rat ration fortified with casein. Fifty per cent of the total ration was replaced with Control Dahi (DC) on wet basis. Cholesterol was added at 0.5 per cent level.

Group IV Basal rat ration fortified with casein. Fifty per cent of the total ration was replaced with Treatment Dahi (DT) on wet basis. Cholesterol was added at 0.5 per cent level.

Group V Basal rat ration fortified with casein. The ration was replaced by 50 per cent, with Control Yogurt (YC) on wet basis. Cholesterol was added at 0.5 per cent level.

Group VI Basal rat ration fortified with casein. The ration was replaced by 50 per cent, with Treatment Yogurt (YT) on wet basis. Cholesterol was added at 0.5 per cent level.

Group I was to know the change in cholesterol level of rats during the experimentation period, by the feeding of basal rat ration. Group II was used to measure the hypercholesteremic effect by the addition of cholesterol in the diet.

Hypocholesteremic effect of DC was estimated from the Group III. The effect of feeding DT, YC and YT on the serum cholesterol levels of rats were measured from the Groups IV, V and VI respectively.

One pre experimental group was used to estimate the normal blood cholesterol levels of rats, just before starting the feeding trial.

Ration required for each rat was calculated as 10 per cent of the body weight and fed daily in the morning. Clean good quality drinking water was made available all the time with dripping bottles. The rats were weighed every week and the weight gain was periodically recorded. Feeding trial was continued for 60 days.

3.5.3 Blood collection

At the end of feeding trial rats under all the six groups were starved overnight, anaesthetised using chloroform, and blood from each group was collected separately, by retrobulbar puncture using heparinized capillary tubes. The blood collecting tubes were thoroughly cleaned and sterilized before use. Serum was collected from each sample and stored in freezer before using for following analysis.

(i) Total serum cholesterol

(ii) Serum triglycerides

(iii) High density lipoprotein cholesterol (HDL-Cholesterol)

(iv) Low density lipoprotein cholesterol (LDL-Cholesterol)

3.5.4 Estimation of total serum cholesterol

Serum cholesterol of all the experimental groups were estimated by the procedure described by Zak (1957). The detailed procedure is given below.

Reagents

(i) Stock ferric chloride solution

Ferric Chloride (840 mg) was dissolved in glacial acetic acid and made upto 100 millilitres with the same, and stored in refrigerator.

(ii) Ferric chloride precipitating reagent

The stock ferric chloride solution was diluted to one in ten with glacial acetic acid.

(iii) Ferric chloride blank

With a help of clean pipette 1.7 millilitres of the stock ferric chloride solution was diluted to 20 millilitres with glacial aceticacid.

(iv) Cholesterol stock standard

Pure dry cholesterol was accurately weighed and 100 mg was dissolved in 100 millilitres of glacial acetic acid and stored in a freezer.

(v) Working standard

Two ml of cholesterol stock standard was mixed with 1.7 ml of stock ferric chloride solution and diluted to 20 ml with glacial acetic acid.

(vi) Final standard

This was always prepared just before the estimation, by mixing two millilitres of the working standard and four millilitres of the ferric chloride blank.

Procedure

Using a clean pipette 0.1 ml of the serum was drawn and added to six millilitres of the ferric chloride precipitating reagent and thoroughly mixed. It was filtered through Whatmann no. 42 filter paper, and the filtrate was collected in a clean dry test tube. Three millilitres each of of the filtrate, final standard, and ferric chloride blank were taken in separate test tubes. Two millilitre of concentrated sulphuric acid was added and mixed by gentle shaking. After cooling the color was read in spectronic-20 colorimeter at 420 nm against blank.

Calculations

$$\text{Total cholesterol in 100 ml serum} = \frac{\text{Reading of test}}{\text{Reading of standard}} \times \frac{0.1}{0.05} \times 100$$

3.5.5 Triglycerides estimation

Serum triglycerides were estimated by making use of commercially available enzymatic kit (New India Chemical Enterprises).

Principle

Triglycerides $\xrightarrow{\text{Lipase}}$ Glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{lycerol kinase}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{Glycerol-3-Phosphate Oxidase}}$ Dihydroxy-acetone phosphate + H_2O_2

Procedure

Using a dry clean pipette 0.01 millilitre of serum was taken and to this one millilitre of reconstituted reagent was added, mixed and incubated for ten minutes at 37°C. The colour developed was read at 500 nm against blank. The colour development of the standard solution was also read against blank at the same wavelength.

Calculation

$$\text{Serum Triglycerides (mg/100 ml)} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 200$$

3.5.6 HDL-cholesterol

Serum HDL cholesterol was estimated by the use of commercially available, HDL-cholesterol precipitating reagent kit (Ortho diagnostic system).

Principle

Polyethylene glycol precipitates chylomicrons, low density lipoproteins (LDL) and very low density lipoprotein fractions. High density lipoproteins (HDL) fraction remains unaffected in the supernatent. The precipitate is removed by centrifugation. The HDL-cholesterol in supernatent is then determined by estimation of cholesterol content enzymatically.

Procedure

Using clean dry pipette 0.5 ml of the Lipogent was mixed with 0.5 ml of serum. It was kept at room temperature for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was aspirated and 100 microlitres of that was mixed with one millilitre of Cholzyme-M working

reagent. The contents were mixed thoroughly, incubated at 37°C for 15 minutes, and four millilitres of distilled water was added. The colour development was read at 515 nm against a blank. A standard was also prepared using the working standard and its colour development was also read in the same wave length.

Calculation

$$\text{HDL-Cholesterol (mg/dl)} = \frac{\text{Optical density of test serum}}{\text{Optical density of standard}} \times 50$$

3.5.7 LDL-Cholesterol

The LDL-Cholesterol was calculated by the following formula

$$\text{LDL-Cholesterol mg/dl} = \text{Total cholesterol} - (\text{HDL Cholesterol} + \frac{\text{Triglycerides}}{5})$$

(Friedewald W.J., 1972).

3.5.8 Cardiac risk factor

Cardiac risk factor was calculated by the following formula (Lin *et al.*, 1989a).

$$\text{Cardiac risk factor} = \frac{\text{Total cholesterol}}{\text{HDL-Cholesterol}}$$

3.5.9 Weight gain of rats

The weight gain of the rats of all the experimental groups were calculated individually.

$$\text{Weight gain} = \frac{\text{Increase in weight in grams}}{\text{Experimentation period in days}}$$

It will give weight gain per day in grams.

Results

RESULTS

The beneficial effects of L. acidophilus as a dietary adjunct in dahi and yogurt was conducted in a well planned experiment. The results obtained were given below.

Control Dahi (DC) was prepared with Lac. lactis and Lac. lactis ssp diacetylactis and treatment Dahi (DT) with Lac. lactis, Lac. lactis ssp diacetylactis and L. acidophilus. Control Yogurt (YC) was fermented with L. delbrueckii ssp bulgaricus and S. salivarius ssp thermophilus and treatment Yogurt (YT) with L. delbrueckii ssp bulgaricus, S. salivarius ssp thermophilus and L. acidophilus, adopting standard procedures and analysed for various parameters mentioned below.

(i) β-galactosidase specific activity

(ii) Antibacterial activity

(iii) Bile tolerance

(iv) Hypocholesteremic effect and

(v) Growth rate in experimental rats

4.1 β-Galactosidase specific activity

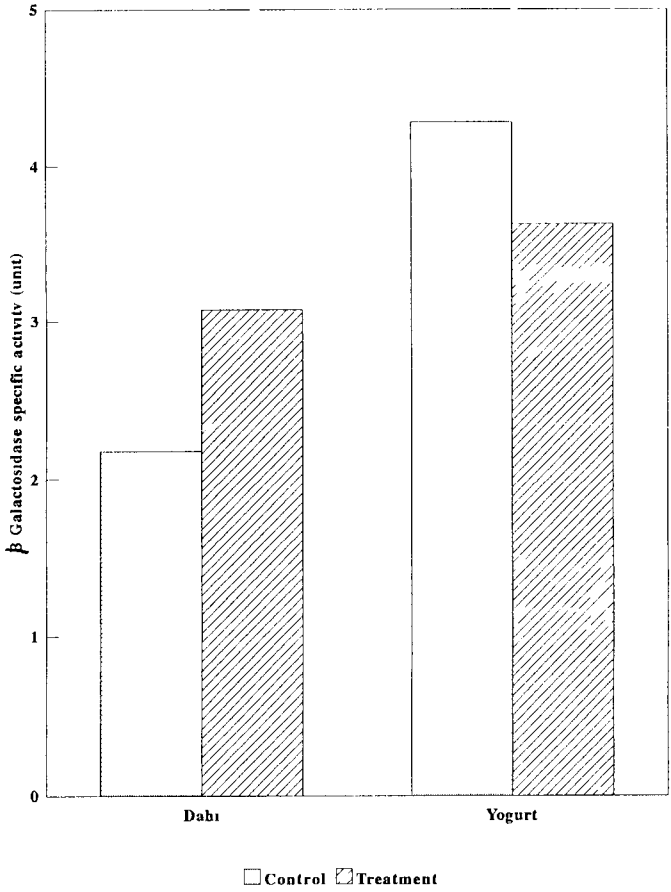
The β-Galactosidase specific activity of control dahi (DC), treatment dahi (DT), control yogurt (YC) and treatment

yogurt (YT) were measured in terms of numbers of units. One unit is defined as the number of μmoles of Ortho nitrophenol (ONP) released/millilitre/minute. The β-Galactosidase specific activities of different treatments are given in the Table 1a. The mean specific activity of DC was 2.179 ± 0.04 with a minimum of 2.083 and maximum of 2.419. For DT a mean value of 3.075 ± 0.034 was obtained. The minimum β-galactosidase

Table 1a. β-Galactosidase specific activities of different treatments (Units)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	2.282	3.064	4.211	3.623
2	2.083	2.956	4.372	3.687
3	2.419	3.137	4.396	3.663
4	2.128	3.029	4.306	3.663
5	2.149	3.003	4.300	3.663
6	2.149	3.267	4.110	3.462
7	2.110	3.089	4.223	3.745
8	2.115	3.053	4.333	3.623
Mean	2.179	3.075	4.281	3.629
SE ±	0.040	0.034	0.033	0.030

Fig.1 β GALACTOSIDASE SPECIFIC ACTIVITY OF DIFFERENT TREATMENTS (UNITS)



specific activity in this treatment was 2.956 and the maximum was 3.267. For the YC and YT the mean specific activities were 4.281 $\pm$ 0.033 and 3.629 $\pm$ 0.030 respectively. The minimum values obtained for YC was 4.110 and for YT 3.462. The maximum values were 4.396 and 3.745 respectively for YC and YT. The mean β-galactosidase specific activity of different treatments is depicted in Fig.1.

The YC showed the highest β-galactosidase specific activity followed by YT, DT and DC. On statistical analysis these differences in the values were found to be highly significant.

Table 1b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	0.066	0.009	0.9828 NS
2.	Treatment	3	19.019	6.340	659.6786 **
3.	Error	21	0.202	0.010	
	Total	31	19.287		

NS - Not significant ** - Highly significant

On pairwise comparison DT was having significantly higher activity than DC. Likewise both YC and YT showed significantly higher activity than DC and DT. However, YC showed significantly higher activity when compared to YT.

4.2 Antibacterial activity

The antibacterial activities of Control Dahi (DC), Treatment Dahi (DT), Control Yogurt (YC) and Treatment Yogurt (YT) were measured against the following test organisms in terms of millimeters of inhibition zones.

(i) Enterobactor aerogenous

(ii) Micrococcus flavous

(iii) Escherichia coli

(iv) Staphylococcus aureus

(v) Bacillus cereus

4.2.1 Antibacterial activity against E. aerogenous

The antibacterial activities of different treatments against E. aerogenous are given in the Table 2a.

The mean inhibition zone (in millimeter) of DC was 10.025 $\pm$ 0.139 with a minimum of 9.6 and maximum of 10.7. For DT a mean inhibition zone of 12.762 $\pm$ 0.386 was obtained. The minimum and maximum inhibition zones were 11.3 and 14.0 respectively.

For YC and YT the mean inhibition zones were 9.262 ± 0.301 and 11.925 ± 0.322 respectively. The minimum value obtained for the YC was 8.2 and for YT 11.0. The maximum values obtained were 10.4 and 13.4 respectively for YC and YT.

Table 2a. Antibacterial activity of Dahi and Yogurt against E. aerogenous (Zone of inhibition in mm)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	10.5	13.5	9.5	11.5
2	10.5	13.0	10.4	12.6
3	9.7	11.4	8.2	11.0
4	10.0	13.7	9.9	11.9
5	10.7	13.4	9.5	12.8
6	10.0	11.3	8.5	11.0
7	9.7	14.0	9.9	13.4
8	9.6	11.8	8.2	11.2
Mean	10.025	12.762	9.262	11.925
SE ±	0.139	0.386	0.301	0.322

The DT showed the highest inhibition zone against E. aerogenous followed by YT, DC and YC. On statistical analysis these differences in the values were found to be highly significant.

Fig.2 ANTIBACTERIAL ACTIVITY OF DIFFERENT TREATMENTS AGAINST E.aerogenous

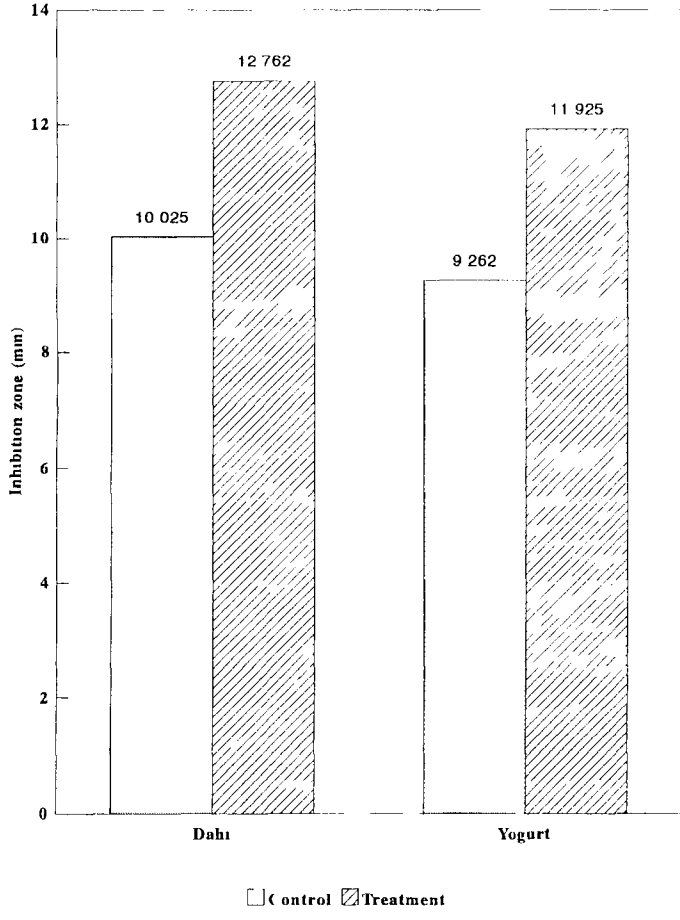


Table 2b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	14.419	2.06	0.3706 NS
2.	Treatment	3	63.451	21.15	75.682 **
3.	Error	21	5.869	0.279	
	Total	31	83.739		

NS - Not significant ** - Highly significant

On pairwise comparison DT was showing significantly higher inhibition than DC. The YT was having inhibition zones, significantly higher than DC, whereas YC exerted significantly lower activity than DC. DT produced significantly high zone of inhibition than YC and YT. The inhibition by YT was significantly higher than YC. The antibacterial activities of different treatments against E. aerogenous is depicted in Fig.2.

