The Immune System

Jill Cline, PhD

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Introduction

The benefits of good immune health go beyond protection from infections and often the immune system is undervalued until a problem occurs. Immune health or the lack thereof, has profound metabolic consequences and new research indicates that it can affect several body systems including cognition, allergic response, gut health and longevity. At a fundamental level, a healthy immune system affords protection by preventing infectious agent/s from entering the host and establishing an active infection. This is the critical 'barrier' function, otherwise called the 'first line of defense' role of the immune system. When the immune system is compromised, this 'barrier' weakens and pathogens invade causing disease. This triggers an active immune response to neutralize and eliminate the infections agent involving physiological changes including fever, inflammation and cellular responses.

The components of the immune system can be divided into two categories: the primary or central lymphoid organs where T and B lymphocytes mature and respond to antigens and the peripheral or secondary lymphoid organs where the majority of immune-surveillance and initiation of the immune response occurs (1). Table 1 contains a truncated list of lymphoid organs and their functions.

Table 1. Major lymphoid organs and cells

Organ	Function
Bone marrow	Cell production
Thymus	Cell production
Spleen	Cell production, antibody production, bacteria removal
Lymph system (lymph nodes, lymph, lymph vessels)	Cell production, pathogen removal
White blood cells (leukocytes)	Defense
Antibodies	Defense
Complement system	Enhances antibody effects
Cytokines	Cell signaling

The body defense mechanisms against invading organisms include physical barriers (i.e. skin or mucus), innate immunity (i.e. inflammation or lysozyme) and specific or adaptive immunity (i.e. antibody production). The time course for initiation of an immune response is varied between individuals but can be as quick as a few minutes to several days or weeks depending on the pathogen and the health status of the dog (Figure 1).

The Innate Immune Response

There are millions of potential pathogens in the environment; however, most dogs are not sick all time and the reason for that is the physical barriers like the gastrointestinal tract and the innate immune response repel the majority of invading organisms. When physical barriers are intact, there are a variety of protective mechanisms available to the dog like vomiting, coughing, saliva and commensal microflora populations on the skin and in the GI tract to prevent pathogen entry into the body.

The innate immune response is the initial defense against infections and is non-specific in nature. The basis of an effective innate response is that the majority of the invading organisms are chemically very different from the dog's cells and tissues. Thus, dogs have developed mechanisms for isolating and neutralizing these foreign organisms by providing epithelial barriers and by specialized cells and natural antibodies present to stop the entry of microbes (2). If pathogens do breach the epithelium, then they are besieged by phagocytes, natural killer cells (a specialized lymphocyte) and a variety of plasma proteins designed to destroy microbes. The innate immune system does not react against non-infectious foreign substances but does enhance the adaptive immune response against infectious agents (1) .

