CNI601 12/97
CLINICAL NUTRITION INSIGHTS

Vol. 6, No. 1

Choosing a Probiotic Supplement

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ABSTRACT: *Probiotics have been used for many years to aid in restoring and maintaining a healthy intestinal balance in favor of healthful bacteria, which is essential in maintaining good health. Probiotics are organisms or supportive substances that improve intestinal microbial balance, and include* Lactobacillus acidophilus, *bifidobacteria, and bioactive proteins such as immunoglobulin A and lactoferrin. A major consideration in the choice of probiotics is to choose a strain that can survive and establish itself under the conditions encountered in the intestinal environment. Therefore, when choosing a probiotic supplement the healthcare professional must consider those qualities and characteristics essential to establishment and activity of* L. acidophilus *in the intestinal environment, such as bile resistance and intestinal adherence. The* L. acidophilus *NCFM™ strain has been thoroughly researched and is recognized for its ability to survive in the presence of bile or stomach acid and adhere to the intestinal mucosa. In addition, studies have demonstrated many beneficial effects of the* L. acidophilus *NCFM strain, such as cholesterol assimilation and reduction of pro-carcinogenic fecal bacterial enzyme activity.*

INTESTINAL MICROFLORA

The human intestinal microflora is complex, with total counts of 10^{11} - 10^{12} bacteria per gram of stool.[1] Among this vast number of organisms are at least 500 species of anaerobes and many facultative organisms, within which are several species of lactobacilli and bifidobacteria.[1] This intestinal microflora is a highly active society of organisms, possessing a diverse complex of enzymes that perform extremely varied functions, both beneficial and detrimental. The delicate, yet critical, balance maintained among this enormous bacterial population plays an important role in maintaining not only intestinal health, but the overall health of the patient.

THE USE OF PROBIOTICS TO PROMOTE INTESTINAL BALANCE

Probiotics are viable organisms and supportive substances that improve intestinal microbial balance, such as *Lactobacillus acidophilus* and bioactive proteins.[2] The empirical evidence that, for many years, linked the use of fermented dairy products such as yogurt and milk with the promotion of intestinal health is today well supported by modern science. The ability of the probiotic *L. acidophilus* to help prevent pathogenic bacteria from proliferating and healthy bacteria from becoming toxic is well documented.[3-8] When the proper strain is chosen, it may help to maintain a population equilibrium, or balance, between the different forms of microorganisms, curtailing their potential overgrowth and pathogenicity.[8-13]

Bifidobacteria is another probiotic naturally occurring in the human intestine, with *Bifidobacterium infantis* being the first flora to colonize the intestines of newborns. Research studies have documented several beneficial effects of bifidobacteria when given to infants, such as its effectiveness against a specific strain of enteropathogenic *Escherichia coli*,[14] in prevention of enteric infections,[15] and in decreasing the growth of *Candida albicans*.[16,17]

What is it that enables *L. acidophilus* and bifidobacteria to help maintain the proper balance between the different forms of microorganisms in the intestine? They produce organic acids that reduce intestinal pH and thereby inhibit the growth of acid-sensitive bacteria, including many pathogenic species. Lactobacilli, which are frequently more acid tolerant than other organisms, produce lactic acid, hydrogen peroxide, and possibly acetic and benzoic acids.[18] Acids produced by bifidobacteria include short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids, as well as lactic and formic acids.[19,20] At optimal pH values they exert several inhibitory influences on bacterial cell growth.[21,22] The most plentiful SCFA produced by bifidobacteria is acetic acid, which exerts a wide range of antimicrobial activity against yeasts and molds as well as bacteria.[20,21]

In addition to lactic and other acids, lactobacilli have the capacity to secrete numerous metabolites, or endotoxins, that kill pathogenic bacteria.[11,23-27] A variety of antibacterial/anti-yeast substances have been isolated such as lactocidin, lactobicillin, lactobreven, and acidolin.[10,11,23,28] Because these substances are difficult to isolate and stabilize, their value can best be obtained through the administration of those strains known to secrete these agents as a part of their life cycle.[28]

