

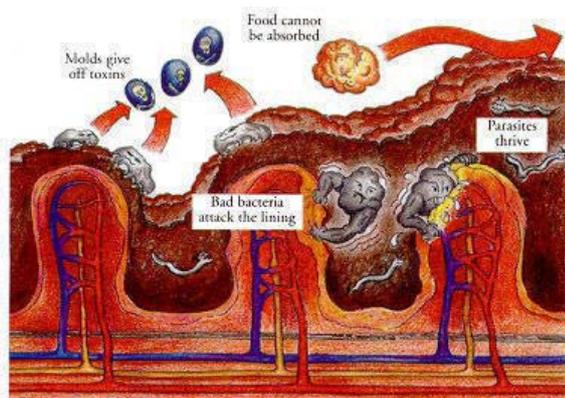
Controlling intestinal microflora with BioHealth™, MicroHealth™ and LactoHealth™

Probiotics are products aimed at delivering living bacterial cells to the guts ecosystem of humans and other animals, whereas prebiotics are non-digestible carbohydrates delivered in food to the large bowel to provide fermentable substrates for selected bacteria. Food scientists and nutritionists have accepted the concepts underlying the use of probiotics and prebiotics in the promotion of health. Knowledge of the gut microbiota has increased dramatically during the past decade thanks largely to the results obtained from the application of nucleic acid-based methodologies. Because of the availability of improved technologies, detailed studies of the two principal kinds of probiotic/prebiotic bacteria, members of the genera *Lactobacillus* and *Bifidobacterium*, can be made. While well-established scientists continue to make important contributions to probiotic/prebiotic

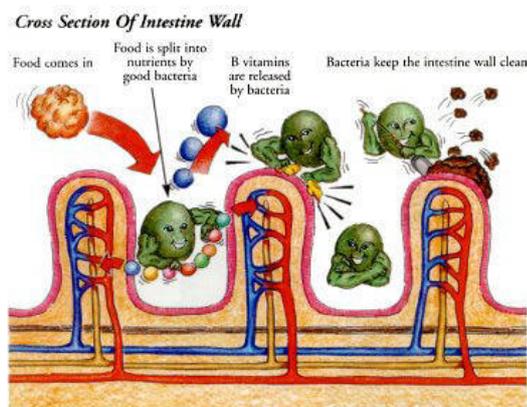
research, it is notable that younger scholars are playing a vital role in developing scientific concepts related to the field representing a state-of-the-art compendium of fundamental science related to early 21st century probiotic/prebiotic research.

The intestinal microbial flora of the animal host is a complex ecologic system that produces a significant impact on the host. Aerobic and anaerobic bacteria of the intestinal flora influence numerous anatomic, physiologic, and immunologic parameters of the host. Healthy intestinal flora represents an important defence mechanism, which suppresses the establishment of enteric pathogenic bacteria, induces immunologic responses, and competes for attachment sites thereby creating a restrictive environment.

Below illustration shows clearly the effects of unbalanced microflora



http://www.frequencyrising.com/images/coloncleanse_cross.jpeg



Good bacteria, such as acidophilus, at work in the small intestine

http://www.frequencyrising.com/images/coloncleanse_cross1.jpeg

Normal microflora of the intestinal tract consists of a diverse population of bacteria. All of these bacteria are in competition for survival. Overgrowth of one, results in undergrowth of the other. Our unique scientific approach to digestion and GI track is unique in the world of petfoods today.

We address the digestive track as an organ on its own, by supporting all its biological functions to achieve superior functionality by including Prebiotics (MOS and FOS) and Probiotics (DSM) to achieve a complete balance to bring full benefits of the feed to the animal.

1. BioHealth™ Prebiotics (MOS)

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. Our BioHealth™ contain beta-glucans and mannaoligosaccharides. Beta Glucan is a powerful immune-enhancing nutritional supplement. This unique compound primes the innate immune system to help the body defend itself against viral and bacterial invaders.

Mannaoligosaccharides protect the GI track from invading toxins and pathogens which may find their way into the dogs stomach, binding them onto them and this way they do not harm the dog

2. MicroHealth™ Prebiotic (FOS)

MicroHealth™ is from inulin, a highly fermentable fiber. Inulin has not only become widely recognized as a superior prebiotic fiber source, it has also been clinically proven to increase calcium absorption.