4.2.2 Antibacterial activity against M. flavous

When the samples were tested against M. flavous for their antibacterial activity the DC showed a mean inhibition zone 8.875 $\pm$ 0.084 with the minimum zone of 8.5 and a maximum of 9.2 (Table 3a).

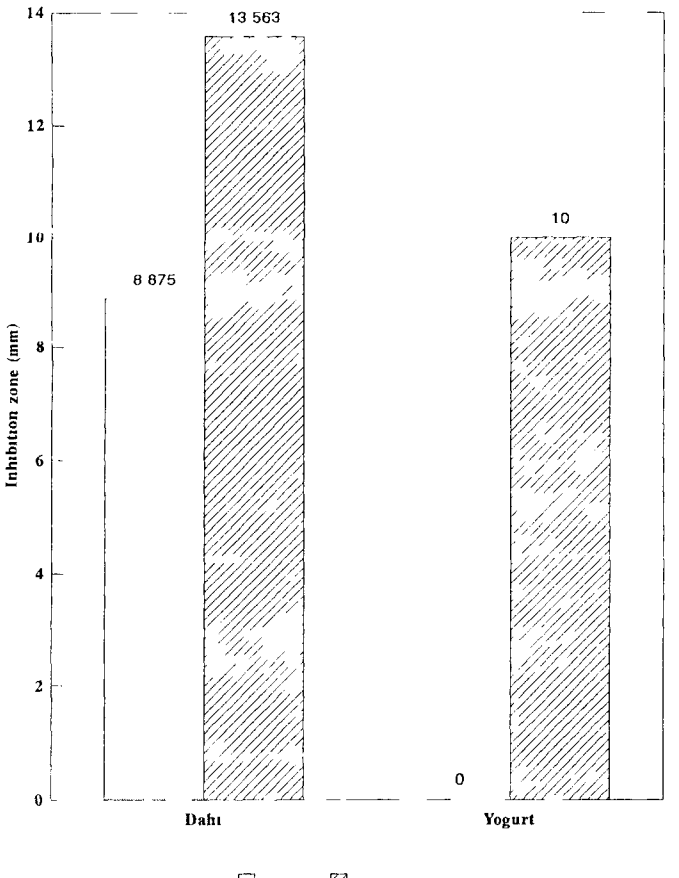
In the case of DT the mean inhibition zone was 13.563 ± 0.367. The minimum and maximum zones being 12.1 and 14.5 respectively.

For YT, the mean zone of inhibition was 10 ± 0.230 with a minimum zone of 9.2 and a maximum zone of 10.9. YC showed no inhibition at all.

Table 3a. Antibacterial activity of Dahi and Yogurt against M. flavous (Zone of inhibition in mm)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	8.5	12.5	0.0	9.5
2	9.1	14.1	0.0	10.1
3	8.8	14.5	0.0	9.2
4	8.6	12.1	0.0	10.0
5	8.9	14.5	0.0	10.5
6	9.2	14.0	0.0	9.2
7	8.9	12.4	0.0	10.6
8	9.0	14.1	0.0	10.9
Mean	8.875	13.563	0.0	10.0
SE ±	0.084	0.367	0.0	0.230

Fig.3 ANTIBACTERIAL ACTIVITY OF DIFFERENT TREATMENTS AGAINST M.flavous



The antibacterial activity of different samples are given in the Table 3a and depicted in Fig.3.

Of all the four samples DT showed the highest inhibition towards M. flavous followed by YT and DC. These difference in the values were found to be highly significant.

Table 3b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	4.333	0.619	1.3168 NS
2.	Treatment	2	95.813	47.906	101.9153 **
3.	Error	14	6.581	0.470	
	Total	23	106.726		

NS - Not significant ** - Highly significant

On pairwise comparison DT was having significantly higher inhibition than DC. The inhibition by YT was also found to be significantly higher than DC. Among DT and YT, the DT showed significantly higher activity than YT.

4.2.3 Antibacterial activity against E. coli

The samples DC, DT, YC and YT were tested against

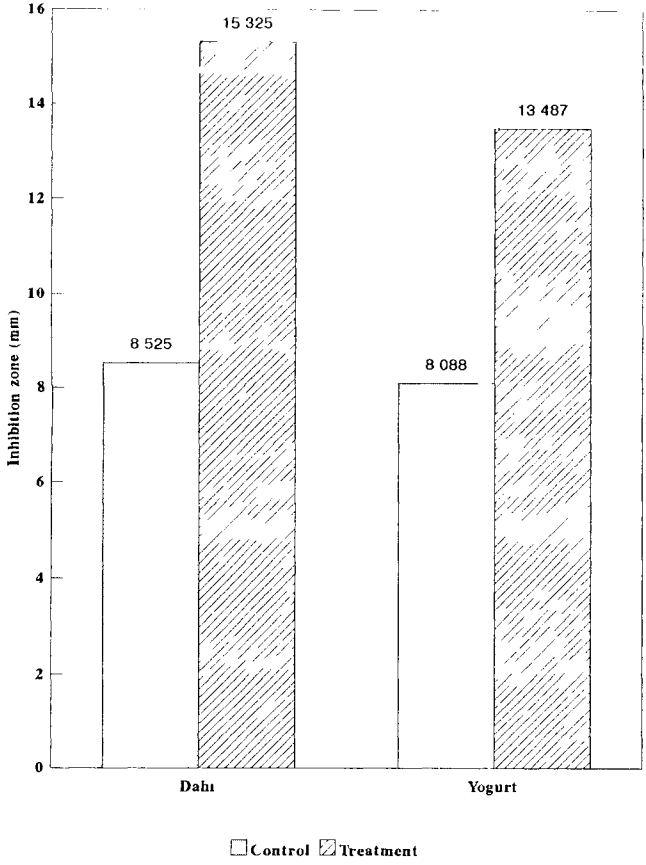
E. coli for their antibacterial activity. The results are given in the Table 4a.

The mean inhibition zone by DC was 8.525 ± 0.139. The minimum zone being 8.0 and maximum 9. For DT the mean zone of inhibition was 15.325 ± 0.246, with minimum and maximum zones of 13.9 and 16.1 respectively. YC and YT showed mean inhibition zones of 8.088 ± 0.055 and 13.487 ± 0.252

Table 4a. Antibacterial activity of Dahi and Yogurt against E. coli (Zone of inhibition in mm)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	8.0	15.0	8.0	13.0
2	8.2	13.9	8.1	13.9
3	9.0	15.0	8.1	13.9
4	8.3	15.9	8.2	13.6
5	8.9	15.7	7.9	14.0
6	8.7	16.1	8.0	12.8
7	8.2	15.6	8.4	13.9
8	8.9	15.4	8.0	14.4
Mean	8.525	15.325	8.088	13.487
SE ±	0.139	0.246	0.055	0.252

Fig.4 ANTIBACTERIAL ACTIVITY OF DIFFERENT TREATMENTS AGAINST E.coli



respectively. YC showed a minimum zone of 7.9 and a maximum zone of 8.4 while the minimum and maximum zones of inhibition by YT were 12.8 and 14.4 respectively.

Among the treatments DT exerted highest inhibition against *E. coli* followed by YT, DC and YC. On statistical analysis, these differences were found to be highly significant.

Table 4b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	2.109	0.301	1.0373 NS
2.	Treatment	3	311.951	103.984	358.0502 **
3.	Error	21	6.099	0.29	
	Total	31	320.159		

NS - Not significant ** - Highly significant

On pairwise comparison it was found that both DT and YT showed significantly higher inhibition than DC and YC. DT produced significantly higher inhibition than YT. However, no differences were noticed between DC and YC regarding their antibacterial activity against *E. coli*. The antibacterial activity of different treatments against *E. coli* is shown in Fig.4.

4.2.4 Antibacterial activity against S. aureus

The samples DC, DT, YC and YT were tested against S. aureus for their antibacterial activity and the results are given in Table 5a and plotted in Fig.5.

DC showed a mean antibacterial inhibition zone of 9.388 ± 0.180. The minimum and maximum zones were 8.6 and 10.1 respectively. The mean zone of inhibition by DT was 12.075 ± 0.125. The minimum being 11.5 and maximum 12.4. For YT, the mean zone of inhibition was 10.125 ± 0.337. The minimum and maximum values were 9.5 and 11.3 respectively. However YC did not showed any inhibition zone against S. aureus.

Among the samples the highest inhibition against S. aureus was exerted by DT followed by YT and DC. The differences between the treatments were found to be highly significant.

On pair wise comparison, it was found that DT showed significantly high inhibition than YT and DC. Eventhough the inhibition zone showed by YT against S. aureus was apparently higher than that of DC, the difference was not statistically different.

Fig.5 ANTIBACTERIAL ACTIVITY OF DIFFERENT TREATMENTS AGAINST S.aureus

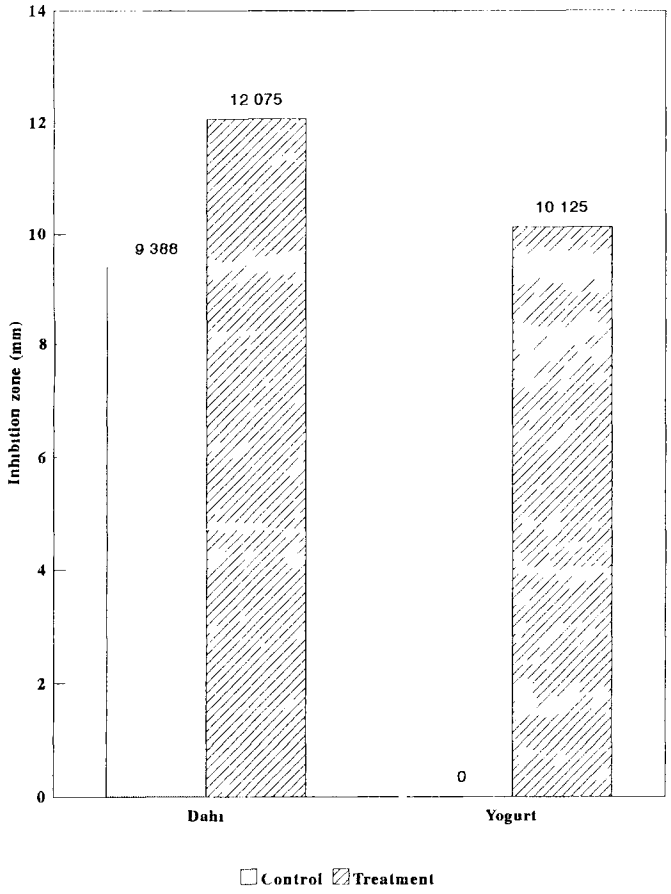


Table 5a. Antibacterial activity of Dahi and Yogurt against S. aureus (Zone of inhibition in mm)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	9.5	11.5	0.0	9.5
2	8.6	12.1	0.0	10.1
3	9.2	12.4	0.0	9.9
4	10.1	11.6	0.0	10.0
5	8.8	12.4	0.0	11.3
6	9.5	12.1	0.0	10.1
7	9.5	12.4	0.0	10.1
8	9.9	12.1	0.0	10.0
Mean	9.388	12.075	0.0	10.125
SE ±	0.180	0.125	0.0	0.337

Table 5b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	1.338	0.191	0.347 NS
2.	Treatment	2	29.433	14.716	26.7077**
3.	Error	14	7.714	0.551	
	Total	23	38.485		

NS - Not significant ** - Highly significant

4.2.5 Antibacterial activity against B. cereus

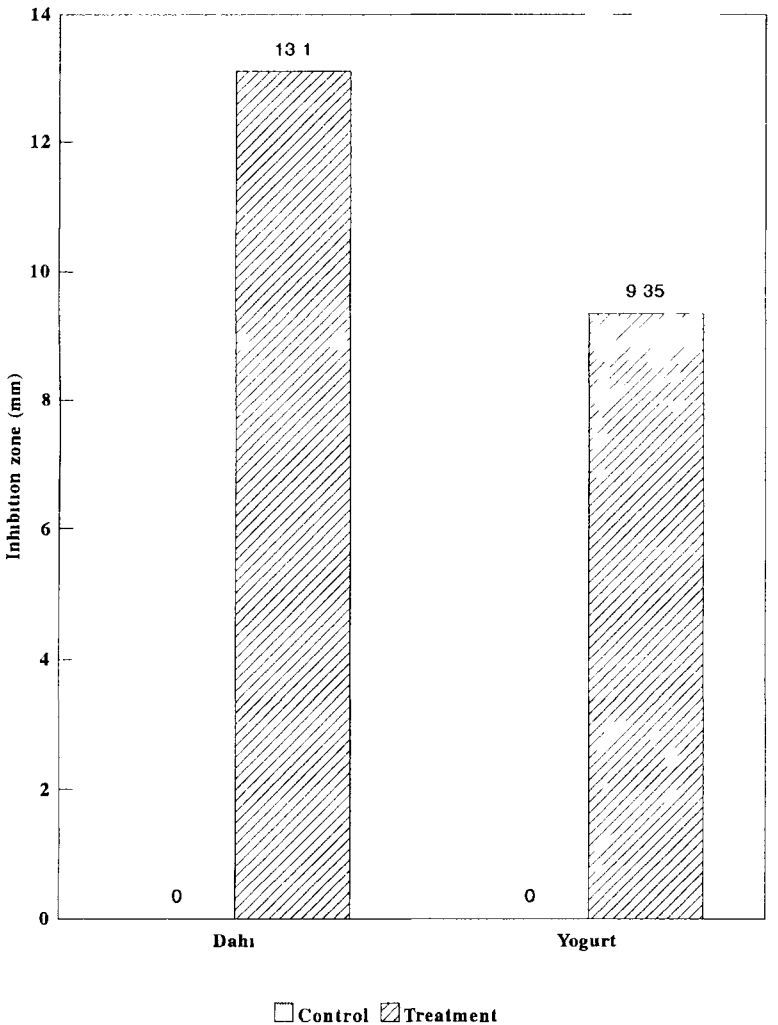
The four samples of DC, DT, YC and YT were tested for their inhibitory activity against B. cereus. The results are given in the Table 6a.