THE NCFM™ STRAIN OF *L. acidophilus*

A great deal has been learned in the last few decades through intense study on many different strains of *L. acidophilus*, bifidobacteria, and other forms of healthful microorganisms. Because probiotic strains

vary greatly, their impact hinges upon the specificity of the strains that are used and the method of culturing, packaging, and handling of the product.[18] The *L. acidophilus* NCFM strain, developed at North Carolina State University, is perhaps the most extensively researched *L. acidophilus* strain available. A multitude of research studies conducted at leading universities have been published documenting the many beneficial properties of the NCFM strain.[1,29,31-52]

A number of factors should be considered when selecting a culture of *L. acidophilus*. Because cultures exhibit host specificity, it is desirable to select an organism originating from the human intestinal tract.[29] The NCFM strain is one that has been isolated from the human fecal flora. In addition, DNA studies have revealed great genetic variability within strains that are designated as *L. acidophilus*.[30] Out of several groups of the organism, researchers determined that only strains from the A1 homology group, which includes the NCFM strain, should be classified as true *L. acidophilus*.

Additional factors that are important in selecting a strain that is able to function by surviving and growing in the intestinal tract include bile tolerance, bacteriocin production, and ability to adhere to the intestinal wall. The NCFM strain has been shown to not only meet the strict criteria of purity and viability, but also to survive and implant in the gut and produce beneficial effects.[1,29,31-52]

• Intestinal Adherence

The primary objective of supplying *L. acidophilus* is to establish the bacterium in the intestinal tract. The stomach and intestinal tract represent a hostile environment that can easily discourage growth or survival of microorganisms. Although many lactobacilli survive selective pressures of this environment, flow rates of digesta through the small intestine would wash out any organism unable either to multiply rapidly enough to avoid dilution or to maintain their residence by physical attachment to the intestinal epithelium.[31]

According to one researcher, many lactobacilli products do not appear to live up to claims regarding human gastrointestinal adherence.[18] Only a limited number of strains have actually been shown *in vitro* to adhere, including the NCFM strain. The ability of the NCFM strain to adhere to the intestinal wall has been demonstrated in several *in vitro* studies utilizing human intestinal cells.[31-33] An *in vivo* study has shown that the NCFM strain, when consumed in milk, is capable of surviving and implanting in the intestinal tract of humans.[34] The researchers determined this by analyzing the fecal flora of healthy males after consuming non-fermented milk containing the NCFM strain of *L. acidophilus*. Highly significant increases in bile-resistant facultative lactobacilli were seen in the group consuming *L. acidophilus*, indicating successful implantation. These numbers decreased after the milk feeding was stopped, although most subjects retained considerably higher counts than they had before feedings.

• Bile Tolerance

Bile tolerance is considered to be an important characteristic of *L. acidophilus* that enables it to survive, grow, and exert its action in the small intestine.[35] Strains that are able to grow and metabolize in the presence of physiological levels of bile should logically be more likely to survive intestinal transit.[36] Although the degree of bile tolerance required for maximum growth of the organism in the intestinal tract is not known, it is important to select one having a high degree of bile resistance.[29] There is a wide range in the ability of cultures to grow in the presence of bile.[29] Studies have shown that the NCFM strain is capable of growing in bile concentrations of up to 3%[29,35-38] and an *in vivo* study demonstrated that the NCFM strain survives in the presence of human gastric juice.[32]

• Bacteriocin Production

Suppression of undesirable intestinal bacteria through antimicrobial action continues to be a major benefit attributed to *L. acidophilus.* This intestinal antagonism is dependent on intrinsic properties of the lactobacilli that enable them to compete for, and maintain, their residence in the intestinal environment. Therefore, the ability to produce effective antimicrobial substances becomes an important consideration when selecting strains.[39] Broad-spectrum inhibition has been clearly demonstrated for organic acids and hydrogen peroxide produced by *L. acidophilus*, including the NCFM strain.[18,40]

Bactericidal proteins with a more specific spectrum of antagonistic activity, termed bacteriocins, are produced by some strains of *L. acidophilus*. The bacteriocins of *Lactobacillus* species usually prove inhibitory to bacteria that are closely related to the producer strain or that compete for the same ecological niche.[29,41,42] It is important to select a culture of *L. acidophilus* that will be able to compete and grow well in the presence of similar bacteria.[29] Some studies have shown the NCFM strain exhibits significant bacteriocin activity against closely related *Lactobacillus* species, which provides it with an advantage in being able to establish and grow in the intestinal tract.[29,40,43] Researchers have even isolated and studied a specific bacteriocin produced by the NCFM strain, called lactacin B, which is active against closely related lactobacilli such as *L. bulgaricus* and *L. helveticus*.[40,44]