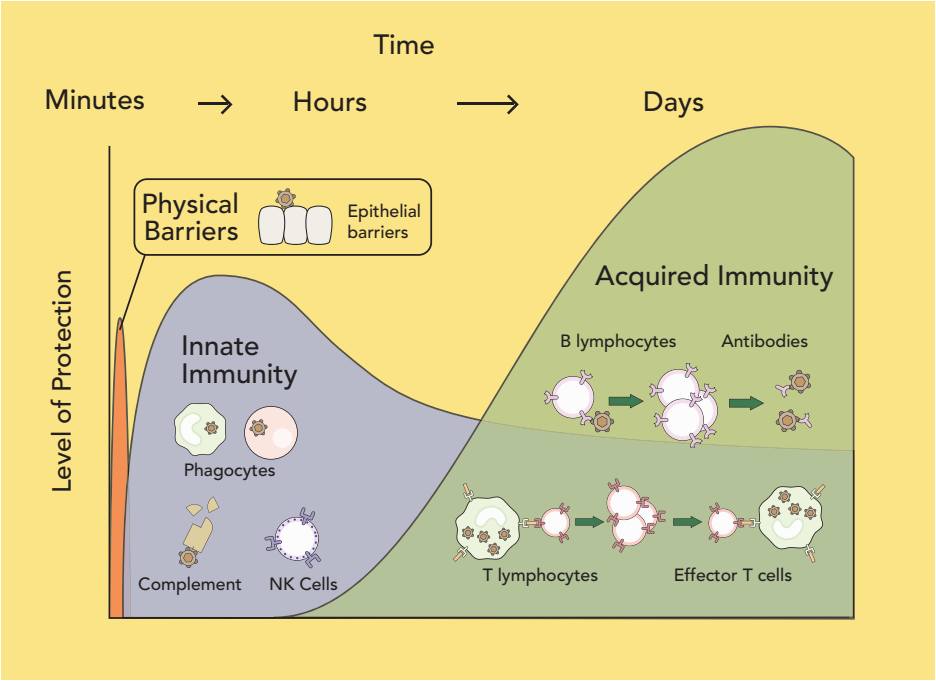


Figure 1 Timeline, cells and level of protection provided by the immune system. Physical barriers are the first line of defense followed by the innate immune response which utilizes non-specific immune cells like natural killer cells (NK cells). The adaptive or acquired immune response takes the longest to initiate, but it is the strongest and most specific protection against pathogens

The Adaptive Immune Response

Adaptive or acquired immune response occurs only if a microbe (including bacteria, fungi, many protozoa and helminthes) passes through the epithelial barrier and is delivered to the lymphoid organs where they are presented to lymphocytes. Lymphocytes determine the specificity of the immune response, orchestrate the effector limbs of the immune system and maintain the immune-memory of the offending pathogen. Lymphocytes are divided into two categories based on site of origin- B lymphocytes which originate in the bone marrow and T lymphocytes which originate in the thymus (Figure1).

The adaptive immune system has two branches, the humoral and cellular mediated systems which are mediated by different cells and mechanisms (Figure 2) (2). The humoral system is directed against extracellular or exogenous invaders and was thus named because it exists in the body fluids or "humors." Circulating antibodies are targeted against specific microbe antigens. The cell-mediated systems are directed against intracellular or endogenous organisms that invade cells (2).

Gastrointestinal tract: the largest immune organ

Besides being the gateway for nutrient intake, the gut is the largest immune organ in the body, containing approximately 70% of all immune cells and over 90% of all Ig producing cells. In addition, vast numbers of bacteria inoculate the gastrointestinal (GI) tract shortly after birth and play an integral part in normal immune system development. The GI tract utilizes all three primary body defenses: physical barriers, innate immunity and adaptive immunity. As the primary gatekeeper against pathogens, the GI tract contains peripheral lymphoid tissue called GALT or gut associated lymphoid tissue. Maintaining a strong local gut immune response is integral not only to local GI tract health in the dog, but also systemic immune health (Figure 3). An obvious yet effective way to alter the local and systemic immune response in dogs is through manipulation of GALT.