It is derived from chicory roots and this becomes a specific foodstuff for the beneficial bacteria found in the intestinal tract.

Benefits from this include firmer stools, reduced risk of colitis and cancers, improvement in glucose and blood lipid metabolism, reduction in gas production (flatulence) and a reduction in fecal odor. This is food for our LactoHealth™, as without our special mix of probiotics, inulin is not fully utilized.

3. LactoHealth™ Probiotics (DSM)

Probiotics are living yoghurt type microbes that can be found in a healthy digestive tract. As cooking would kill these microbes, we add them to the outside of the kibble after the dry food has been gently cooked.

Our special mix of 5 different yoghurt type bacteria, called LactoHealth™, help in keeping the intestines in optimum state, increasing the digestibility, evicting pathogens from the gut, alleviating diarrhea and other stomach upsets.

Our LactoHealth™ containing different living yoghurt type bacterias, considerable increasing our foods digestibility which is reflected by our suggested daily feeding amounts. Your dog eats less, but gets more vital nutrients to support its health and vitality. Our bacteria drives in the gut as we feed it with MicroHealth™ and BioHealth™

The biological activity of probiotic bacteria is due in part to their ability to compete for the enterocytes. They compete for attachment sites and nutrients from the ingested feed passing through the intestine. Each species has specific requirements for growth and is affected by relative acidity or alkalinity (pH) of their environment and by products produced by other bacteria. One of the advantages of the early gut colonization is that, by occupying all the available receptor sites in the gut, there is inhibition of the binding of enteric pathogens and this is due to the by a process of competitive exclusion.

Probiotic bacteria also exert an influence on commensal micro-organisms by the production of lactic acid and bacteriocins. These substances inhibit growth of pathogens

and also alter the ecological balance of enteric commensals. A probiotic is a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. Several health-related effects associated with the intake of probiotics, including alleviation of lactose intolerance and immune enhancement, have been reported.

The fact that *E. Faecium* is protected in LactoHealth™ by means of polysaccharide layers allows it to pass through the stomach without affecting the acid pH. On reaching the intestine, it multiplies at a rapid rate (doubling in CFU every 20 minutes), and adheres to the intestine lining by means of a membrane which makes it possible for it to form the “biological barrier” that protects the body against disease-causing germs.

LactoHealth™ produces different enzymes (proteases, amylases etc...) in sufficient amounts to make the feed more digestible. LactoHealth™ also helps to restore the intestinal flora: the balance of intestinal flora changes following treatments with antibiotics and also in situations of stress. Some bacteria are sensitive to antibiotics and with their application the microflora profile is modified and certain microorganisms grow more than others depending on the antibiotic used; usually the endogenous lactobacillus and bifidobacteria are more sensitive to antibiotic and their recuperation time is slower than E. coli strains. So in this case - and stress situations - is very important to help the intestinal lactic acid bacteria with the inclusion of probiotics with fast growing capacity to make the environment comfortable for their development.

It is well known and researched that during weaning and in the immediate post-weaning period, the puppies suffer an important alteration on the intestinal wall. Stress, withdrawal from the mother and the new diet, destroy the enterocytes involved in nutrient absorption. During the first days after weaning the animals lose weight and only afterwards increase feed intake; the effect of this high intake on the damaged intestinal wall produces an increase of none digested

feed that is used by the pathogens to grow in the distal part of the intestine. The final result is an increase in the incidence of diarrhoea.

Including all our nutraceuticals in the starter feed protects the enterocytes and stimulates feed intake the day after weaning, avoiding enteric diseases, helping the regeneration of enterocytes and improving enzyme production and nutrient absorption. So, it can reduce the losses in weight and development and also facilitate adaptation to the diet.

Diarrhoea prevention: Nutraceuticals contribute to improve the intestinal wall protection; the damage to enterocytes is lower when they are protected by lactic bacteria. Diarrhoea incidence is the result of bad nutrient digestibility and absorption and it is accentuated by the increasing permeability of the intestinal wall that produces water and electrolyte losses.