The DT showed a mean inhibition zone of 13.1 ± 0.177. The minimum and maximum inhibition zones were 12.3 and 14.1

Table 6a. Antibacterial activity of Dahi and Yogurt against B. cereus (Zone of inhibition in mm)

Treatment	Dahi		Yogurt	
Replication	Control (DC)	Treatment (DT)	Control (YC)	Treatment (YT)
1	0.0	13.0	0.0	8.5
2	0.0	12.3	0.0	9.4
3	0.0	13.0	0.0	10.5
4	0.0	14.1	0.0	9.4
5	0.0	13.2	0.0	9.9
6	0.0	12.9	0.0	8.6
7	0.0	13.0	0.0	9.0
8	0.0	13.3	0.0	9.5
Mean	0.0	13.1	0.0	9.35
SE ±	0.0	0.177	0.0	0.234

Fig.6 ANTIBACTERIAL ACTIVITY OF DIFFERENT TREATMENTS AGAINST B.cereus



respectively. For YT the mean of inhibition zone was 9.35 ± 0.234. The minimum zone of inhibition being 8.5 and maximum being 10.5. Both DC as well as YC did not showed any zone of inhibition against B. cereus.

DT gave higher zone of inhibition than the YT. The difference between the treatments were found to be highly significant. The antibacterial activity of different treatments against B. cereus is shown in Fig.6.

Table 6b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	7	2.660	0.380	1.2315 NS
2.	Treatment	1	56.250	56.250	182.2917 **
3.	Error	7	2.160	0.309	
	Total	15	61.070		

NS - Not significant ** - Highly significant

The results of the antibacterial activity against test organisms namely E. aerogenous, M. flavous, E. coli, S. aureus and B. cereus indicated that DT showed the highest antibacterial activity against all the test organisms. YT showed the second highest inhibition against E. aerogenous,

M. flavous, E. coli and B. cereus. Eventhough the inhibition zone of YT against S. aureus was apparently higher than that shown by DC, the difference was not statistically significant.

DC showed the third highest inhibition against E. aerogenous and M. flavous. However, the antibacterial activity of DC and YC were not significantly different against E. coli. DC failed to show any inhibition against B. cereus.

YC showed the weakest inhibition against E. aerogenous, among the other four treatments. It did not showed any inhibition against M. flavous, S. aureus and B. cereus.

4.3 Bile tolerance

The bile tolerance of all the lactic acid bacteria used in the study namely L. acidophilus, S. salivarius ssp thermophilus, L. dulbrueckii ssp bulgaricus, Lac. lactis and Lac. lactis ssp diacetylactis were studied. The growth of these organisms in MRS broth containing 0.3 per cent of oxgall, was compared with the growth in the same media, with out oxgall. The results are given in the Table 7.

The mean time taken for L. acidophilus to reach an optical density of 0.3 nm in ordinary MRS broth was 2.53 hours. The minimum and maximum being 2.30 and 3.15 hours

Table 7. Growth of lactic acid bacteria in MRS broth with and without 0.3 per cent oxgall

Replication	Time taken to reach O.D - 0.3 at 600 nm (hours : Minutes)									
	L. acidophilus		*S. Salivarius* ssp *thermophilus*		*L. delbrueckii* ssp *bulgaricus*		*Lac. lactis*		*Lac. lactis* ssp *diacetylactis*	
	MRS	MRS + oxgall	MRS	MRS + oxgall	MRS	MRS + oxgall	MRS	MRS + oxgall	MRS	MRS + oxgall
1	2.30	3.55	3.06	*	2.54	+	3.30	*	3.41	*
2	2.47	3.55	3.11	*	2.48	+	3.25	*	3.33	*
3	3.00	4.00	3.02	*	2.52	+	3.47	*	3.20	*
4	2.47	4.08	3.16	*	2.56	+	3.19	*	3.50	*
5	2.55	3.55	3.15	*	3.00	+	3.24	*	3.27	*
6	2.55	3.50	3.08	*	3.07	+	3.28	*	3.29	*
7	3.15	4.00	3.22	*	2.55	+	3.25	*	3.40	*
8	2.54	3.47	3.10	*	2.50	+	3.31	*	3.35	*
Mean	2.53	3.56	3.11	-	2.55	-	3.29	-	3.34	-

* - Organisms did not show any growth at the end of six hours of incubation

\+ - *L. debrueckii* ssp *bulgaricus* showed only a mild growth and the increase in the optical density was only 0.11 even after six hours of incubation

respectively. The same organism took a mean time of 3.56 hours to each the same optical density when grown in MRS media containing 0.3 per cent oxgall. The minimum and maximum time taken were 3.47 and 4.08 hours respectively.

S. salivarius ssp thermophilus took a mean time of 3.11 hours to reach an optical density of 0.3 nm in ordinary MRS broth. The minimum time taken was 3.02 hours and the maximum was 3.22 hours. However, the organism did not showed any growth when grown in the MRS broth containing 0.3 per cent oxgall, even after six hours of incubation.

In the case of L. delbrueckii ssp bulgaricus, the mean time taken to reach an optical density of 0.3 was 2.55 hours when grown in ordinary MRS broth. The minimum and maximum time taken were 2.48 and 3.07 hours respectively. Eventhough, the organism showed a mild increase in the optical density when grown in MRS medium containing 0.3 per cent oxgall, the increase in optical density was only 0.11 even after six hours of incubation.

The mean growth time for Lac. lactis to reach an optical density of 0.3, when grown in ordinary MRS broth was 3.29 hours. The minimum time taken was 3.19 and the maximum being 3.47 hours. Nevertheless, the organism failed to show any increase in the optical density, when grown in MRS broth

containing 0.3 per cent oxgall at the end of six hours incubation period, Lac. lactis ssp diacetylactis took a mean time of 3.34 hours to reach the optical density of 0.3, in ordinary MRS broth. The minimum and maximum time taken were 3.20 and 3.50 hours respectively. In the MRS broth containing 0.3 per cent oxgall, however, this organism did not showed any increase in the optical density, even after incubation for six hours.

Among the lactic acid bacteria used in this study, only L. acidophilus attained a satisfactory growth in the presence of 0.3 per cent oxgall, to reach an optical density of 0.3 with in the period of six hours.

The results indicated that L. acidophilus was capable of growing in the presence of 0.3 per cent of oxgall and reach an optical density of 0.3 within six hours. Eventhough L. delbrueckii ssp bulgaricus showed a slight increase in the optical density to 0.11, in the presence of 0.3 per cent oxgall, it failed to reach the optical density of 0.3 even after incubating for six hours. The other cultures S. salivarius ssp thermophilus, Lac. lactis and Lac. lactis ssp diacetylactis were not at all showing any increase in the optical density when grown in the presence of 0.3 per cent oxgall, at the end of six hours of incubation period.

4.4 Hypocholesteremic effect

The Hypocholesteremic effect of samples of control Dahi (DC), Treatment Dahi (DT), Control Yogurt (YC) and Treatment Yogurt (YT) were determined through a feeding trial. The different samples were incorporated in the rat ration and fed to the rats. After a feeding period of 60 days, the blood collected from the individual animals was analysed for the following parameters.

(i) Serum Total Cholesterol

(ii) Serum Triglycerides

(iii) Serum HDL-Cholesterol

From the above values the following parameters were calculated.

(i) Serum LDL-Cholesterol

(ii) Cardiac risk factor

In addition, the growth rate of the animals per day in grams was calculated using the feed intake and body weight records.

4.4.1 Serum total cholesterol

The serum total cholesterol levels of rats belonging to the groups Normal Feed (NF), Normal Feed with Cholesterol

(NFC), Control Dahı wıth Cholesterol (DC), Treatment Dahı wıth Cholesterol (DT), Control Yogurt wıth Cholesterol (YC) and Treatment Yogurt wıth Cholesterol (YT) are gıven ın the Table 8a.

The mean Total Cholesterol level of the rats fed wıth Normal Feed (NF) was 62.567 ± 2.263. The range of serum Total Cholesterol ın thıs group ıs from 53.1 to 69.3 mg/100 ml. When Cholesterol was added ın normal ratıon ın the NFC group. The mean total cholesterol has been elevated to 91.817 ± 4.046 wıth a range of 81.6 to 106.1 mg/100 ml. Incorporatıon of Control Dahı wıth Cholesterol (DC), ın the rat feed resulted ın a mean serum total cholesterol level of 94.533 ± 3.395 mg/100 ml, wıth a mınımum value of 85.7 and maxımum of 102 mg/100 ml.

A drastıc reductıon occurred ın the serum total cholesterol levels of rats fed wıth Treatment Dahı and Cholesterol. The mean value obtaıned under thıs treatment group was 75.533 ± 1.744 mg/100 ml, wıth a range from 69.4 to 81.6 mg/100 ml.

The group fed wıth control yogurt wıth cholesterol showed a mean serum total cholesterol level of 84.350 ± 2.277 mg/100 ml. The mınımum value was 77.6 and the maxımum beıng 93.9 mg/100 ml.

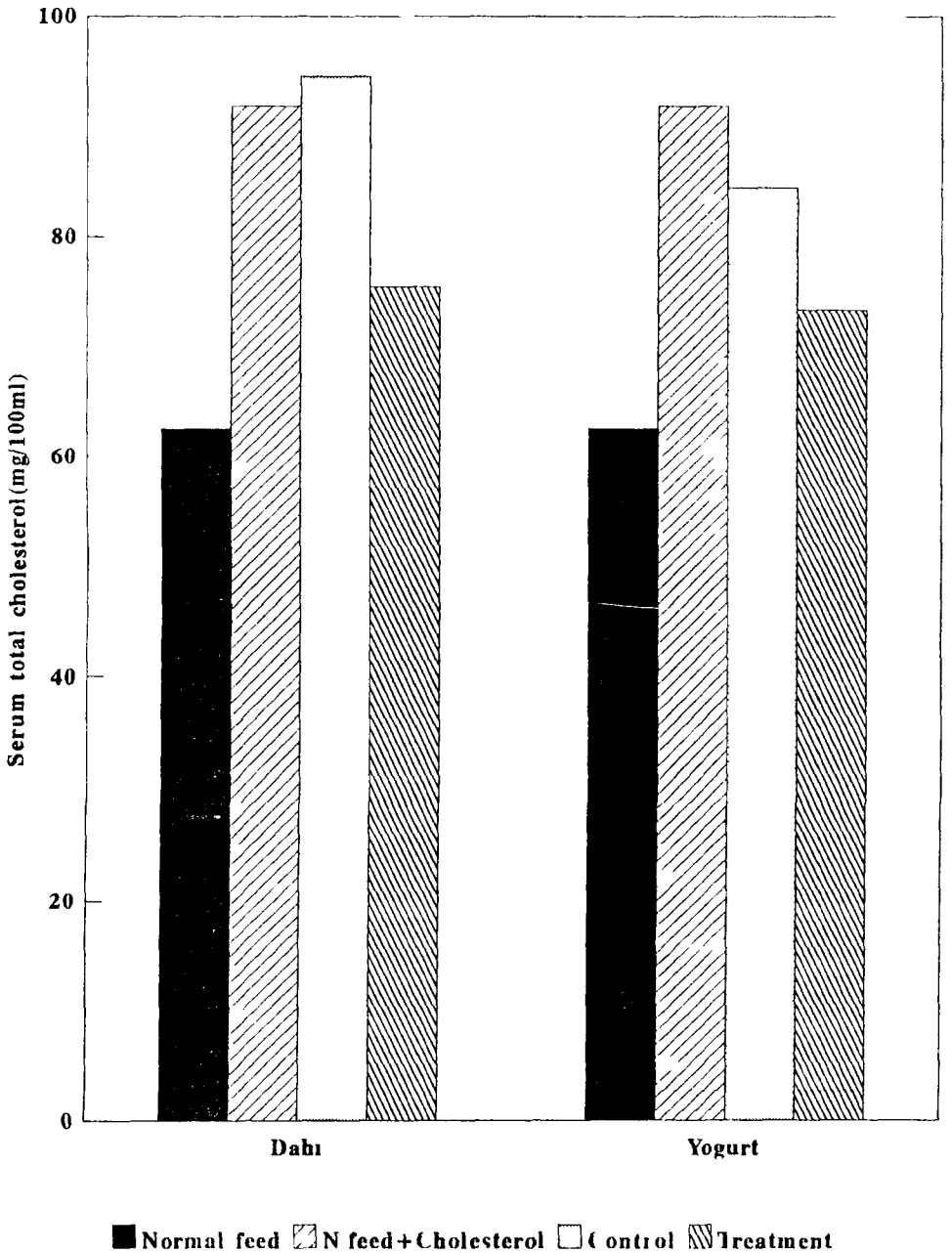
The treatment yogurt with cholesterol fed rats also showed a similar reduction in serum total cholesterol levels, like the treatment Dahi group. The treatment yogurt with cholesterol fed rats had a mean serum total cholesterol level of 73.50 ± 1.497 mg/100 ml, with a range between 69.4 and 77.6 mg/100 ml.

Table 8a. Serum Total Cholesterol levels of rats fed on different treatments (mg/100 ml).