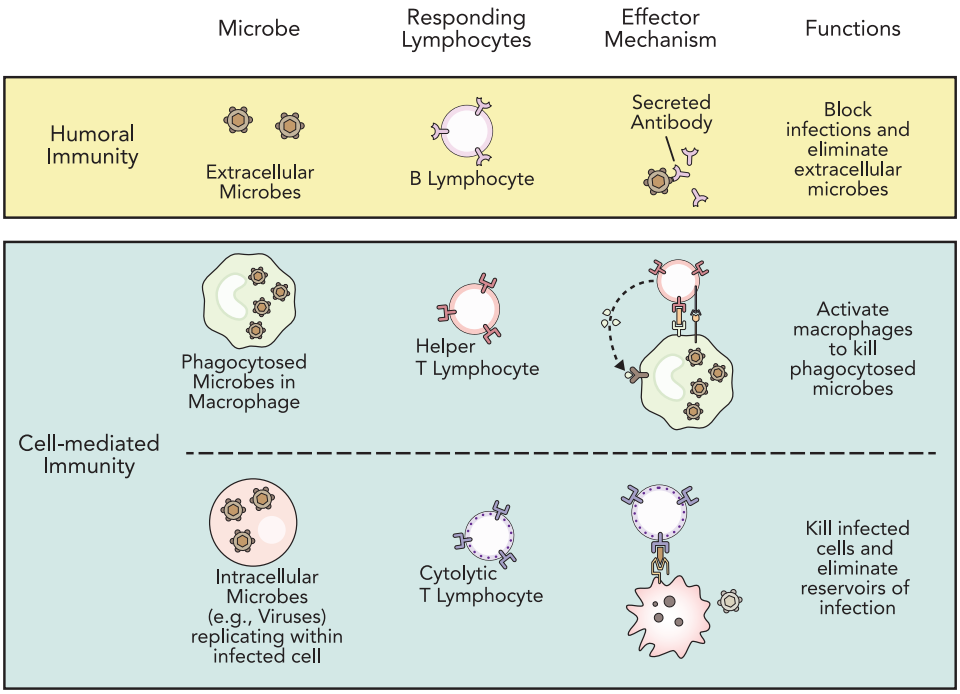


Figure 2. Two branches of the adaptive or acquired immune system. Humoral immunity relies on B-lymphocytes to neutralize exogenous pathogens while the cell mediated immune response uses T-lymphocytes to target endogenous pathogens and infected cells.

The GALT offers unique opportunity for immunomodulation via diet or dietary supplements. Most dietary immune modulating strategies involve targeting specialized receptors of GALT using appropriate ingredients. The enhanced immune activity induced by specialized ingredients which interact directly with the GALT spread to the entire immune system, by the shuttling of activated immune cells and the signaling substances like cytokines which give information to the rest of the body , improving immune response. Table 2 lists examples of enhanced immune activity to dietary supplements.

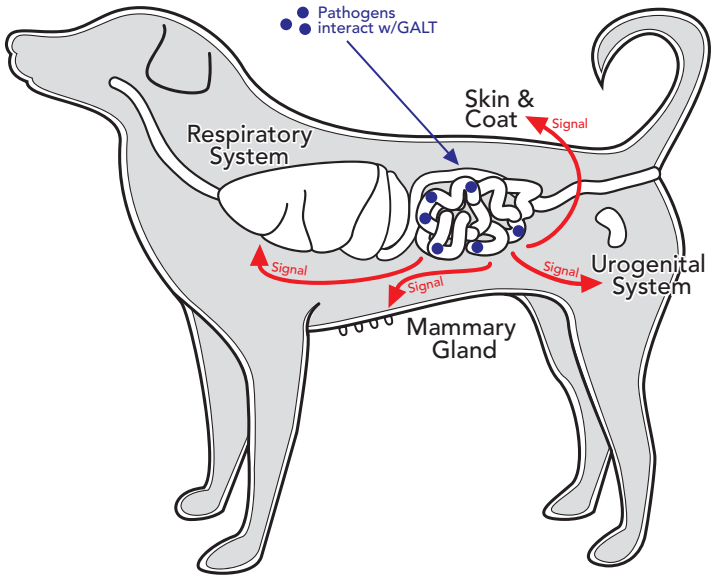


Figure 3. GALT evaluates all material enters the body via the GI tract. If the material is determined to be a pathogen, GALT initiates a cascade of reactions which results in the engagement of the immune response in other areas of the body with the final aim to provide systemic protection against invading pathogens.

Functional dietary supplements for immune health

When there is an infection or other initiation of the immune system resulting in clinical symptoms, the traditional treatment is to actively eradicate the pathogen and then presume that the immune system will "right itself" after the treatment is finished. However, re-inoculation with

suboptimal microflora from the environment along with daily stress like travel or separation anxiety can delay the restoration of status quo in the immune system.

Table 2. Dietary supplements enhance immune health

Activity	Function
Increases in phagocytic activity	Component of the innate immune system
Increase in lymphocytic proliferation, which increases T and B cell activity	Improve adaptive cellular immune response
Increase in natural antibody or immunoglobulin (Ig) production	To prime the adaptive humoral immune response
Tightening of the junctions between gut epithelium	Improve the physical barriers of the gut

New studies indicate that prophylactically managing the immune system by "priming" or training it through dietary supplement manipulation will strengthen the immune system and prevent pathogen invasion or lesson the severity of the infection. Dietary supplements like Prudence Immune Health are designed to contain natural ingredients supported by cutting edge research which when combined target a broad spectrum of metabolic processes in the immune system primarily through interaction with the GALT and ultimately the rest of the body (Figure 4).

Hyper-Immunized Egg: A Natural Source Of Antibodies For Immune Health

Hyper-immunized egg or Hyper Egg® is dried egg powder that contains natural antibodies or immunoglobulins (Ig) and immune regulatory factors which modulate immune cell functions in the GALT and systemically in animals and humans (3). Studies in humans and animal models show that hyper-immunized egg has positive effects on gastrointestinal upset and inflammatory response in the gut, skin and joints (4) (5).