Because many bacteriocins have been identified for lactobacilli, the practice of mixing *L. acidophilus* products together with other species groups (i.e., *L. bulgaricus, L. leichmannii*) may hinder therapeutic efforts due to significant bacteriogenic activity by these species groupings.[18,41,42] By combining various strains together, as in a broad-spectrum probiotic, the beneficial strains may be rendered innocuous.[18]

• Assimilation of Cholesterol

Several studies have reported that ingestion of *L. acidophilus* can result in decreased serum cholesterol levels in humans and animals.[35,45] Certain strains of *L. acidophilus* have the ability *in vitro* to assimilate cholesterol, as shown by the appearance of cholesterol in the cells during growth and decreases in the concentrations of cholesterol in the growth medium.[29,45] This uptake of cholesterol occurred only when the culture was growing anaerobically in the presence of bile. These conditions required in the *in vitro* system for cholesterol uptake by *L. acidophilus* would also be expected to occur in the human intestinal tract. Such assimilation of cholesterol in the small intestine may be important in reducing the absorption of dietary cholesterol from the digestive system into the blood.[35,45]

If the purpose in using the dietary culture is to provide a beneficial influence on serum cholesterol levels, the culture should very actively assimilate cholesterol during growth under conditions existing in the intestinal tract.[29] Data from one study showed wide variation in the ability to assimilate cholesterol among *L. acidophilus* cultures of human origin.[29] Among 13 isolates of *L. acidophilus*, the NCFM strain ranked first in cholesterol assimilation over 16 hours of growth.

• Fecal Enzyme Activity and Antimutagenicity

There is considerable interest in the metabolic activities of the intestinal microflora, especially in relation to the etiology of colon cancer.[46] Epidemiological studies indicate a correlation between regular consumption of fermented dairy products and low incidence of colon cancer.[1,46,47] To investigate this, several studies have involved measurements of fecal bacterial enzymes, such as ß-glucuronidase, nitroreductase, and azoreductase, which are known to catalyze reactions that convert procarcinogens to carcinogens.[1,46-48] In one study, oral administration of *L. acidophilus* NCFM to meat-fed rats substantially reduced the activities of these fecal bacterial enzymes.[47] Similarly, in a study with 7 human subjects, it was found that supplementing the diet with *L. acidophilus* NCFM for one month significantly reduced fecal ß-glucuronidase and nitroreductase activities.[1] In a larger study with 21 human subjects, reductions of 2- to 4-fold in the activities of the three fecal enzymes were observed during a 4-week period of *L. acidophilus* NCFM supplementation.[46] Whether these changes in bacterial enzyme activity directly affect colon cancer risk is a matter of speculation.

To investigate the role of *L. acidophilus* NCFM in prevention of chemically induced colon tumors in rats, two groups of rats were challenged with a colon cancer inducing agent.[49] The experimental group, which was fed a supplement of the *L. acidophilus* NCFM strain, showed a lower incidence of colon cancer after a 20-week induction period than the control group. This difference was not observed after 36 weeks, indicating that *L. acidophilus* may play a role in delaying the initiation of colon cancer.

• Small Bowel Bacterial Overgrowth

Small bowel bacterial overgrowth (SBBO), well known to occur in end-stage kidney failure, is responsible for producing toxic amines such as dimethylamine (DMA).[50] These toxic amines cause general chronic renal failure symptoms as well as target organ dysfunction, especially in the brain.[51] In a recent study, *L. acidophilus* NCFM was administered to hemodialysis patients to determine if supplementation could modify SBBO, thereby reducing the toxic levels of amines in the blood.[50] The patients treated with NCFM for at least one month showed significant reductions in serum DMA levels as well as levels of nitrosodimethylamine, a potent carcinogen. The researchers concluded that *L. acidophilus* NCFM changed small bowel pathobiology by modifying metabolic actions of SBBO, reducing generation of toxins and carcinogens with no adverse side effects.