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed plus Cholesterol			
			Dahi control (DC)	Dahi treatment (DT)	Yogurt control (YC)	Yogurt treatment (YT)
1	65.3	85.7	102.0	81.6	85.7	69.4
2	69.3	81.6	85.7	77.6	93.9	77.6
3	53.1	89.8	102.0	73.5	81.6	77.6
4	61.2	106.1	102.0	77.6	77.6	73.5
5	65.3	102.0	85.7	69.4	81.6	69.4
6	61.2	85.7	89.8	73.5	85.7	73.5
Mean	62.567	91.817	94.533	75.533	84.35	73.50
SE ±	2.263	4.046	3.395	1.744	2.277	1.497

Among the samples tested, YT showed the highest reduction in serum total cholesterol levels of rats followed

Fig.7 SERUM TOTAL CHOLESTEROL OF RATS UNDER DIFFERENT TREATMENTS (mg/100ml)



by DT and YC in that order. The difference between the treatments were found to be highly significant. However there was no significant difference in the serum total cholesterol levels of DC groups when compared with the NFC group. The serum total cholesterol level of rats under different treatments is shown in Fig.7.

Table 8b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	5	93.211	18.642	0.3925 NS
2.	Treatment	6	6036.499	1006.083	21.1844 **
3.	Error	30	1424.752	47.492	
	Total	41	7554.463		

NS - Not significant ** - Highly significant

A pairwise comparison was made between the individual groups. A drastic increase in the serum total cholesterol level was observed when cholesterol was incorporated in the normal ration of rats.

The statistical analysis showed a highly significant reduction in serum total cholesterol levels in the groups DT, YC and YT, in spite of adding cholesterol in their diet.

However, the values obtained for DC group were in par with the groups fed with Normal ration and added Cholesterol (NFC). Treatment Dahi (DT) and Yogurt Treatment (YT) showed a profound effect in reducing the serum total cholesterol levels. Though apparently YT had got a higher reduction, their effect was almost same, since their values were statistically not significant.

Though YC was showing hypocholesteremic effect, its effect was statistically different from both DT and YT.

The results of comparison of total serum cholesterol reduction, showed the marked ability of DT and YT in lowering the total cholesterol levels in serum. YC also produced a significant reduction in serum total cholesterol, eventhough the reduction was not as much as that of both treatment groups.

4.4.2 Serum Triglycerides

The serum triglyceride levels of rats fed with different treatment rations are given in the Table 9a.

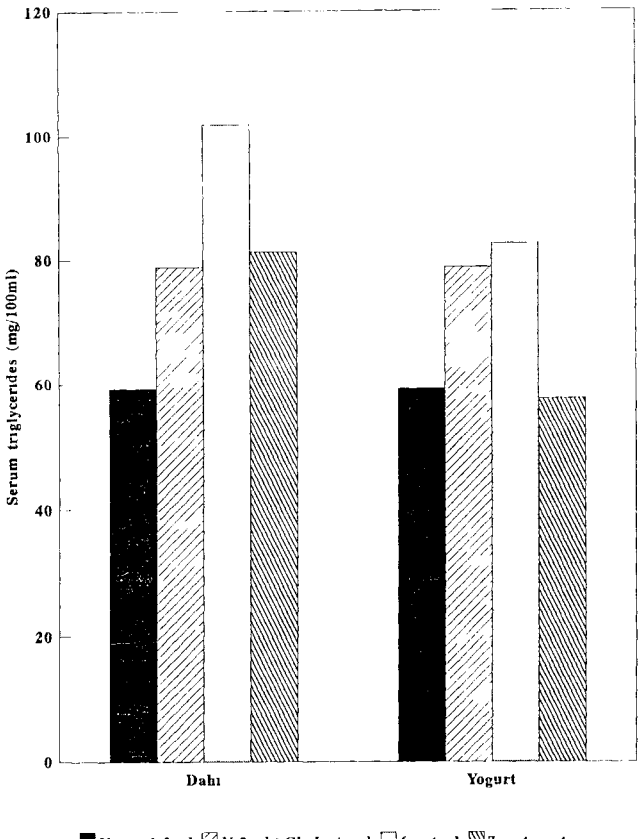
The mean triglyceride level of rats fed on normal feed (NF) was 59.2 $\pm$ 5.313 mg/100 ml, with a range between 44.4 and 74.0. In the rats fed with normal feed added with cholesterol, the triglyceride levels shot up to a mean level

of 78.933 ± 2.274. The minimum and maximum values in this group was 74.0 and 88.8 mg/100 ml respectively. Incorporation of control Dahi and cholesterol (DC) in the rat feed resulted in a mean serum triglyceride level of 101.75 ± 3.542 mg/100 ml, with a minimum value of 88.8 and a maximum value of 111.0 mg/100 ml. In the rats fed on treatment dahi with cholesterol along with the feed, the mean serum triglyceride level was 81.4 ± 2.136 mg/100 ml, with a range of 74.0 to 88.8. For the group which fed on control yogurt and cholesterol along with

Table 9a. Serum triglyceride levels of rats under different treatments (mg/100 ml)

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed plus Cholesterol			
			Dahi control (DC)	Dahi treatment (DT)	Yogurt control (YC)	Yogurt treatment (YT)
1	70.3	81.4	88.8	81.4	74.0	62.9
2	55.3	77.7	103.6	85.1	70.3	66.6
3	74.0	74.0	111.0	77.0	81.4	55.5
4	66.6	88.8	99.9	81.4	77.7	62.9
5	44.4	77.7	111.0	74.0	103.6	48.1
6	44.4	74.0	96.2	88.8	88.8	51.9
Mean	59.2	78.933	101.75	81.4	82.633	59.967
SE ±	5.313	2.274	3.542	2.136	4.933	2.97

Fig.8 SERUM TRIGLYCERIDES OF RATS UNDER DIFFERENT TREATMENTS (mg/100ml)



normal feed, the mean serum triglyceride was estimated as 82.633 ± 4.933 mg/100 ml. The minimum and the maximum values were 70.3 and 103.6 respectively.

A sharp decline in the serum triglyceride levels of rats fed with treatment yogurt (YC) with cholesterol in addition to normal feed was noticed. The mean triglyceride level in the serum of this group was 59.967 ± 2.97 mg/100 ml with the values ranging from 48.1 to 66.6.

Among the treatment groups YT fed group showed a drastic reduction in serum triglyceride level. The reduction was highly significant. However there was no significant difference in the serum triglyceride levels of rats in DT and YC group. But a sharp increase in the triglyceride level in

Table 9b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	5	187.388	37.478	0.4263 NS
2.	Treatment	6	8326.026	1387.671	15.7839 **
3.	Error	30	2637.502	87.917	
	Total	41	11150.916		

NS - Not significant ** - Highly significant

serum was noticed in the rats fed with DC. The serum triglyceride level of different treatments is shown in Fig.8.

A pairwise comparison was made between the individual groups. The increase in the triglycerides in the rat serum was drastic when cholesterol was added along with normal ration.

The statistical analysis revealed a highly significant reduction in the serum triglyceride levels, in the group fed with Treatment Yogurt (YT) and cholesterol along with normal feed. Nevertheless there was no significant change in the serum triglyceride levels of rats fed with DT and YC when compared to the NFC group. In the case of Control Dahi (DC) group there was a highly significant increase in serum triglyceride level.

The results showed that the se||um triglycerides were markedly decreased in the YT group and there was no significant change in the DT and YC group while in the DC group it elevated to significantly high level.

4.4.3 HDL-Cholesterol

The Serum HDL-Cholesterol levels of the rats under different treatments were estimated. The values are given in the Table 10a.

The mean serum HDL-Cholesterol level of the rats fed with normal feed was 8.133 ± 0.364 mg/100 ml, ranging between 6.9 and 9.4. The serum HDL-Cholesterol sharply increased when the diet was added with cholesterol. In the normal feed with added cholesterol (NFC) group the mean serum HDL-Cholesterol level was 11.033 ± 0.446. The minimum value was 10.0 mg/100 ml whereas the maximum value was 13.1 mg/100 ml.

The increase was more pronounced in DC, DT, YC and YT groups. For the group which fed on DC along with cholesterol and normal feed, the mean HDL-Cholesterol level was 13.45 ± 0.478 mg/100 ml. The minimum and the maximum values were 11.9 and 15.0 mg/100 ml respectively. In the group which received DT and cholesterol along with normal ration, the mean serum HDL-Cholesterol level was 14.833 ± 0.477 mg/100 ml, with the values ranging from 13.8 to 16.3.

As far as the group maintained on YC, cholesterol and normal feed was concerned, the mean HDL-Cholesterol levels in serum was 16.567 ± 0.422 mg/100 ml, the minimum being 15.0 and the maximum 17.5 mg/100 ml. Rats fed on diet containing YT, Cholesterol and Normal Feed recorded a mean HDL-Cholesterol level of 17.617 ± 0.637 mg/100 ml. The minimum and maximum values were 15.0 and 19.4 respectively.

Table 10a. Serum HDL-Cholesterol levels of rats under different treatments (mg/100 ml)

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed plus Cholesterol			
			Control Dahi (DC)	Treatment Dahi (DT)	Control Yogurt (YC)	Treatment Yogurt (YT)
1	8.1	10.6	14.4	14.6	15.0	16.9
2	6.9	10.0	13.1	16.3	16.9	19.4
3	7.5	11.3	11.9	16.3	15.6	18.1
4	8.8	10.6	13.8	13.8	16.9	15.0
5	9.4	13.1	12.5	13.8	17.5	17.5
6	8.1	10.6	15.0	14.4	17.5	18.8
Mean	8.133	11.033	13.450	14.833	16.567	17.617
SE ±	0.364	0.446	0.478	0.477	0.422	0.637

Among the treatment groups the YT fed rats showed the highest serum HDL-Cholesterol levels, followed by YC, DT, DC and NFC in that order. The statistical analysis revealed, that the differences between the treatments were highly significant. The serum HDL cholesterol level of rats under different treatments is plotted in Fig.9.

Fig.9 SERUM HDL - CHOLESTEROL OF RATS UNDER DIFFERENT TREATMENTS (mg/100ml)

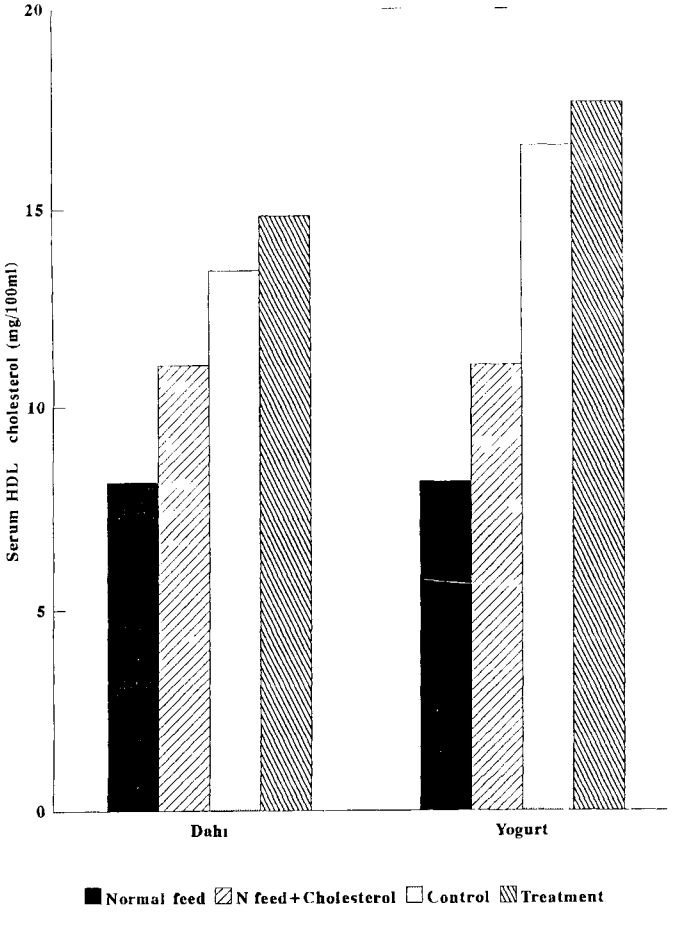


Table 10b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	5	2.393	0.479	0.3222 NS
2.	Treatment	6	552.036	92.006	61.9536 **
3.	Error	30	44.552	1.485	
	Total	41	598.981		

NS - Not significant ** - Highly significant

A pairwise comparison was made among the treatments. A significantly high increase in the HDL-Cholesterol levels of rats fed with DC and DT, was observed when compared to the NFC group. A similar trend but more pronounced increase of HDL-Cholesterol levels were seen in groups YC and YT. However, no significant differences were observed between the DC and DT groups. Likewise the differences in the HDL-Cholesterol levels of YC and YT were statistically not significant, though there was an apparent increase in the HDL-Cholesterol level of YT fed group.

Both yogurt fed groups (YC and YT) showed significantly high levels of HDL-Cholesterol than DC as well as DT fed groups.

4.4.4 LDL-Cholesterol

The serum LDL-Cholesterol levels of all the treatment groups are given in the Table 11a.

Rats fed with normal feed were having a mean serum LDL-Cholesterol level of 42.6 ± 2.879 mg/100 ml. The minimum level was 30.8 and the maximum being 51.3. In the NFC groups where cholesterol was added in the normal feed there was a drastic increase in the LDL-Cholesterol level. The mean level being 64.983 ± 3.536 mg/100 ml, with a range from 56.0 to 77.7. The mean LDL-Cholesterol level of rats under DC feed group was 60.767 ± 3.625 mg/100 ml. The minimum and maximum levels were 51.0 and 69.9 respectively.

A sharp decline in the LDL-Cholesterol levels was found in the DT, YC and YT groups, where the reduction is more pronounced in DT and YT groups. The mean LDL-Cholesterol level of rats under DT group was 44.433 ± 1.664 mg/100 ml, with a range between 40.8 and 51.

In the YC fed group, the mean LDL-Cholesterol was 51.267 ± 2.936 mg/100 ml. The values ranges from 43.4 to 62.9. For the rats under YT group, mean LDL-Cholesterol level was 44.283 ± 1.187 mg/100 ml. The minimum and the maximum levels were 39.9 and 48.3 respectively.

Table 11a. Serum LDL-Cholesterol levels of rats under different treatments (mg/100 ml)

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed plus Cholesterol			
			Control Dahi (DC)	Treatment Dahi (DT)	Control Yogurt (YC)	Treatment Yogurt (YT)
1	43.1	58.8	69.9	51.0	55.9	39.9
2	51.3	56.0	51.9	44.3	62.9	44.9
3	30.8	63.7	68.0	41.7	49.7	48.3
4	39.2	77.7	68.3	47.5	45.2	45.9
5	47.0	73.4	51.0	40.8	43.4	42.3
6	44.2	60.3	55.5	41.3	50.5	44.4
Mean	42.6	64.983	60.767	44.433	51.267	44.283
SE ±	2.879	3.536	3.625	1.664	2.936	1.187

Among the treatment groups, the YT fed group was having the lowest LDL-Cholesterol level followed by DT, YC, DC and NFC in that order. Statistical analysis revealed that the differences were highly significant. The serum LDL cholesterol level of rats under different treatments is shown in Fig.10.