Hyper-immunized egg is produced using a specialized process by which laying hens receive multiple vaccines against a variety of pathogens and the resulting eggs harvested and processed to preserve the Igs (Figure 5). The nutrients contained in hyper-immunized egg or their intestinal metabolites may serve as either substrates for the microflora or as regulators of the endogenous gut microflora through a variety of mechanisms. Feeding antigen-specific hyper immunized egg increased survival rates, improved weight gain and reduced diarrhea in calves, piglets and lambs (3). Currently, hyper-immunized egg is seen as an alternative to low doses of antibiotics to improve livestock health and growth rate.

Figure 4. Proposed benefits of immune modulating ingredients in Prudence Immune Health

Absolute Immune Health - High Potency

5 Probiotics Advanced with Hyper-Egg™ plus Prebiotics

Body

Enzymes
Acts as an anti-inflammatory

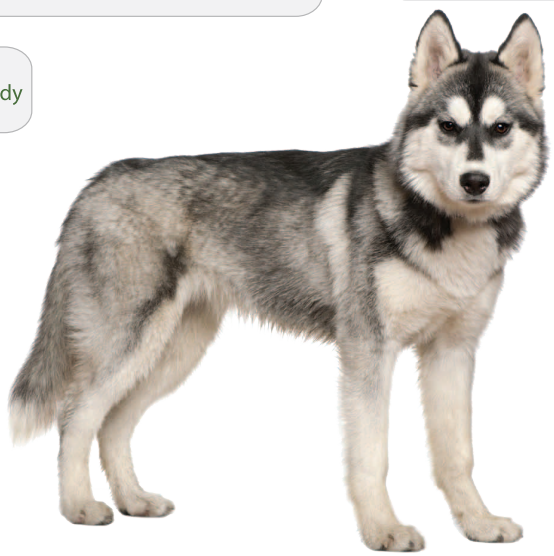
Probiotics
Improves vaccine response

Whey
Boosts immune status and antibody level

Deglycerrhized Licorice
decreases inflammation

Gamma Oryzanol
decreases stress by blocking stress hormones

Zinc
Acts as an antiviral agent



Hyper Egg™
Improves tissue repair

Previda®
Decreases pathogen growth

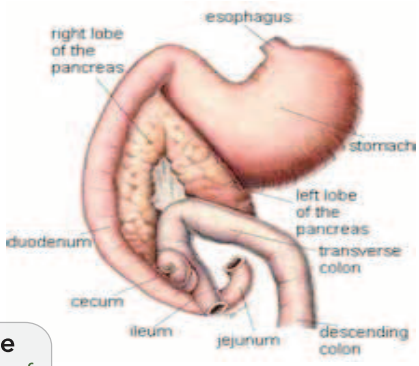
Cu
Acts as an antibacterial agent

Gut

Enzymes
Improves digestion even when the gut is upset

Whey
Inhibits bad bacteria from growing

Deglycerrhized Licorice
Promotes the protective barrier of the gut



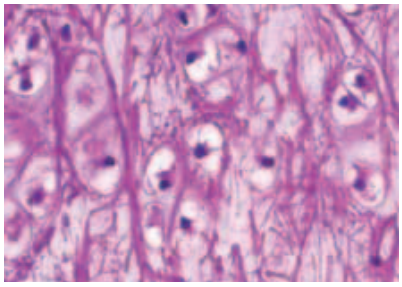
Gamma Oryzanol
Protects sensitive stomachs against upset

Sodium Copper Chlorophllin
Increases immune cell production

Cellular

Previda®
Can boost immune cell production

Minerals
Increases the number of immune cells produced



Hyper Egg™
Increases natural antibodies

Probiotics
Changes gene expression to Increase immune cell production
Increase natural antibodies

