Nutritional and Therapeutic aspects

Fermented milk products have been used therapeutically in the ancient systems of medicine in the Near and Middle East for centuries. However, the nutritional and therapeutic value of lactic acid organisms is still controversial. Several preclinical and clinical studies have been performed, showing that fermentation of food with lactobacilli increases the quantity, availability, digestibility and assimilability of nutrients. A number of studies also show that ingestion of preparations containing lactobacilli lowers the serum cholesterol level in humans and animals. Fermented dairy products have also been claimed to inhibit tumor proliferation and to enhance the immune functions. Fermented dairy products and lyophilized lactobacilli preparations have also proved to be useful in the prevention and treatment of gastrointestinal disorders such as constipation and infections such as salmonellosis, shigellosis and antibiotic-induced diarrhea. Lactobacillus GG has been useful in the treatment of recurring diarrhea caused by toxin produced by Clostridium difficile. A preparation of Lactobacillus brevis has been found to be effective in the treatment of recurrent headache. Lactobacilli have also been successfully used as adjuvants in the treatment of fungal and protozoal vaginitis and in the treatment of non-specific bacterial vaginitis. The nutritional and therapeutic benefits of lactobacilli are summarized in Figure 2.1.

Nutritional and Therapeutic Benefits of Lactobacilli

Nutritional benefits
- Vitamins B production
- Improved digestibility of food components and enhanced bioavailability of nutrients.

Therapeutic benefits
- Restoration of the ecological balance of intestinal microflora
- Alleviation of lactose intolerance
- Enhancement of immunity
- Detoxification of harmful products
- Elimination of carcinogenic end products
- Suppression of food-borne pathogens

Figure 2.1

Nutritional Benefits:
Studies on rats have shown improved growth rate and increased feed efficiency when the rats were fed with yogurt containing lactobacilli22. Improved feed efficiency in rabbits fed diets supplemented with *L. sporogenes* has been reported.27 Although several lactobacilli require B-vitamins for growth, some of these organisms are capable of synthesizing B-vitamins16. The levels of some of the B-vitamins in yogurt are shown in the Figures 2.3 (a,b).28 Similarly, bioavailability of copper, iron, calcium, zinc, manganese and phosphorus was increased in yogurt fed rats 4.

**Figure 2.2(a)**

**Figure 2.2(b)**

**Therapeutic Benefits**
Earlier research on indigenous microflora in animals and humans has shown their host-specificity and location-specificity, complexity in composition and their beneficial effects on the hosts. The important effects of probiotics as described in recent literature are summarized in Table 2.3:

**CLAIMED APPLICATIONS OF PROBIOTICS IN ANIMALS AND HUMANS**

<table>
<thead>
<tr>
<th>Applications In Animals</th>
<th>Applications In Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disturbance of indigenous microflora due to non-infectious disbacteriosis, antibiotic therapy, stress</td>
<td>Disturbance of indigenous microflora due to non-infectious disbacteriosis, antibiotic therapy, radiation therapy</td>
</tr>
<tr>
<td>Health Promotion:</td>
<td></td>
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</tbody>
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Table 2.3

Preparations containing lactobacilli have been shown to be effective in the treatment of a variety of disorders and infections including colitis, constipation, diarrhea, recolonization of the intestine with pathogens after treatment with antibiotics, flatulence, acidity, hepatic encephalopathy, tumorogenesis, hypercholesterolemia, headache and vaginitis.

### 1. Hypercholesterolemia

Coronary heart disease is often related to elevated serum cholesterol levels. Preclinical studies with laboratory rats fed fermented milk mixed with animal feed showed lower serum cholesterol levels as compared to rats fed with skim milk-supplemented feed. Pulusani and Rao showed that this difference could not be attributed to simple redistribution of cholesterol between the plasma, liver and other body pools. Another study showed that the consumption of L. acidophilus fermented milk supplemented with a commercial feed lowered serum cholesterol levels in weanling rats. In a study by Gilliland et al., pigs were fed a high cholesterol diet which increased their serum cholesterol levels. When these animals were simultaneously fed L. acidophilus, the rise in serum cholesterol levels was inhibited. Since in vitro studies showed that the organism assimilated cholesterol from the culture medium, the authors concluded that the Lactobacillus bound cholesterol to the intestinal lumen, thereby reducing its absorption into the blood stream. Compounds such as orotic acid, lactose, casein, and hydroxymethyl glutaric acid have been suggested to be hypocholesterolemic factors. Yogurt was also found to reduce dietary
cholesterol-induced hypercholesterolemia in rabbits. Administration of L. sporogenes to rabbits fed high cholesterol diets to increase the serum cholesterol level resulted in 90% inhibition of rise in serum cholesterol. L. sporogenes in in vitro studies was found to assimilate cholesterol from the culture medium, suggesting that this organism could assimilate cholesterol directly from the gastrointestinal tract.