A comparison was made among the treatment pairwise. A highly significant reduction in the LDL-Cholesterol levels of rats fed with DT, YC and YT was noticed. However, the LDL-

Fig.10 SERUM LDL - CHOLESTEROL OF RATS UNDER DIFFERENT TREATMENTS (mg/100ml)

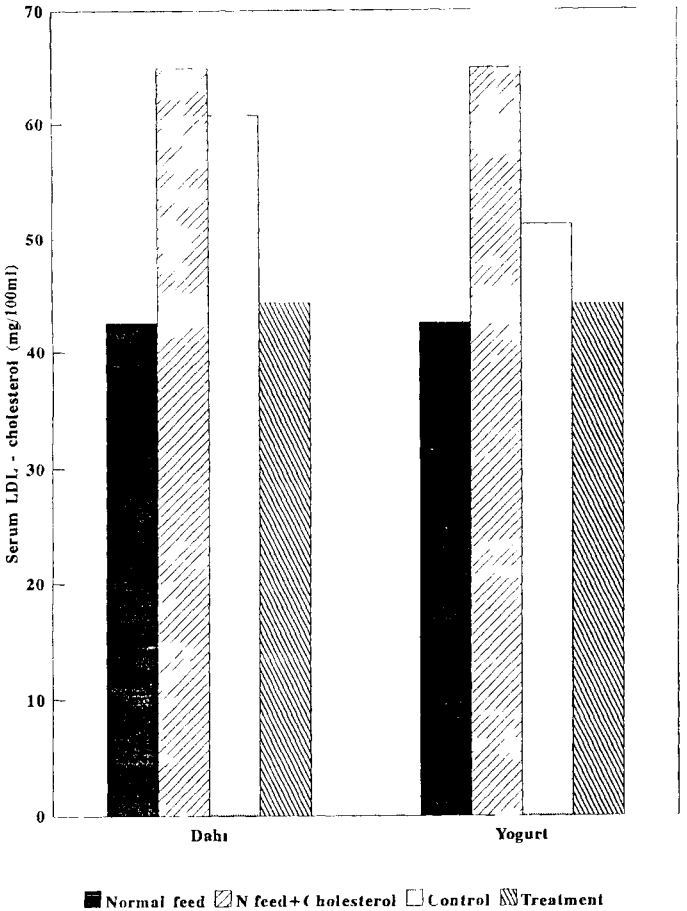


Table 11b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	5	77.313	15.463	0.3094 NS
2.	Treatment	6	3271.736	545.289	10.9112 **
3.	Error	30	1499.252	49.975	
	Total	41	4848.301		

NS - Not significant ** - Highly significant

Cholesterol level of DC fed group was not statistically different from that of NFC fed group. There was no significant difference in the LDL-Cholesterol levels of DT, YC and YT fed groups, eventhough the levels in YT and DT groups were apparently lower than the YC fed group.

4.4.5 Cardiac Risk Factor (CRF)

The cardiac risk factor (CRF) of all the feeding groups of rats were calculated and given in the Table 12a.

The mean CRF of the rats under normal feed group was 7.773 ± 0.487 with a range between 6.95 and 10.04. When cholesterol was added to normal feed, there was a sharp increase in cardiac risk factor. The mean CRF of the groups

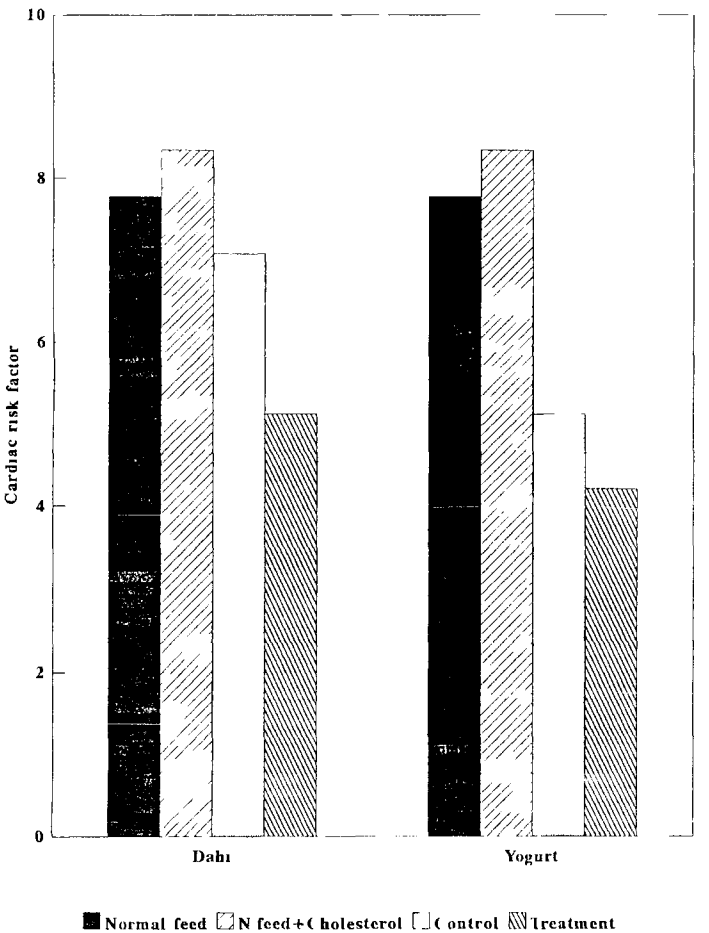
fed with normal feed and cholesterol was 8.343 ± 0.336. The minimum and maximum values were 7.79 and 10.00 respectively.

There was a general reduction in the CRF of rats fed under DC, DT, YC and YT groups. The mean cardiac risk factor of DC group was 7.072 ± 0.358. The minimum value obtained was 5.99 and the maximum was 8.57. For the rats under DT group, the mean CRF was 5.115 ± 0.188, with minimum and maximum values of 4.51 and 5.67 respectively.

Table 12a. Cardiac risk factor of rats under different treatments (Total cholesterol/HDL-Cholesterol)

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed with Cholesterol plus			
			Control Dahi (DC)	Treatment Dahi (DT)	Control Yogurt (YC)	Treatment Yogurt (YT)
1	8.06	8.08	7.08	5.67	5.71	4.11
2	10.0||	8.16	6.54	4.76	5.56	4.00
3	7.08	7.95	8.57	4.51	5.23	4.29
4	6.95	7.79	6.86	5.03	4.66	3.97
5	6.95	10.00	7.39	5.62	4.59	4.90
6	7.56	8.08	5.99	5.10	||.90	3.91
Mean	7.773	8.343	7.072	5.115	5.108	4.197
SE ±	0.487	0.336	0.358	0.188	0.191	0.151

Fig.11 CARDIAC RISK FACTOR OF RATS UNDER DIFFERENT TREATMENTS



The mean cardiac risk factor of YC fed rats was 5.108 ± 0.191, with the values ranging from 4.59 to 5.71. A more drastic reduction in CRF was found in the YT fed rat groups. Which had a mean cardiac risk factor of 4.197 ± 0.151. The minimum value was 3.91 and the maximum being 4.9.

Of all the treatments it was the YT fed group which showed the lowest cardiac risk factor. It was followed by YC, DT and DC groups in that order. Normal feed with cholesterol (NFC) group of rats showed the highest CRF. Statistical analysis showed highly significant differences between treatments. The cardiac risk factor of rats under different treatments is depicted in Fig.11.

Table 12b. Analysis of variance

Sl. No.	Source	Degrees of freedom	Sum of squares	Mean squares	F value
1.	Replication	5	1.773	0.355	0.4229 NS
2.	Treatment	6	103.575	17.262	20.5797 **
3.	Error	30	25.1640	0.839	
	Total	41	130.513		

NS - Not significant ** - Highly significant

A pairwise comparison was made between the groups. The rats under feed groups DC, DT, YC and YT all showed a significant reduction in the cardiac risk factor when compared to the NFC group.

The CRF of the rats fed with DT, YC and YT was significantly lower than that of the rats under DC group. Eventhough the YT groups was having apparently the lowest cardiac risk factor, statistical analysis revealed that there was no significant difference in the CRF of DT, YC and YT fed groups.

4.4.6 Growth rate

The growth rate of all the rats under different feeding groups were calculated and the values are given in the Table 13a and plotted in Fig.12.

The mean growth rate of the rats belonging to the normal feed group was 0.414 $\pm$ 0.019 gm/day. The minimum value was 0.375 and maximum was 0.5. In the NFC group where the normal feed was added with cholesterol there was a sharp reduction in growth rate. The mean growth rate in this group was 0.375 $\pm$ 0.018 gm/day. The range was between 0.321 and 0.446.

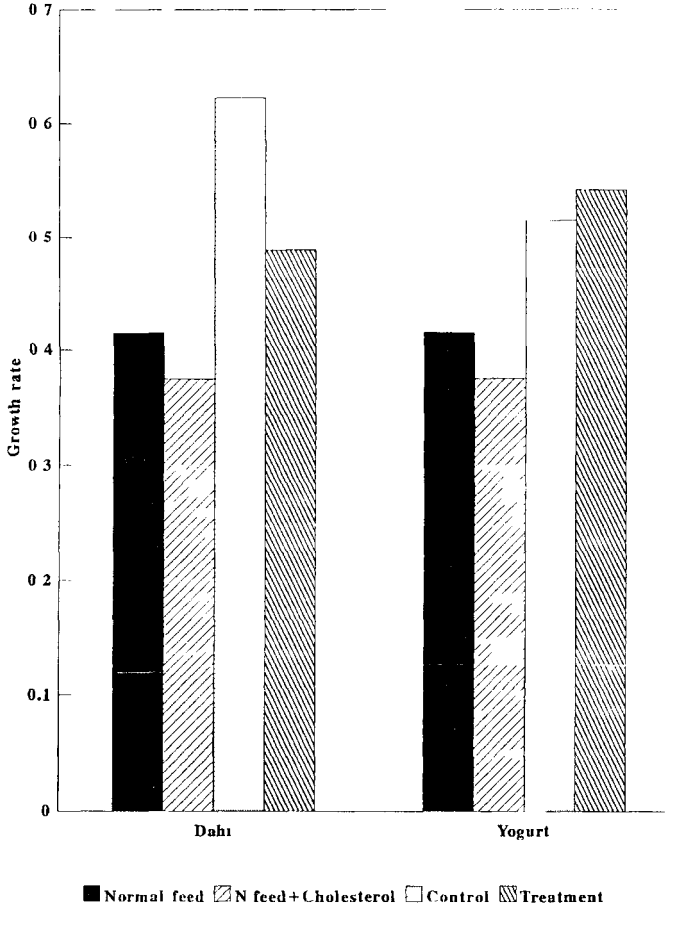
In all the other groups namely DC, DT, YC and YT, there was a drastic increase in the growth rate. In the DC fed group, which has achieved the highest growth rate, the mean growth rate was 0.622 ± 0.045 gm/day. The minimum and the maximum values were 0.554 and 0.839 respectively.

For the rats fed with DT the mean growth rate was 0.488 ± 0.026 gm/day. The minimum value was 0.429 and the maximum 0.571. The mean growth rate for rats fed with YC was 0.515 ± 0.032 gm/day. The value ranged from 0.429 to 0.607.

Table 13a. The growth rate of rats under different treatments (g/day)

Treatment / Replication	Normal Feed (NF)	Normal Feed with Cholestrol (NFC)	Normal feed with Cholesterol plus			
			Control Dahi (DC)	Treatment Dahi (DT)	Control Yogurt (YC)	Treatment Yogurt (YT)
1	0.500	0.393	0.554	0.571	0.464	0.571
2	0.375	0.339	0.571	0.500	0.589	0.607
3	0.429	0.375	0.839	0.429	0.429	0.536
4	0.393	0.446	0.643	0.554	0.446	0.804
5	0.375	0.375	0.571	0.446	0.607	0.357
6	0.411	0.321	0.554	0.429	0.554	0.375
Mean	0.414	0.375	0.622	0.488	0.515	0.542
SE ±	0.019	0.018	0.045	0.026	0.032	0.064

Fig.12 GROWTH RATE OF RATS UNDER DIFFERENT TREATMENTS (g/day)



The growth rate was highest in the DC fed group. The growth rate of this group was significantly higher than DT and YC groups. However, there was no significant difference between the DC and YT groups in growth rate. Likewise there was no significant difference in growth rates of DT and YC groups.

Discussion

DISCUSSION

Dahi is the most important fermented milk product of India. Yogurt on the other hand is fastly gaining ground in the expanding organised dairy sector. The fermented milk products have many beneficial effects than the raw material milk from which they are made. Still these products have a drawback, that the starter culture organisms used for manufacture of dahi and yogurt are incapable of colonising in the intestine (Speck, 1977; Gilliland and Kim, 1984 and Gandhi and Rao, 1989), thus making the beneficial effect temporary.

On the other hand L. acidophilus which is capable of intestinal implantation is not widely used in India due to the lack of popular vehicle. In the present study an effort is made to incorporate L. acidophilus in dahi and yogurt, and their beneficial effects are estimated in terms of β-Galactosidase specific activity, antibacterial activity, bile tolerance and hypocholesteremic effect.

Control dahi (DC), Treatment dahi (DT), Control yogurt (YC) and Treatment yogurt (YT) were prepared by inoculation of two per cent of mixed culture in the pretreated milk.

All the products under each treatments were analysed for β-galactosidase specific activity, antibacterial activity

and hypocholesteremic effect and also weight gain in rats. In addition, the individual cultures were tested for their ability to grow in the presence of bile. The results obtained are discussed below.

5.1 β-Galactosidase specific activity

The β-galactosidase specific activity of different treatments is given in Table 1a. The mean β-galactosidase specific activity of Control dahi (DC) was 2.179 ± 0.04, whereas that of Treatment dahi (DT) was 3.075 ± 0.034. The results suggest that there was an increase in the β-galactosidase activity for DT. It could be due to the incorporation of L. acidophilus. The results come in line with the findings of Kim and Gilliland (1983), who reported that L. acidophilus added in milk could effectively reduce lactose intolerance due to the production of more bacterial β-galactosidase.