There are different products, such as pectins in inulin, to prevent these symptoms, but they improve neither the feed efficiency nor the microflora. The inclusion of all our nutraceuticals BioHealth™, MicroHealth™ and LactoHealth™ contribute to reducing the effects like diarrhoea and improving the feed efficiency simultaneously reducing the pathogenic bacteria.

Bacillus Licheniformis and Bacillus Subtilis in LactoHealth™

The condition of Bacillus Licheniformis and Bacillus Subtilis used as Microbial Ecological Agents in animal feeds and agriculture

In 1989, FDA and AAFCO issued a list of the microbial strains which can be fed directly and are considered safe. Bacillus Licheniformis and Bacillus Subtilis were included. Bacillus Subtilis has been used in for a long time. Thanks to their significant physiological function, Bacillus Licheniformis and Bacillus Subtilis are widely used in breeding industry.

Table1: the characteristics of various probiotics

	Ability of intestinal colonization	Thermal stability	Colonization time(minute)	Survival method	Inhibit pathogenic bacteria	Help of digestion
Bacillus Subt.	+++	+++++	20	Facultative anaerobic	++++	+++++
Lactobacillus	++++	+	50	Anaerobic	-	++

Bacillus, as the feed additive for the microbial ecological agents, it processes characteristics such as acid resistance (resist the erosion from gastric acid), salt tolerance, high temperature resistance (100Degr) and extrusion resistance. Besides the activities of protease, lipase and

amylase, it also contains various amino acids and can grow only in upper intestine quickly and become the cells of nutrition type with metabolic function, instead of multiplying in intestinal environment.

The mechanism of Bacillus as intestinal available bacteria

Deprive oxygen biologically and adjust the micro-ecological balance of intestinal:
The research shows that when young animal is born, its digestive tract is aseptic. Invasive bacterium can be found in gastrointestinal tract 3 hours after the birth, in colorectal 12 hours after the birth. Then, through the alternation of aerobic bacteria, facultative anaerobes and strict anaerobes, the flora in which lactobacillus and other anaerobic bacteria are in majority is formed finally. Bacillus Licheniformis belongs to aerobic bacteria, and needs abundant of oxygen in its growth. It enters animal intestine, consumes a large quantity of free oxygen, decreases the oxygen concentration and redox potential in intestine, improves the growth environment for lactobacillus and other anaerobic bacteria which will be benefit for the growth of them,

and maintains the balance of the micro ecosystem in intestine. Meanwhile, Bacillus Licheniformis inhibits the growth of the aerobic bacteria, enterobacter and etc. which have been in intestine under the condition of hypoxia, improves antiviral ability of animals, and decreases the incidence of gastrointestinal diseases.

Antagonize pathogenic microorganism and improve the ecological environment in vitro and in vivo (Antibiotics) As probiotics, compared with antibiotics, Bacillus has native advantages. Probiotics occupy binding sites in intestine actively and colonize, form dominant flora, and substitute or inhibit harmful flora. Antibiotics, however, inhibit or kill bacteria passively, which will cause the resistance from pathogenic bacteria.

Affect effect (different from antibiotics)

- Protect alimentary canal
- Improve the content of IgA in alimentary canal
- Strengthen complement system of alimentary canal (anti-inflammatory, dephlogisticate)
- Contact head affects on endothelial system and strengthen the activity of carrier cell
- Stimulus immune reaction, induce the production of interferon, antagonize the invasion of virus

The research shows that after feeding the animals with Bacillus, the quantity of Escherichia Coli, Clostridium perfringens and Salmonella will be significantly decreased, the quantity of beneficial bacteria will be increased while that of potential pathogenic bacteria decreased. Through increasing the quantity of beneficial bacteria in excrement and secretion and decreasing the quantity of

pathogenic microorganism, the environment in vivo and in vitro will be purified and incidence of diseases will be reduced. Ammonia, amine, indole, hydrogen sulfide and etc. have specific poisoning effect on cells of intestinal mucosa, but Bacillus can inhibit harmful microbe, reduce the existence of harmful substances so as to benefit the healthy growth of animals.