Studies by Hepner et al. on healthy human volunteers (with no history of cardiovascular disease) showed that dietary supplementation with yogurt decreased serum cholesterol. Mann and Spoerig surmised from clinical trials that the lower serum cholesterol levels in individuals from the Masai tribe of Africa could be attributed to high consumption levels of fermented milk.

**Mechanism of action:**

Emulsification of dietary fat is an intermediate process in fat absorption. Bile salts together with phospholipids and cholesterol form micelles which helps in the absorption of cholesterol. Lactobacilli deconjugate the bile salts in the intestine to form bile acids and thereby inhibit micelle formation. This leads to decreased absorption of cholesterol. Cholesterol entering the intestine through the enterohepatic circulation is similarly treated. Lactobacilli elaborate the enzyme conjugated bile acid hydrolase (CBH), which hydrolyzes bile salts, and hydroxy steroid dehydrogenase (HSDH) which degrades bile acids and interrupts the enterohepatic circulation of bile acids. Another factor thought to be elaborated by lactobacilli is hydroxy methyl glutarate CoA (HMG CoA) which inhibits HMG CoA reductase, the rate limiting enzyme in endogenous cholesterol synthesis. All these factors collectively contribute to the hypocholesterolemic effects of lactobacilli as illustrated in Figure 2.3.
2. Lactose intolerance:

Individuals with deficiency of the enzyme b-galactosidase (lactase) suffer from abdominal distress when they consume milk or dairy products. These individuals can, however, tolerate yogurt, as more than 50% of the lactose in yogurt is converted into lactic acid by the starter cultures during fermentation. The enzyme can also be released from the organism in the gastrointestinal tract of the consumer and bacterial lactase would be present in the intestine after consumption of the yogurt.

Alm monitored the increase in serum glucose (derived from lactose) in control subjects and in lactose intolerant subjects who were given a 500 ml. dose of milk or yogurt. When given milk, the lactose-intolerant subjects had a much lower rise in serum glucose as compared to the controls. This difference was marginal in the case of yogurt. In a separate study, it was observed that administration of fermented acidophilus milk markedly decreased the breath hydrogen level in lactose-intolerant subjects when compared with the high breath hydrogen levels when taking unfermented milk. Another researcher noted that lactose-intolerant subjects given 18 grams of lactose in yogurt had only about one-third as much hydrogen excretion as in the case of the same amount of lactose in milk or water. There was significant lactase activity in the intestine one hour after ingestion of yogurt. Lactobacillus sporogenes was found to possess considerable b-galactosidase activity when tested in vitro. In vivo studies on the effect of yogurt and fermented milks on lactose digestion have been performed. The results revealed that all samples of yogurt tested dramatically and similarly improved lactose digestion regardless of their b-galactosidase activity. The response to fermented milks varied from marginal improvement with B. bifidus milk to nearly complete lactose digestion with L. bulgaricus milk.

Mechanism of action:

Lactobacilli provide the enzyme b-galactosidase which hydrolyzes lactose. The hydrolyzed lactose is converted to lactic acid as explained in an earlier section describing the metabolism of lactose in lactic acid bacteria.

3. Hepatic encephalopathy:

Hepatic encephalopathy is a neurologic disorder associated with liver failure and elevated blood ammonia levels. The enzyme urease from intestinal proteolytic bacteria acts on amino acids, urea and other nitrogenous compounds leading to the production of ammonia. Under normal circumstances ammonia is absorbed and detoxified in the liver. However, in patients suffering from liver failure, the detoxification mechanism is impaired and the ammonia levels rise in the circulating blood. This depresses the nerve functions leading to hepatic coma and precoma. L. acidophilus was found to be effective in decreasing fecal urease level. The use of lactobacilli in patients on long term treatment with neosporin produced an improvement in EEG and clinical status of 71% of these patients and a fall in blood ammonia in 60% of the patients tested.
Mechanism of action:

Lactobacilli produce lactic acid and other substances creating a gastrointestinal environment which is not conducive for the growth of putrefactive organisms. This results in lower intestinal urease levels and consequently lower blood ammonia levels. In addition, the low pH due to lactic acid production disfavors the absorption of ammonia from the gut into the tissues and facilitates excretion of ammonia from the blood into the gut. This explains the usefulness of lactobacilli in the treatment of hepatic encephalopathy.

4. Carcinogenesis

Several preclinical and clinical trials have shown that fermented dairy products or the starter cultures used in their manufacture inhibit transplantable animal and human tumor lines.

These anti-tumor properties are based on:

1. Inactivation or inhibition of carcinogenic compounds produced in the gastro-intestinal tract by specific microorganisms.