VIABILITY ASSAYS

For *L. acidophilus* to establish and react within the intestinal environment, the bacteria must be in a viable condition. The viability of the selected strain can be determined through third-party laboratory analysis of the number of colony forming units (cfu) per unit weight (G) and bile resistance (oxgall bile test) for each batch produced. The laboratory assay results should be provided by the culture supplier, expressed as the ratio of bile resistant cfu/G to total cfu/G. A high ratio, above 90%, indicates high viability of the organism in the gastrointestinal tract.[18] The importance of this analysis cannot be understated, as a study conducted in 1990 suggests that there are serious problems associated with some commercial probiotic preparations.[53] Researchers analyzed 11 products claiming to contain *L. acidophilus* in powder, capsule, and tablet form for number and type of bacteria present. Only two of the products were found to contain *L. acidophilus*, while the remainder contained *L. casei*. Problems with culture viability and contamination with *Enterococcus* and *Clostridium* were also found.

In addition to selecting viable strains, the method of packaging and storing of the product is important in maintaining viability.[18] Temperature, moisture, light, and air can all adversely impact viability. These variables can be controlled through the use of amber glass containers to prevent entry of oxygen, moisture, and light. Most importantly, refrigeration of the product from the time of manufacture through delivery and storage is critical in ensuring the potency of the bacterial strains.

BIOACTIVE PROTEINS

Bioactive proteins are another class of supportive substances that qualify as probiotics because they may beneficially affect intestinal microbial balance. Secretory immunoglobulin A (IgA) is the predominant antibody, or immune protein, the body manufactures and releases in external secretions such as saliva, tears, and milk, and through the epithelial cells lining the intestine out into the lumen.[54,55] It plays a major role in the defense mechanism on the surface of the intestine by preventing the absorption of, and/or by disposing of, microbial antigens.[54,55] Mucosal IgA also neutralizes viruses and, in the case of bacterial infections, blocks the attachment of pathogens to mucosal tissues and cells.[54,55] Other immune proteins, such as IgM and especially IgG, may also be helpful because they are known to have remarkably similar specificities.[54,56]

The benefits of these bioactive immune proteins can be experienced when given orally, such as in mother's milk; however, human colostrum and milk are not the only source.[57] A concentrate of bioactive immune proteins from the milk of cows hyperimmunized with four types of human rotavirus showed neutralizing activities against all four types that were 100 times higher than that produced in human samples and 10 times higher than specific commercial samples.[57] Laboratory tests showed these bioactive proteins had powerful antiviral activity, even against very high doses of infectious rotavirus.

In human studies it has been shown that a concentrate of bioactive immune proteins from the milk of cows immunized with human rotavirus could provide passive immunity and prevent rotavirus gastroenteritis when added to an infant's diet.[57,58] Similar results have been achieved in infants and adults against enteropathogenic *E. coli*.[59,60]

Cows' milk is a rich source of IgG and other immune proteins. Because the functionality of certain types of IgA and IgG are similar, IgG from milk can be considered a valuable nutritional contribution to the health of the intestinal tract.[56]

• Lactoferrin and Lactoperoxidase

Lactoferrin is a bioactive protein that is similar to secretory IgA, is found in external secretions, and is very plentiful in milk.[61] An iron-binding protein, it has been speculated to play a role in the primary defense system against invading pathogenic organisms, probably by depriving them of iron.[62] Its effectiveness has been demonstrated against a variety of microorganisms, including *E. coli*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *S. aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, while it does not affect *Lactobacillus caseii* and actually may promote the growth of *Bifidobacterium*.[61-67]

Lactoperoxidase is another enzyme occurring in various secretions and is the second most prominent enzyme in bovine milk.[61,68] It has no antibacterial activity itself but forms, with hydrogen peroxide and thiocyanate, a potent natural antibacterial system known as the LP-system. The antimicrobial activity of the LP-system has been studied extensively, with a wide range of microorganisms being inhibited such as *Staphylococcus aureus*, *Campylobacter jejuni*, which is recognized as a cause of acute enteritis, *Campylobacter coli*, *Streptococcus* species, *Bacillus* species, *E. coli*, *Salmonella* species, and *Pseudomonas* species.[68-73] Some lactic acid bacteria are unaffected because they contain a "reversal enzyme," which prevents the antimicrobial activity of the LP-system.[68]

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