Alm (1982) also reported that L. acidophilus was more effective in reducing the lactose content of milk when compared to S. lactis.

The mean β-galactosidase activity of control yogurt (YC) was 4.281 ± 0.033. But the incorporation of L. acidophilus in the yogurt culture resulted in significant decline in the B-galactosidase specific activity to a level of

3.629 ± 0.03. Lin et al. (1991) reported that the β-galactosidase specific activity of L. acidophilus was lower than that of L. bulgaricus and S. salivarius spp thermophilus. Savaiano and Levitt (1987) reported that the β-galactosidase specific activity of yogurt was higher than acidophilus milk.

Lower level of β-galactosidase specific activity in YT in the present study could be explained by the lower level of S. salivarius ssp thermophilus and L. delbrueckii ssp bulgaricus used in the inoculum (0.5 per cent each) when compared to the YC, whereas the inoculum level of the starters were one per cent each. So the total number of the S. salivarius ssp thermophilus and L. delbrueckii ssp bulgaricus in the products might have influenced the total enzyme produced in the treatments. Another possible reason that can be attributed for the low level of B-galactosidase specific activity in YT could be the liberation of inhibitory substances by L. acidophilus against yogurt cultures, though pairing of organisms did not reveal any such inhibitory effect.

Eventhough there was a decline in the β-galactosidase specific activity in YT, the implantation of L. acidophilus in the intestine may produce better results in vivo, since they are capable of colonising in the gastrointestinal tract. Similar findings were reported by Kim and Gilliland (1983).

5.2 Antibacterial activity of lactic cultures

5.2.1 Antibacterial activity against E. aerogenous

The antibacterial activity was measured in terms of inhibition zones shown by the particular sample. The mean inhibition zone of DC against E. aerogenous was 10.025 ± 0.139. Dahi cultures are capable of inhibiting the growth of E. aerogenous by producing antibacterial metabolites. The findings are in correlation with the reports of Gandhi and Nambudripad (1975).

The antibacterial activity of different treatments against E. aerogenous is given in Table 2a. The mean inhibition zone exerted by DT against E. aerogenous was 12.762 ± 0.386. The increase in the inhibition zone of DT against the test organism may be due to the incorporation of L. acidophilus. The result is in agreement with the earlier reports, regarding the ability of L. acidophilus to cause potential inhibition against a wide range of bacteria including E. aerogenous, P. fluorescens, B. pumulis, B. subtilis, P. aeruginosa and S. aureus (Pulusani et al., 1979; Dubois et al., 1982; Prasad and Gandhi, 1987 and Varadharaj et al., 1990).

The mean inhibition zone produced by YC against E. aerogenous was 9.262 ± 0.301. The finding is in line with

the report of Schaack and Marth (1988 b), that Yogurt is capable of inhibiting a variety of pathogenic and spoilage organisms including E. aerogenous. The YT produced a mean inhibition zone of 11.925 ± 0.322. The incorporation of L. acidophilus may be the reason why the YT gave a higher inhibition. Prasad and Gandhi (1987) reported the potential ability of L. acidophilus in exhibiting a strong inhibition against E. aerogenous. Shahani reported that the bacteriocins produced by L. acidophilus like acidolin, acidophilin, Lactocidin and Lactocin-B were capable of exerting strong inhibition against a wide range of pathogenic and spoilage bacteria, Reddy et al. (1984) also reported a similar finding.

5.2.2 Antibacterial activity against M. flavous

The antibacterial activity of different treatments against M. flavous is shown in Table 3a. The mean inhibition zone produced by Control dahi (DC) against M. flavous was 8.875 ± 0.084. Branan et al. (1975) reported that the cell free fermentation liquors from S. diacetylactis showed inhibition against a range of pathogens including M. flavous. The DT containing L. acidophilus showed a significant increase in the inhibition against the same pathogen. It showed a mean inhibition zone of 13.563 ± 0.367. The findings are in correlation with the reports of Gandhi and Nambudripad, who stated that L. acidophilus produced a high degree of

inhibition against M. flavous. The antibacterial bacteriocins and other inhibitory metabolites produced by the L. acidophilus may be the reason for increased inhibition by DT. Mikolajcik (1975 a,b) identified a strong inhibitory agent in the cultures of L. acidophilus called Acidophilin. Collins and Aramaki (1980) reported that the antagonistic activity of L. acidophilus towards enteric pathogens and psychrophilic spoilage organisms was in part due to the production of hydrogen peroxide.

M. flavous did not showed any inhibition when tested against YC. Maciejska and Czarniakowa (1985) reported that Micrococcus sp were able to resist and grow in the presence of yogurt and cheese starter cultures. However, YT, which was incorporated with L. acidophilus show inhibition against the same test organism. The mean inhibition zone produced was 10.0 $\pm$ 0.23. The result showed similarity with the earlier findings of Gandhi and Nambudripad (1980), who demonstrated the potential ability of L. acidophilus to inhibit M. flavous. The inhibitory effect of bacteriocins, and high acidity produced by L. acidophilus might be the reason for the inhibition produced by YT (Shahani et al., 1976, 1977 and Reddy et al., 1984).

5.2.3 Antibacterial activity against *E. coli*

The antibacterial activity of different treatments against *E. coli* is shown in Table 4a. All the treatments DC, DT, YC and YT showed inhibition against *E. coli*. The mean inhibition zone exerted by DC was 8.525 ± 0.139. Daly *et al.* (1970, 1972) in their report, mentioned that *S. lactis* ssp *diacetylactis* significantly reduced the growth of *E. coli* and other test organisms. Dahi samples when tested against *E. coli*, showed significant inhibition (Gandhi and Nabudripad, 1975). A significant increase in the inhibition zone was noticed when the *L. acidophilus* incorporated DT, was used. DT gave a mean inhibition zone of 15.325 ± 0.248. The possible explanation may be the higher ability of *L. acidophilus* to inhibit *E. coli*. Vincent *et al.* (1959) reported that *E. coli* and other pathogens failed to survive in the presence of lactocidin, a bacteriocin produced by *L. acidophilus*. Singh and Laxminarayana (1973) also came out with similar reports.

When *E. coli* was tested against control yogurt (YC), a mean inhibition zone of 8.088 ± 0.055 was obtained. The results are in accordance with the earlier reports. Pulusani *et al.* (1979) in their reports, mentioned that *L. bulgaricus*, *S. thermophilus* and *L. acidophilus* strongly inhibited the growth of *E. coli*. Varadharaj *et al.* (1990) when testing the inhibitory activity of extra cellular filtrates obtained from

L. bulgaricus found that it produced inhibition against E. coli.

The L. acidophilus supplemented YT gave a significantly high zone of inhibition against E. coli. The mean value was 13.487 ± 252. It is quiet likely that the enhanced antibacterial activity of YT against E. coli may be due to the incorporation of L. acidophilus. Sharma and Prasad (1986) reported that the antibacterial activity of yogurt was enhanced when supplemented with L. acidophilus. Geetha (1992) also demonstrated the increase in the antagonistic activity of yogurt against E. coli, S. aureus and B. cereus, when L. acidophilus was incorporated.

5.2.4 Antibacterial activity against S. aureus

The antibacterial activity of different treatments against S. aureus is shown in Table 5a. The results of antibacterial activities of DC, DT, YC and YT against S. aureus are discussed below.

The mean inhibition zone produced by DC against S. aureus was 9.388 ± 0.18. Daly et al. (1970; 1972) reported that S. lactis ssp diacetylactis strongly inhibited S. aureus. Gandhi and Nambudripad (1975) when studying the antagonistic activity of dahi, found out that, it showed a high inhibition against S. aureus. DT showed a significantly high inhibition

zone against the same pathogen. The mean inhibition zone of DT was 12.075 ± 0.125. The elevated antibacterial activity of DT when compared to DC may be due to the antibacterial activity exerted by L. acidophilus. The ability of L. acidophilus to strongly inhibit S. aureus was well documented (Vincent et al., 1959; Singh and Laxminarayana, 1973 and Gandhi and Nambudripad, 1980).

The YC did not showed any inhibition against S. aureus. Reddy et al. (1984) reported that, some of the L. delbrueckii ssp bulgaricus, strains failed to show any inhibition against S. aureus. Arnott et al. (1974) reported that some strains of S. aureus can survive in commercial yogurt. On contrary to the above reports Varadharaj et al. (1990) reported that L. delbrueckii showed inhibition towards S. aureus. The probable reason may be, the variations in the strains of L. bulgaricus used.

However, when L. acidophilus was incorporated in the yogurt, it showed inhibition against S. aureus. The mean zone of inhibition exerted by YT was 10.125 ± 0.337. The result suggested that the antibacterial activity of YT might be due to the production of inhibitory metabolites and bacteriocins produced by the L. acidophilus. Vincent et al. (1959) reported that lactocidin a bacteriocin produced by

L. acidophilus strongly inhibited the growth of S. aureus. Khedkar et al. (1990b) also published a similar report.

The inhibitory activity of acidophilus milk prepared using L. acidophilus, against S. aureus, was reported by Gandhi and Nambudripad (1980).

5.2.5 Antibacterial activity against B. cereus

The antibacterial activity of different treatments against B. cereus is given in Table 6a. All the treatments DC, DT, YC and YT were tested against B. cereus for their antibacterial activity. While both controls did not show any inhibition, there was considerable inhibition by both the treatments.

The DC did not bringforth any inhibition against B. cereus. Gandhi and Nambudripad (1975) reported that, out of 100 dahi samples tested only 40 showed inhibition against B. cereus, while the remaining 60 did not showed any inhibition. When DT was tested against B. cereus, it gave a mean inhibition zone of 13.1 $\pm$ 0.501. The probable cause may be the strong inhibitory activity of L. acidophilus against the test organism. Khedkar et al. (1990b) reported that L. acidophilus significantly inhibited B. cereus, S. typhosa, S. aureus, E. coli and P. aeruginosa. Neutralised extracellular culture filtrates obtained from isolates of

L. acidophilus, showed inhibition against a long list of bacteria including B. cereus (Varadharaj et al., 1990). This suggested that the antibacterial compounds, produced by the L. acidophilus could be the possible factor for inhibition. Shahani (1982), reported the L. acidophilus produced a wide variety of bacteriocins namely acidolin, acidophilin, lactocidin and lactocin-B, which were capable of exerting inhibition against a wide range of pathogenic and spoilage microorganisms.

In the case of YC, there was no inhibition against the B. cereus. Reddy et al. (1984) reported that some of the L. delbrueckii ssp bulgaricus when tested against B. cereus failed to show any inhibition against the organism. In contrary to this report, Varadharaj et al. (1990) reported that L. bulgaricus showed a weak to moderate inhibition against B. cereus. The possible reason may be, due to the strain variation of the cultures used in the respective studies. The yogurt incorporated with L. acidophilus (YT) showed a mean inhibition zone of 9.35 $\pm$ 0.661. The inhibition may be brought about by the antibacterial substances and other compounds, produced by the L. acidophilus. Prasad and Gandhi (1987) reported that L. acidophilus showed a strong inhibition against B. cereus. The reports of Geetha (1992) suggested that incorporation of L. acidophilus in the yogurt resulted in

a significant increase in the antibacterial activity of the product against B. cereus, E. coli. and S. aureus.

The results suggested that both DT and YT produced a high inhibition zone when compared to their respective treatments against E. coli, M. flavous, B. cereus, E. aerogenous and S. aureus in that order. Among the DT and YT the inhibition was higher in DT. The possible reason may be the production of maximum antibacterial substance by L. acidophilus at 37°C. Thus the treatment dahi incubated at room temperature produced higher inhibition than treatment yogurt. Shahani et al. (1976) reported that optimum temperature for L. acidophilus to produce antibacterial substances was 37°C, and the milk medium has been indicated to be essential for production of these substances.

The possible reason for an increase in the antibacterial activity in both DT and YT was, the production of bacteriocins and other metabolites, produced by L. acidophilus, which were detrimental to the succeptible organisms. Shahani (1982) reported the production of bacteriocins like acidolin, acidophilin, lactocidin and lacticin-B by L. acidophilus. The acid and pH might not have any effect in the inhibition because these are same in the controls and treatments. Gilliland and Speck (1977b) reported that antagonistic activity of L. acidophilus against S. aureus

and E. coli was not directly related to the amount of acid produced by the lactobacilli.

On the contrary to this reports Patkal et al. (1977) established a relationship between rate of acid production and degree of inhibition of coliforms by lactic acid bacteria. Gilliland and Speck (1977a) and Collins and Aramaki (1980) reported that inhibition of enteric pathogens and psychrophilic spoilage organisms, by L. acidophilus, was in part, due to the hydrogen peroxide produced by the bacteria.

5.3 Bile tolerance

The bile tolerance of different lactic cultures used in the present study is shown in Table 7. Bile tolerance is an important prerequisite for an organism which is expected to colonise in the intestine. The bile tolerance of L. acidophilus and all other lactic acid bacteria used in this study were measured by cultivating them in MRS-broth which was added with 0.3 per cent of oxgall. The growth if any, was compared with the growth of same organism in MRS-broth without bile.

L. acidophilus took a mean time of two hours and 53 minutes to reach the optical density of 0.3 in MRS-broth without oxgall. But the growth was slowed down in the presence of 0.3 per cent oxgall. The mean time taken to reach

an optical density of 0.3 was three hours and 56 minutes. The results are in accordance with the previously reported results. Walker and Gilliland (1993) reported that out of 19 strains of L. acidophilus tested, all of them exhibited some degree of bile tolerance. In all the cases, the growth in the presence of bile was slower than, that obtained without bile. They suggested that there existed a wide difference among the strains in their ability to tolerate and grow in the presence of bile. Overdahl and Zottola (1991) and Hoier (1992) also found similar results in their research.

All the other lactic acid bacteria used in the present study, were not able to grow in the presence of 0.3 per cent oxgall.

S. salivarius ssp thermophilus took a mean time of three hours and 11 minutes to reach an optical density of 0.3, in MRS-broth without bile. But the organism did not showed any increase in the optical density when grown in MRS-broth containing 0.3 per cent oxgall even after six hours of incubation. The inability of S. salivarius ssp thermophilus to survive the bile environment of intestinal tract was reported by Hargrove and Alford (1978). Khattab and Abour-Donia (1987) also reported the complete absence of growth of S. thermophilus when grown in media containing 0.3 per cent bile salt.