2. Stimulation or enhancement of the immune response

3. Reduction of intestinal bacterial enzyme activities: Some of these enzymes may convert procarcinogens into carcinogens.

Friend et al.45 investigated the inhibitory effect of yogurt on the proliferation of Ehrlich ascites tumor cells in male Swiss mice. They observed that feeding yogurt resulted in a 28% to 35% reduction of tumor cells when compared to control groups fed milk. DNA synthesis in the tumor line of animals receiving yogurt was only 75% of that found in animals fed a commercial diet. Subsequently, Reddy et al. determined the antitumor effects of various yogurt components. Shahani et al. reported that feeding milk and colostrum fermented with L. acidophilus resulted in 16% to 41% reduction in tumor proliferation.

In an earlier study, Bogdanov et al. observed that L. bulgaricus possessed potent antitumor activity. They isolated three glycopeptides which showed biological activity against sarcoma-180 and solid Ehrlich ascites tumor. In the etiology of colon cancer, the conversion of procarcinogens to carcinogenic compounds by intestinal bacterial enzymes plays an important role, a phenomenon observed in predominantly meat-eating populations. These enzymes are β-glucuronidase, azoreductase and nitroreductase. One group of researchers found a marked decrease in the levels of these enzymes in rats fed diets orally supplemented with L. acidophilus. These studies were extended in an animal model of colon cancer induced by the chemical carcinogen 1,2 dimethyl hydrazine (DMH). This compound is activated into a proximate carcinogen in the large bowel by the β-glucuronidase produced by intestinal flora50. DMH-treated animals were given L. acidophilus in powdered form and compared with controls. At 20 weeks, 40% of the L. acidophilus- treated animals had tumors as against 77% of the control.
animals (P < 0.2) while at 36 weeks, 73% of the L. acidophilus animals and 83% of the control animals had tumors.

These results show that L. acidophilus can prolong the induction of colon tumors. In a more recent study4, the same authors found that oral L. acidophilus supplementation to the diet in rats lowered the amount of carcinogenic amines excreted in the feces after feeding procarcinogen precursors to these animals. The evidence to date suggests that lactobacilli may slow tumor development in laboratory animals. However, there is no conclusive evidence to suggest that lactobacilli or their fermented products can prevent cancer in humans.

**Mechanism of action:**

Putrefactive colonic microflora produce the enzymes b- glucuronidase, azoreductase and nitroreductase which convert procarcinogens to carcinogens. Lactobacilli, by competitive inhibition and the production of non-conducive acidic environment, suppress the metabolic activity of colonic microflora and in this manner may reduce the formation of carcinogens in the large intestine.

Lactic acid bacteria suppress carcinogen-induced mutations. In vitro studies with mutagens such as 4-nitroquinoline-N-oxide revealed that the bacterial cells themselves as well as their metabolites possessed anti-mutagenic action. It has been suggested that some bacterial cell wall fractions possess anti-mutagenic activity.

5. Intestinal Infection

A variety of studies have examined the proposition that lactobacilli and dairy products fermented with lactobacilli can alleviate gastrointestinal disorders. The results however, are inconsistent, probably due to differences in Lactobacillus strains used as well as variation in conditions of preparation and storage of cultures or fermented products. Results showing significant amelioration of diarrheal symptoms of salmonellosis in weanling rats; salmonellosis and shigellosis in children have been reported. Antibiotic-induced diarrhea was prevented by use of Lactobacillus. The Lactobacillus species used in these studies were L. acidophilus and/or L. bulgaricus. Administration of another strain of Lactobacillus, which produces a broad spectrum bacteriocin, Lactobacillus GG, helped relieve symptoms of relapsing Clostridium difficile colitis resulting from antibiotic treatment for an infection.

Lactobacilli, particularly L. acidophilus and L. Sporogenes* have also been used in the treatment of chronic constipation and flatulence.

**Mechanism of action:**

Lactobacilli, through the production of lactic acid and bacteriocins create an intestinal environment which is not conducive for the growth of pathogens. Lactic acid also helps relieve constipation by improving the bowel movement.
6. Immune response system:

Antibodies against intestinal bacteria are commonly detected in healthy humans. These antibodies are produced when the host is stimulated by the antigens of the intestinal bacteria. Defense tissues such as the thymus, lymph nodes, spleen and bone marrow are well developed in conventional mice, but poorly developed in germ free mice, indicating the influence of intestinal flora on the host's immune response. In more detailed studies with germ-free animals that were fed yogurt, an increase in the levels of immunoglobulins, IgG1, IgG2a, IgG2b and IgM were detected in the serum.