Improving the immune function of animals

The recent research shows that Bacillus can bring the related lymphoid tissue of animal intestine to be on a high degree of immune preparation, accelerate the growth of immune organs and system, increase the quantity of T/B lymphocyte and improve the immune level of animal's cells and body fluid. Some scholars suggest that as non-special immune factors, bacillus stimulates the organic immunocyte through cells and cell wall constituents, activates them and produces mitogenic factor, promotes cells activity or plays its role as adjuvant. What's more, Bacillus can also play specific immune function and promote cell B to produce antibody ability. Other researchers suggested

after animals taking feed bacillus orally, the bacillus can help to adjust intestinal flora and let the intestinal micro ecosystem keep the best balance condition. Meanwhile, other normal floras, together with bacillus, play a role as immune adjuvant in the lymphoid tissue of intestine which has antigen recognition, activate the related lymphoid tissue in intestinal mucosa, improve the secretion of SIgA and immune discrimination power, induce lymphocyte T/B and macrophage to produce cytokine, and strength nonspecific and specific immune function of the body through the re-cycle of lymphocytes to activate general immune system.

Through tests and research, our company has found that our products of LactoHealth™ together with BioHealth™ and MacroHealth™ have certain effects on the promotion of immune function in the process of animal feeding.

Production of various digestive enzymes:

That Bacillus can improve the performance of animals is an important aspect to reflect its production of various digestive enzymes. It can also improve daily gain and feed efficiency, and decrease feeding costs. The research shows that bacillus can produce various digestive enzymes and help animals digest and absorb nutrients. Bacillus processes strong activity of protease, amylase and lipase. Meanwhile, it also has the enzymes which can degrade the complex

carbohydrate in feed, such as pectin, dextran and cellulose. Many of these enzymes are not processed by animal originally. Our company has made the preliminary identification for the characteristics of enzyme production of Bacillus, which is the effect component of the products of Bacillus and can produce enzymes in animal's intestine, and the observation results of the single carbon source medium have shows the Bacillus can produce gelatinase, amylase and casease.

The application of Bacillus to human drugs

Nowadays, under the condition of frequent medical accidents caused by antibiotics, medication safety has been concerned by the whole society. Safety is required from ingredients to therapeutic mechanism of drugs. Bacillus breeds quickly in intestinal tract through its function of sterilization and oxygen deprive so as to form hypoxia environment in intestinal tract. With its promotion of the breed of anaerobic bacteria which is good for health and inhibition of the breeds of pathogenic bacteria, Bacillus improves the balance of intestinal flora and maintains the balance of intestinal

microecology so as to treat and prevent intestinal diseases.

Bacillus Licheniformis and Bacillus Subtilis have been used in human drugs as microbial ecological agents. 30~40 microbial ecological agents for human have been sold on the market such as Laxban which are used for treatment of chronic or acute enteritis, dysentery, alteration of intestinal flora, diarrhea and infant dyspepsia caused by other reasons. It also has some therapeutic efficacy on pseudomembranous enterocolitis, diarrhea caused by cirrhosis and gaseous distention.

About our product of LactoHealth™

Nomenclature

Probiotic bacteria are very strain dependent, not species dependent. A strain is a type of a bacterial species, similar as to the example below:

Everybody knows that

Bacterial **group** = **German car** = **lactic acid bacteria**

Bacterial **genus** = **Volkswagen** = **Lactobacillus**

Bacterial **species** = **VW Golf** = **Lactobacillus acidophilus**

Bacterial **strain** = **VW Golf 2.0I TURBO** = **Lb. acidophilus DSM13241**

Everybody knows that a Volkswagen Golf 2.0i turbo has other characteristics than a Volkswagen Golf 1.4 D, but you can't see the difference on the outside.

The same is true for bacteria; they all look the same, but the biological characteristics ('the engine') are different. Hence, claims on health effects of a certain probiotic are only valid for that specific strain, not species.

For our finished formulation of LactoHealth™, we choose highly enriched spore of Bacillus which is produced through pure culture in the bio-fermentation reactor. The spore has the characteristics of acid and alkali resistance, high temperature resistance and high growth activity. Thanks to excellent production process, LactoHealth™ can resist hostile environment, meanwhile, it can be stored in a long period without the survival problems. What's more, LactoHealth™ can fully exert the physiological function above mentioned because of its easy colonization in the body of animals as well as of human with high germination.

Our LactoHealth™ contains Bacillus Licheniformis, Bacillus Subtilis, Bacillus Acidophilus, Enterococcus Faecium and Phytase for maximum health benefits.