The L. delbrueckii ssp bulgaricus took a mean time of two hours and 55 minutes to reach an optical density of 0.3, in ordinary MRS broth. However, the organism did not show desirable growth, in the MRS broth, with 0.3 per cent oxgall, in which, even after six hours of incubation, the rise in the optical density was only 0.11.

Pettersson et al. (1983a) reported that L. bulgaricus survived poorly, when grown in gastric juice, containing bile. The results in the present study also showed a similar trend. The poor survival and growth of L. bulgaricus in the presence of bile was also reported by Lindwall and Fonden (1984), and Khattab and Abour-Donia (1984).

In the case of Lac. lactis, the mean time taken to reach an optical density of 0.3, in MRS broth, without bile was three hours and 29 minutes. But it failed to grow in the presence of 0.3 per cent of oxgall. At the end of six hours of incubation there was no increase in the optical density. The result is in agreement with the previous results. S. lactis failed to grow in the presence of bile salts at any of the concentration between 0.15 to 0.3 per cent (Khattab and Abour-Donia, 1987). Lac. lactis ssp diacetylactis took a mean time of three hours and 34 minutes to reach an optical density of 0.3, when grown in the MRS broth without oxgall. But it did not showed even slightest increase in optical density,

when grown in the same broth containing 0.3 per cent oxgall, at the end of six hours incubation period. Khattab and Abour-Donia (1987) reported similar findings, in their research. Gandhi and Rao (1989) reported that the dahi culture organisms were incapable of tolerating bile.

The results suggested that, among the Lactic acid bacteria tested, only L. acidophilus showed a satisfactory growth in the presence of bile. By virtue of its bile tolerance, L. acidophilus may be able to establish itself in the intestine, and colonise, thus extending the beneficial effects for longer duration.

5.4 Hypocholesteremic effect

The hypocholesteremic effect of the different treatments were studied through a feeding trial involving rats.

5.4.1 Serum total cholesterol

The serum total cholesterol level of rats under different treatments is shown in Table 8a. The mean total cholesterol level in the serum of rats fed with normal feed was 62.567 $\pm$ 2.263 mg/100 ml. When the cholesterol was added to the normal feed in the NFC group of rats a significant increase in serum total cholesterol levels were found. The

mean total cholesterol in the serum of NFC group was 91.817 ± 4.046 mg/100 ml. This clearly showed that incorporation of cholesterol in the diet definitely elevated the serum total cholesterol levels. The mean total serum cholesterol level of rats fed with normal feed containing control dahi (DC) and cholesterol was 94.533 ± 3.395 mg/100 ml. This value was not significantly different from the value of NFC group. The result suggests that DC has got no significant effect on the serum total cholesterol levels. Nakajama et al. (1992) reported that the hypocholesteremic effect of ropy fermented milk containing Lac. lactis ssp cremoris was due to the slime formation. A non slime forming variant of the same organism did not show hypocholesteremia. The presence of some unidentified compounds in the slime material may be the factor regulating the cholesterol level by this organism. The absence of slime formation in our study may be the reason why the serum cholesterol levels were not reduced.

The mean serum cholesterol level of the rats fed with normal feed containing treatment dahi (DT) and cholesterol were 75.533 ± 1.744 mg/100 ml. The significant reduction in total serum cholesterol levels of this group may be due to the hypocholesteremic activity of L. acidophilus. The result suggested that the reduction in the total serum cholesterol levels of DT fed rats might only be due to L. acidophilus,

with no contribution from Lac. lactis and Lac. lactis ssp diacetylactis.

Tortuero et al. (1975) reported that serum cholesterol levels of laying hens were considerably reduced by L. acidophilus. Gilliland and Speck (1977a) demonstrated that L. acidophilus could deconjugate both taurocholic and glycolic acids under anaerobic conditions. They suggested that an increased excretion of bile acid might lead to a faster rate of catabolism of cholesterol by bile acids.

The rats fed with YC also showed significant decrease in the serum cholesterol levels when compared to the NFC group. The mean value obtained in this group was 84.35 $\pm$ 2.277 mg/100 ml. This finding is in par with the earlier reports. Manon (1977a) in his experiment on human volunteers reported a significant reduction in serum total cholesterol levels of persons fed with yogurt.

Hepner et al. (1979) and Thakur and Jha (1981) also reported similar findings. While the DC did not show any hypocholesteremia, the YC showed a significant reduction in the serum cholesterol levels of the rats. The possible reason may be, the absence of slime material in the DC could have contributed to its inability to reduce the cholesterol level in the serum. On the other hand the hypocholesteremic effect

of yogurt cultures were reported by many scientists (Mann, 1977a and Hepner et al., 1979).

The mean serum cholesterol levels of rats fed with normal ration, YT and Cholesterol was 73.50 ± 1.497 mg/100 ml. The significant reduction in serum cholesterol level in this group, when compared to YC might be due to the ability of L. acidophilus to implant in the intestine and deconjugate bile acids (Gilliland and Speck, 1977a). Nelson and Gilliland (1984) reported that the ability of bile resistant strains of L. acidophilus to remove cholesterol was increased in the presence of bile. Danielson et al. (1989) reported that the serum cholesterol levels of hypercholesteremic pigs were significantly reduced by the feeding of acidophilus yogurt.

The above results suggested that both dahi and yogurt when incorporated with L. acidophilus, resulted in a considerable decrease in serum total cholesterol when compared to the respective controls. Among the treatments, YT showed more hypocholesteremia than the DT. The possible explanation may be, that in the YT, the hypocholesteremia might have been contributed in part by L. acidophilus and in part by the yogurt cultures themselves.

5.4.2 Serum triglycerides

The mean serum triglyceride level of rats under different treatments is shown in Table 9a. The serum triglyceride levels of different groups of rats were estimated. Feeding normal ration to the rats resulted in a mean serum triglyceride level of 59.2 ± 5.313 mg/100 ml but the level got elevated when cholesterol was added in the diet. The NFC group showed a mean serum triglyceride level of 78.933 ± 2.274 mg/100 ml. The increase in the triglyceride level could be the result of added cholesterol.

The mean serum triglyceride level of DC fed rats were 101.75 ± 3.542 mg/100 ml. The increase in the triglyceride level might have been influenced by the feed components fed to the DC group. Wang *et al*. (1982) reported that the triglyceride levels of milk products were much higher than the meat products.

The rats fed with DT, showed a significant reduction in serum triglycerides. The mean value in this group was 81.4 ± 2.136 mg/100 ml. It is evident that the incorporation of *L. acidophilus* resulted in the reduction of serum triglycerides. Pulusani and Rao (1983) reported the ability of *L. acidophilus* to reduce the serum cholesterol level as well as the total lipids.

In the rats fed with YC, the mean serum triglyceride level was 82.633 ± 4.933. The triglyceride levels of YC fed groups showed a significant reduction when compared to the DC fed groups. The possible explanation may be, the effect due to the yogurt cultures.

Pulusani and Rao (1983) reported the ability of L. bulgaricus and S. thermophilus, to decrease the total lipid content in experimental rats.

The serum triglyceride levels of rats fed with YT were significantly reduced. The mean serum triglyceride levels of YT fed group was 59.967 ± 2.97 mg/100 ml. The reduction in this group is more pronounced than the reduction in the DT fed group due to the combined effect of L. acidophilus and the yogurt culture. The result is in accordance with the report of Pulusani and Rao (1983).

5.4.3 HDL-Cholesterol

The serum HDL-Cholesterol is an important indicator of the state of circulatory system. The HDL-Cholesterol acts as a scavenger, to remove the cholesterol flakes, that are partially obstructing the blood vessels. The cholesterol molecules are then taken to the liver for metabolism. That is why it is also called as good cholesterol (Robins and Cotron, 1981).

The serum HDL-cholesterol level of rats under different treatments is given in Table 10a. The mean serum HDL-Cholesterol level of the normal feed group was 8.133 ± 0.364 mg/100 ml. In the NFC group where the feed was supplemented with cholesterol. Kurski and Narayana (1976) reported that the chickens fed with cholesterol in their feed, showed an elevated HDL-Cholesterol level of 77.1 ± 13.9 mg/dl when compared to controls having 65.5 ± 16.4 mg/dl.

The mean serum HDL-Cholesterol levels of DC group was 13.45 ± 0.478 mg/100 ml. The significant increase in HDL-Cholesterol levels in this group may in part be attributed to the added cholesterol. Casein would also have played a part in the elevation. Lefevre and Schneeman (1984) reported that the casein had got a high ability to increase HDL-Cholesterol, when compared to soyprotein. A part may also be played by the dahi cultures.

The DT group of rats, showed a mean serum HDL-Cholesterol level of 14.833 ± 0.477 mg/100 ml. The increase in the level may be attributed to the combined effect of L. acidophilus and yogurt cultures. Lin et al. (1989a), reported that the HDL-Cholesterol levels of human Volunteers fed on L. acidophilus containing tablets showed a sharp increase in their HDL-Cholesterol fraction.

The mean HDL-Cholesterol levels of rats fed with YC diet was 16.567 ± 0.422 mg/100 ml. The HDL-Cholesterol level in this feed group had been significantly increased when compared to the NFC group and DC group. The probable reason may be the effect of yogurt cultures to elevate the HDL cholesterol level. The results are in line with the findings of Lin et al. (1989a). The rats fed with YT showed the mean HDL-Cholesterol level of 17.617 ± 0.637 mg/100 ml. A combination of effects would have caused the increase. Lin et al. (1989a) when conducted studies on the effect of L. acidophilus and L. bulgaricus, on HDL-Cholesterol level, reported that the level showed an increase of 1.8-3 mg/100 ml when the patients were given tablets containing these lactobacilli. The combined effect of L. acidophilus and yogurt cultures would have caused the increase in HDL-cholesterol level.

Other factors such as the cholesterol added in the feed and casein would also have played their part in the increased HDL-Cholesterol levels. The results are in line with the reports of Kurski and Narayana (1976) and Lefevre and Schneeman (1984).

The results suggested that there was a significant increase in the HDL-Cholesterol fraction of DT and YT when compared to their respective controls. Incorporation of

L. acidophilus along with the other factors might have caused that increase. Among the treatments, YT showed a significantly high HDL-Cholesterol level than DT. The probable cause may be, the combined effect of L. acidophilus and yogurt cultures, that would have caused the increased HDL-Cholesterol level.

5.4.4 LDL-Cholesterol

The serum LDL-cholesterol level of rats under different treatments is given in Table 11a. The mean LDL-Cholesterol level of the normal feed group was 42.6 ± 2.879 mg/100 ml. When cholesterol was added to normal feed, the LDL-Cholesterol level was elevated. The mean value in NFC group was 64.983 ± 3.536 mg/100 ml. Kruski and Narayan, (1976) fed the chickens cholesterol containing feed, found that the LDL-Cholesterol levels shot up to 152.3 ± 41.3 mg/100 ml, when compared to the controls containing 25.6 ± 6.7 mg/100 ml.

The rats fed with Control dahi (DC) showed a mean serum LDL-Cholesterol level of 60.767 ± 3.625 mg/100 ml.

Eventhough there was an apparent decrease in the LDL-Cholesterol level of DC when compared to NFC, the difference was not statistically significant.

The mean value for the DT group was 44.433 ± 1.664 mg/100 ml. The possible reason would be the supplementation of L. acidophilus in the DT. While studying the effect of L. acidophilus, on human volunteers, Khedkar (1990d) reported a similar trend. In addition to this, the dahi cultures, and the other components such as casein would also have caused some reduction.

The mean LDL-Cholesterol levels of the rats fed with YC was 51.267 ± 2.936 mg/100 ml. A significant, reduction occurred when compared to the NFC fed rats, and DC fed rats. The possible reason could be the ability of yogurt cultures in reducing the serum LDL-Cholesterol levels when compared to the dahi cultures. Rasic et al. (1992) reported the high ability of yogurt cultures to assimilate cholesterol.

The mean LDL-Cholesterol level of the rats fed with YT was 44.283 ± 1.187 mg/100 ml. The significant decrease in the LDL-Cholesterol level of YT might be due to the combined effect of L. acidophilus and yogurt cultures along with other unidentified hypocholesteremic factors. Lin et al. (1989b) reported a similar result in his study with human volunteers.

The LDL-Cholesterol is responsible for the deposition of cholesterol flakes in the arterial walls, which subsequently leads to coronary insufficiency and other heart

related diseases (Brown and Goldstein, 1984). Thus the reduction of LDL-Cholesterol gets more emphasis.

Above results suggested that the incorporation of L. acidophilus along with some other factors in dahi and yogurt drastically decreased the serum LDL cholesterol levels.

The effect was slightly more in YT when compared to DT because in the YT the reduction might have been caused by the combined effects of L. acidophilus and the yogurt cultures used.

5.4.5 Cardiac risk factor

The cardiac risk factor is the ratio between the total cholesterol and HDL chloresterol (Lin et al., 1989a).

The cardiac risk factor gives an idea about how likely a particular person is having chance for heart ailments. The higher the cardiac risk factor, the higher the chance for cardiac diseases.

The cardiac risk factor of rats under different treatments is given in Table 12a. The mean cardiac risk factor of rats fed with normal diet was 7.773 $\pm$ 0.487. When the diet was incorporated with cholesterol in the diets of NFC groups, the mean value was increased to 8.343 $\pm$ 0.336.

When compared to the NFC group, all the other groups DC, DT, YC and YT produced a low cardiac risk factor. This report is in line with earlier reports that the consumption of fermented milk produced a decrease in cardiac risk factor (Mann and Spoerry, 1974).

The mean cardiac risk factor of the DC fed rats were 7.072 ± 0.358. But when L. acidophilus was incorporated in the dahi, the risk factor further declined to a mean of 5.115 ± 0.188. The possible reason for this reduction may be in two ways. First the L. acidophilus containing DT significantly reduced the serum total cholesterol level, while on the other hand, the HDL cholesterol level was increased. This ultimately caused the decrease in cardiac risk factor of DT over DC. The ability of L. acidophilus to decrease the total cholesterol level was well documented (Gilliland and Waker, 1989, Gilliland, 1989 and Khedkar, 1990 d).

The mean cardiac risk factor of YC fed rats was 5.108 ± 0.191. The reason in the reduction in CRF could be due to the ability of yogurt cultures to reduce the serum total cholesterol. However, YT fed rats resulted in a further reduction in the CRF. The mean value was 4.197 ± 0.151. Walker and Gilliland (1993) reported that because of its ability to deconjucate bile salts, L. acidophilus was capable of high hypocholesteremic effect. They also found a positive

correlation between the bile salts deconjugation and cholesterol assimilation.