Recent studies have shown that Lactobacillus brevis sub-species coagulans, may enhance the body's capacity to produce alpha interferon, natural killer (NK) cell activity and 2-5 A-synthase enzyme activity, each important aspects of the body’s natural defenses. Scientists at the Institut Pasteur de Kyoto showed that when 10 healthy adults consumed this bacterial supplement, their average producing capacity of alpha interferon increased 65% after two weeks and 59% after four weeks. In the same time frame, natural killer cell activity increased 68% and 47%, as shown in Figure 2.4.

![Figure 2.4](image)

The US Food and Drug Administration has approved alpha interferon for use in treating certain types of cancer, hepatitis and genital warts.

7. Treatment of recurring headaches:

In many patients suffering from various forms of migraine headache, prophylaxis by antiserotonin agents, although successful in 60%-70% of the cases, may result in the serious complication of retroperitoneal fibrosis. The use of orally administered capsules containing Lactobacillus acidophilus was tried by one group of researchers in a series of 20 patients. Of 16 patients followed up over a period of 1-2 months, 9 reported complete relief or considerable reduction in frequency of attacks. In three others, in whom the frequency did not decrease, there was definite reduction in the severity of the headaches. No adverse symptoms were noted.
8. Treatment of aphthous stomatitis and glossitis:

Lactobacilli have been effectively employed in treatment of aphthous stomatitis and glossitis. These conditions arise due to imbalance in intestinal flora resulting in B vitamins deficiency. The administration of lactobacilli to colonize the gut can cure this condition.

9. Treatment of vaginitis:

Vaginal infections can be caused by a variety of organisms of which Trichomonas vaginalis, a protozoan parasite, and Candida albicans, a yeast-like fungus are the chief non-bacterial organisms responsible. No single bacterial species is responsible for vaginal infection and hence this type of infection is called non-specific vaginitis (NSV). Symptoms include a gray vaginal discharge, an unpleasant amine smell and "clue" cells, viz., vaginal epithelial cells coated with Gram-variable bacteria. NSV is the most frequent type of vaginal infection. A notable symptom is a significant reduction in the numbers of lactobacilli present and a proliferation of other bacteria in the vaginal environment, including Gardnerella vaginalis and anaerobes.

Lactobacilli are natural inhabitants of the vaginal mucosa. The predominant species is L. acidophilus. Lactobacilli maintain the vaginal pH in the range of 4.0-4.5 through glycogen fermentation to lactic acid. This establishes an environment unfavorable for the growth of pathogens. The level of glycogen in the epithelial cells of the vagina is controlled by circulating estrogens.

Prophylaxis by oral administration of L. acidophilus as well as treatment by intravaginal application of lactobacilli have proved to be effective in the treatment of vaginitis. A commercial formulation using L. sporogenes*, trade marked MYCONIP®, is successfully marketed for this indication.

10. As adjuvant to antibiotic treatment:

The microecological balance of the gut flora is disturbed by treatment with antibiotics. Some of the beneficial flora are killed and on stopping treatment, pathogens begin to re-establish themselves in the intestine. Overgrowth of these organisms and the subsequent invasion of the system by yeast like Candida albicans cause inflammatory, immunologic, neurologic and endocrinologic problems. This occurs due to proliferation and toxin production by these organisms in the host tissues. Administering lactobacilli along with antibiotics helps to prevent this syndrome. The lactobacilli through their metabolic activities establish themselves in the gut, vaginal or oral environment and provide conditions which are non-conducive to the growth of pathogens. Lactobacillus therapy is essential after treatment with anti-amebic drugs.

Drugs such as estrogens and oral contraceptives, if administered during antibiotic treatment, have a significant failure rate. If lactobacilli are administered concurrently, they provide essential intestinal microflora which can correct this situation, by deconjugating drug complexes and keeping the drug in circulation.
11. Growth-promoting effect of probiotics:

Lactobacilli have been recommended for veterinary use, being effective in restoring the gastrointestinal microecological balance and helping in the establishment of healthy rumen flora. This in turn results in improved health and growth of farm animals.

**Mechanism of action:**

By reducing the intestinal ammonia concentration and by preventing intestinal infections caused by putrefactive organisms, lactobacilli, particularly L. sporogenes* are effective growth promoters for chicks and domestic animals. This explains the use of probiotics in animal and poultry feeds. In experimental trials with probiotics, it has been found that the effectiveness of treatment depends upon factors such as type, viability and composition of the implanted lactobacilli; type of dosing; and type and age of the recipient animal.

12. Anti-HIV activity of lactic acid bacteria:

A journal article69 hypothesizes that live Lactobacillus cultures may be used therapeutically in patients suffering from AIDS. This hypothesis is based on the enhancement of antimicrobial resistance, immunomodulatory action and anabolic effect caused by the consumption of live lactobacteria, as described in earlier sections of this review.

* The taxonomical classification was revised in 1939 in the seventh edition of the Bergey’s Manual of Determinative Bacteriology to B. coagulans, although some researchers continued to use the original name.