The above discussed results suggested that the dahi as well as yogurt when supplemented with L. acidophilus showed a further reduction in cardiac risk factor, when compared to their respective controls.

5.4.6 Growth rate

The growth rate was calculated and the values obtained are discussed below.

The growth rate of rats under different treatments is given in Table 13a. The rats fed with a normal feed showed a mean growth rate of 0.414 ± 0.019 g/day. In the NFC group, the growth rate declined to 0.375 ± 0.018 m/day.

All the rats fed under groups DC, DT, YC and YT showed a high growth rate when compared to the NFC group.

The mean growth rate achieved in the DC fed groups were 0.622 ± 0.045 g/day. But the growth rate actually decreased when L. acidophilus was incorporated. The mean growth rate for the rats under DT were 0.488 ± 0.026 g/day. Some unknown factors might have influenced in reducing the body weight in this group.

The mean growth rate of rats under YC feeding group was 0.515 ± 0.032 g/day. The growth rate of YT feeding group showed an increase with a mean of 0.542 ± 0.064 g/day. The increase in the growth rate of YT fed groups, may be due to the ability of L. acidophilus to colonise in the intestine and exert inhibition towards the enteropathogenic organisms, normally present in the gut (Tomar and Prasad, 1989). Since L. bulgaricus is moderately resistant to bile, the cell proteins would not have been utilised by the rats for digestion.

The above discussed results suggested that the incorporation of L. acidophilus in dahi and yogurt along with the normal cultures resulted in better beneficial effects. In the dahi, the incorporation of L. acidophilus resulted in an increased β-galactosidase specific activity and antibacterial activity. It also showed better hypocholesteremic activity when compared to the control dahi and significantly reduced the cardiac risk factor.

When L. acidophilus was incorporated in yogurt, it too showed enhanced beneficial effects. Eventhough, the β-galactosidase specific activity was less than the control yogurt, because of its inherent ability of bile tolerance the treatment yogurt may be more beneficial, in vitro. The treatment yogurt showed more inhibition against the test

organisms than the control yogurt. As in the case of dahi, in yogurt also, Supplementation of L. acidophilus resulted in better hypocholesteremic effect than the control yogurt.

Among the treatment yogurt and treatment dahi, the treatment yogurt was having higher β-galactosidase specific activity, and it also showed a higher degree of hypocholesteremia, while treatment dahi exhibited higher antibacterial activity. This difference would have been caused by the dahi and yogurt cultures which were used along with L. acidophilus.

In future, biological studies involving human volunteers, can be conducted to know the effect of L. acidophilus incorporated dahi or yogurt in lactose intolerance. A comparison may be made between the in vitro antibacterial activity of L. acidophilus incorporated fermented milk products and the effect of the same product, on the fecal count of pathogenic and putrifactive bacteria. This may give a correlation between the antibacterial activity of that product in vitro and in vivo. Another possible field of exploration may be the usefulness of L. acidophilus incorporated dahi and yogurt in the control of blood cholesterol and atherosclerotic lesions, in the hypercholesteremic human patients. This may give a cost effective, reliable remedy for hypercholesteremia without any side effects.

Summary

SUMMARY

An experiment was designed to find out the beneficial effect of using L. acidophilus in Dahi and Yogurt as a dietary adjunct in terms of β-galactosidase specific activity, antibacterial activity and hypocholesteremic effect. An attempt was also made to study the bile tolerance of the lactic acid bacteria used in the present study.

Control dahi, treatment dahi, control yogurt and treatment yogurt were prepared under laboratory conditions.

Control Dahi was prepared using Lac. lactis and Lac. lactis ssp. diacetylactis. Treatment Dahi was prepared using Lac. lactis, Lac. lactis ssp. diacetylactis and L. acidophilus. Control yogurt was prepared using the starters S. salivarius ssp. thermophilus and L. delbrueckii ssp. bulgaricus. Starter cultures S. salivarius ssp. thermophilus, L. delbrueckii ssp bulgaricus and L. acidophilus were used for the preparation of treatment yogurt. The mixed inoculum was used at two per cent level.

Samples from each treatment were analysed for β-galactosidase specific activity, antibacterial activity and hypo-cholesteremic effect.

The mean β-galactosidase specific activity of control dahi was 2.179. The value significantly increased in the treatment Dahi with a mean of 3.075. The increase may be due to the addition of L. acidophilus which has got a higher β-galactosidase specific activity than dahi starter culture organisms. Mean β-galactosidase specific activity of control yogurt was 4.281. The value for treatment yogurt was 3.629. The reduction in the β-galactosidase specific activity of treatment yogurt may be due to the low β-galactosidase specific activity of L. acidophilus when compared to the yogurt cultures and the low level of normal yogurt starter cultures in the inoculum.

Incorporation of L. acidophilus in dahi and yogurt resulted in a significant increase in their antibacterial activity against the test cultures.

The mean inhibition zone exerted by control dahi against E. aerogenous, M. flavous, E. coli and S. aureus were 10.025, 8.875, 8.525 and 9.388 respectively. It did not showed any inhibition against B. cereus. The inhibition shown by the treatment dahi against the test organisms were significantly higher than that of control dahi. The mean zones of inhibition shown by treatment dahi against E. aerogenous, M. flavous, E. coli, S. aureus and B. cereus were 12.762, 13.563, 15.325, 12.075 and 13.1 respectively.

The high antibacterial activity of treatment dahi samples might have been due to the inhibitory substances produced by L. acidophilus along with the dahi culture organisms.

The control yogurt exhibited a mean inhibition zone, of 9.262, and 8.088 against E. aerogenous and E. coli respectively. It failed to show inhibition against M. flavous, S. aureus and B. cereus. The treatment yogurt exerted significantly high antibacterial activity when compared to the control. The mean inhibition zones against E. aerogenous, M. flavous, E. coli, S. aureus and B. cereus were 11.925, 10.0, 13.487, 10.125 and 9.35 respectively. The elevated antibacterial activity in treatment yogurt may be due to the combined effect of L. acidophilus and yogurt cultures in the production of bacteriocins and other antibacterial substances.

All the lactic cultures used in the study grow satisfactorily in the MRS-broth with out Oxgall. But in the presence of 0.3 per cent Oxgall, S. salivarius ssp. thermophilus, Lac. lactis and Lac. lactis ssp. diacetylactis failed to grow. The L. delbrueckii ssp. bulgaricus showed a poor growth. It reached only an optical density of 0.11 even after six hours of incubation. However L. acidophilus was able to grow satisfactorily in the presence of 0.3 per cent

oxgall. It took a mean time of three hours and 56 minutes to reach the desired optical density.

The dahi and yogurt, incorporated with L. acidophilus showed a significant hypocholesteremia in rats, in the feeding trials, when compared to the rats fed with control dahi and yogurt.

The mean serum total cholesterol level of control dahi fed rats was 94.533 mg/100 ml. The value was significantly low in the treatment dahi fed rats. The mean was 75.533 mg/100 ml. The reduction may be due to the assimilation of cholesterol by the L. acidophilus.

The rats fed with control yogurt showed a mean total serum cholesterol level of 84.35 mg/100 ml. The level was significantly reduced to a mean of 73.50 mg/100 ml in treatment yogurt fed rats. The reduction might have been produced by the combined effect of L. acidophilus and yogurt cultures.

The mean serum triglycerides of control dahi fed rats was 101.75 mg/100 ml. The level was significantly reduced in the treatment dahi fed rat groups to a mean of 84.1 mg/100 ml. The hypolipidemic factors of the L. acidophilus might have contributed to this reduction in serum triglyceride levels of treatment yogurt fed rats.

The mean serum triglyceride level of control yogurt fed rat group was 82.633. The level was reduced to a mean of 59.967 mg/100 ml in the treatment yogurt fed rats. The reduction in the serum triglyceride levels of treatment yogurt fed rat groups may be attributed to the combined effects of L. acidophilus and yogurt culture organisms.

The rats under dahi control group had a mean HDL-cholesterol of 13.45 mg/100 ml. The level was significantly increased to a mean of 14.833 mg/100 ml. The elevation may be due to the influence of L. acidophilus.

The mean HDL-cholesterol levels of control yogurt fed rat group was 16.567 mg/100 ml. The level showed a significant increase in treatment yogurt fed rat group. The mean was 17.617 mg/100 ml. A combination of effects would have caused this elevation. The L. acidophilus and yogurt culture organisms along with the milk protein would have bringforth the increased HDL-cholesterol level in treatment yogurt fed rat groups.

The LDL-cholesterol level of both dahi and yogurt treatments showed a significant decline, when compared to the respective control fed rat groups. The control dahi fed rats showed a mean LDL-Cholesterol level of 60.767 mg/100 ml. There was a significant reduction in the level of LDL

cholesterol of treatment dahı fed rat groups wıth a mean of 44.433 mg/100 ml. Lıkewıse the mean value ın control yogurt fed group was 51.267 mg /100 ml, wıth a sıgnıfıcant reductıon ın treatment yogurt fed rat group, havıng a mean of 44.283 mg/100 ml. The decrease ın the LDL ın both the treatments may be due to the hypocholesteremıc substances produced by the L. acıdophılus as well as normal dahı or yogurt cultures as the case may be. The mean cardıac rısk factor of rats fed wıth control dahı was 7.072. The value sıgnıfıcantly reduced ın the treatment dahı fed rats, wıth a mean of 5.115. The reductıon may be due to two factors. One, an ıncrease ın the HDL-Cholesterol level, and the second beıng the reductıon ın total serum cholesterol level. The mean cardıac rısk factor for control yogurt fed rats were 5.108. There was a sıgnıfıcant decrease ın the rats fed wıth treatment yogurt. The mean was 4.197. The reductıon ın total serum cholesterol level and ıncrease ın HDL-Cholesterol level of treatment yogurt fed rat groups may be the reason for the decreased cardıac rısk factor. The above saıd effects mıght have brought out by the combıned actıon of L. acıdophılus and yogurt culture organısms.

The rats fed wıth control dahı showed a mean growth rate of 0.622 g/day. The rate was 0.488 g/day ın treatment dahı fed rats. The hıgh growth rate ın the control dahı fed

rats may be due to the high bile sensitivity of dahi cultures, thus making cell protein available for digestion in the intestinal tract of rats. The mean growth rate of rats fed with control yogurt was 0.515 g/day. There was an apparent increase in the mean growth rate of treatment yogurt fed rats with a mean of 0.542 g/day. The increase may be due to the ability of L. acidophilus to colonise in the intestine and suppress the pathogenic microflora of the gut.

From the results of the experiments it was concluded that dahi and yogurt could be a good vehicle for L. acidophilus to enhance the therapeutic properties in terms of improved lactose digestion and hypocholesteremic conditions. The antibacterial activities against some common pathogens were also found to be increased by the presence of L. acidophilus in these products. Since the tested strain of L. acidophilus was found to be bile resistant it may help the organism to colonise in the gastrointestinal tract, and thus prolonging the beneficial effects.

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Lactobacillus acidophilus AS A DIETARY ADJUNCT IN *Dahi* AND YOGURT

By

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ABSTRACT OF A THESIS

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ABSTRACT

An experiment was conducted to study the beneficial effect of incorporating L. acidophilus in dahi and yogurt as a dietary adjunct. An attempt was also made to find out the bile tolerance of L. acidophilus and other lactic acid bacteria.

An exhaustive review of literature has been presented on the issues of lactose intolerance and hypercholesteremia and the beneficial effects of lactic acid bacteria in alleviating these drawbacks, with a special emphasis on bile tolerance and intestinal colonisation.

The methods of analysis of some important components of dahi and yogurt have been detailed.

Treatment dahi was prepared by inoculation with L. acidophilus in addition to normal dahi cultures. This was compared with control dahi prepared with normal cultures alone.

Treatment yogurt was prepared with inoculating L. acidophilus along with normal yogurt cultures. This was compared with control yogurt prepared using normal yogurt cultures. The samples were then analysed for various parameters.

There was an increase in the β-galactosidase specific activity of treatment dahi when compared to the control dahi. But in the case of yogurt, the treatment yogurt was having a low β-galactosidase specific activity when compared to the control yogurt.

Control dahi showed inhibition against E. aerogenous, M. falvous, E. coli and S. aureus. It did not showed any inhibition against B. cereus. Treatment dahi exerted a significantly high inhibition zone against all the test organisms in comparison to control dahi. Control yogurt inhibited only E. aerogenous and E. coli. Treatment yogurt exerted a significantly high antibacterial activity against all the organisms tested. Of, all the lactic acid bacteria tested for their ability to grow in the presence of 0.3 per cent of Oxgall, only L. acidophilus grew satisfactorily. L. delbrueckii ssp bulgaricus showed a poor growth, whereas S. salivarius ssp thermophilus Lac. lactis and Lac. lactis ssp diacetylactis failed to grow in the presence of Oxgall.

Both the dahi and yogurt treatments showed higher hypocholesteremia when compared to their respective controls. The total serum cholesterol level, serum triglyceride, LDL-Cholesterol and cardiac risk factor of the treatment groups were significantly lower than the respective controls. The

HDL-Cholesterol was high in both the treatments when compared to the respective controls.

The growth rate of treatment dahi group was low when compared to the control dahi group. But the treatment yogurt group showed a higher growth rate as compared to the rats fed on control yogurt.

From the above study the following conclusions were made.

1. The incorporation of L. acidophilus in dahi, increases its β-galactosidase specific activity. However, in yogurt it resulted in a decreased β-galactosidase specific activity when compared to normal yogurt.

2. In both dahi and yogurt, the incorporation of L. acidophilus resulted in an elevated antibacterial activity against E. aerogenous, M. falvous, E. coli, S. aureus and B. cereus.

3. L. acidophilus was able to grow satisfactorily in the presence of Oxgall. While the other lactic acid bacteria tested either showed a poor growth or no growth at all in the presence of Oxgall.

4. Rats fed with dahi and yogurt incorporated with L. acidophilus showed a lower serum total cholesterol, serum triglyceride, and LDL-Cholesterol levels, when compared to the control groups.

5. The HDL-Cholesterol level was high in both the treatment dahi and treatment yogurt fed rat groups.

6. The treatment dahi and treatment yogurt fed rats showed a significant decrease in the cardiac risk factor when compared to their respective controls.

7. The treatment dahi showed a lower growth rate, and treatment yogurt showed a high growth rate when compared to their controls.