Enzymes
Breaks down bad bacteria

Rev. A 01/12

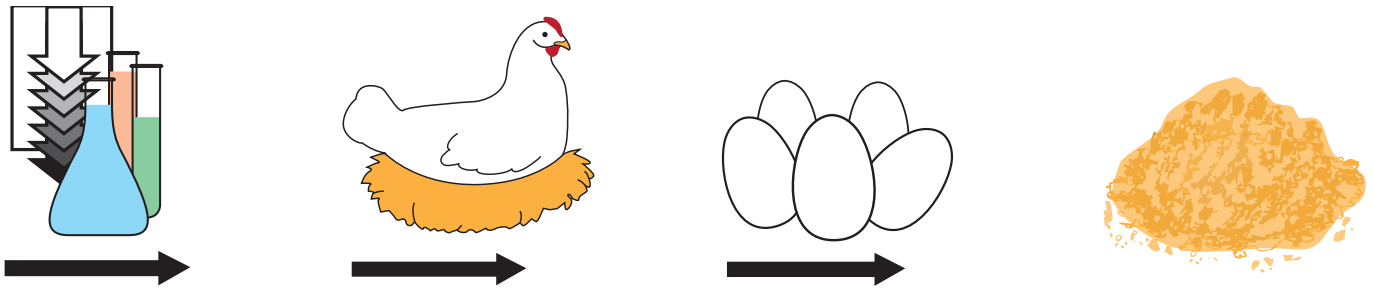


Figure 5. Hyper immunized egg is produced by immunizing laying hens against certain pathogens and collecting the resulting eggs. Each egg has the same immunoglobulin profile as that of the inoculated hen. Eggs are processed into a powder for human and animal consumption.

Hyper-immunized egg supplementation also improves quality of life parameters for immune compromised humans. In a trial of 17 men with AIDS- induced wasting syndrome, there was significant improvement in weight gain and maintenance as well as physician-evaluated improved physical functioning (6) and in a trial with 50 immuno-compromised people, there was improvement in anorexia, weight gain, incidence of diarrhea, and mental acuity (7).

Orally administered hyper-immunized egg has proven successful for treatment of a variety of GI infections including those caused by rotaviruses, coronavirus, Yersinia ruckeri, enertoxigenic E. coli and others. In one study, hyper-immunized egg was administered to mice with acute castor oil onset diarrhea. There was a dose dependent inhibitory effect of hyper-immunized egg on clinical symptoms of acute diarrhea (8). The mechanism by which hyper immunized egg acts is unclear but could be related to several processes like those listed in Table 3. It is interesting to note that while antigen specific hyper-immunized egg, for example immunized against bovine rotovirus, is highly effective in cattle and it is also effective at improving the immune response in other species that are not sensitive to that particular pathogen. The Igs from hyper-immunized egg may be partially degraded by digestive enzymes but the fragments retain a significant portion of their neutralizing activity (9).

Table 3. Possible mechanisms for Hyper Egg® function

Hyper Egg® functions
Prevent adhesion of enteric pathogens to epithelium of GI tract
Bind specific receptors to induce mucosal immune response
Alter receptor interaction
Prevent formation of cell messengers induced by pathogens

The immune system of puppies does not fully develop until the puppy is approximately 22 weeks of age and even after maturation, the immune system is assailed by various stressors. Stress, like moving from one home to the next, learning to interact with other dogs, or being boarded can cause changes in the immune response in puppies and adults alike. In one study comparing 12-16 week old puppies in mildly stressful conditions, those fed hyper-immunized egg had lower pre-stress fecal pH indicating a more acidic environment that correlated with increased levels of beneficial bacteria (bifidobacteria, lactobacilli, and enterococci). Control dogs had higher fecal pH, which favors the growth of potentially more undesirable microflora like coliforms and clostridia. Furthermore, puppies fed the hyper-immunized egg powder had higher microflora stability before, during and after stress when compared to puppies that were not hyper-immunized egg thus demonstrating a more

stable gut microflora population. Finally, there was an increase in fecal IgA in the puppies fed hyper-immunized egg powder, which indicates a stimulation of the mucosal immune system (10). IgA is a GI specific immunoglobulin or antibody which can be stimulated by a variety of ingredients to help "prime" the immune response for future action and it also acts as a communicator to the humoral and cell mediated immune systems (Figure 6).

Hyper-immunized egg is reported to have anti-inflammatory effects equal to ibuprofen in inflammation protocols in dogs and mice (11) and is gaining momentum as a potential dietary supplement for arthritis due to its anti-inflammatory effects (11) and its reported ability to counter, prevent or reduce NSAID induced GI damage (12).

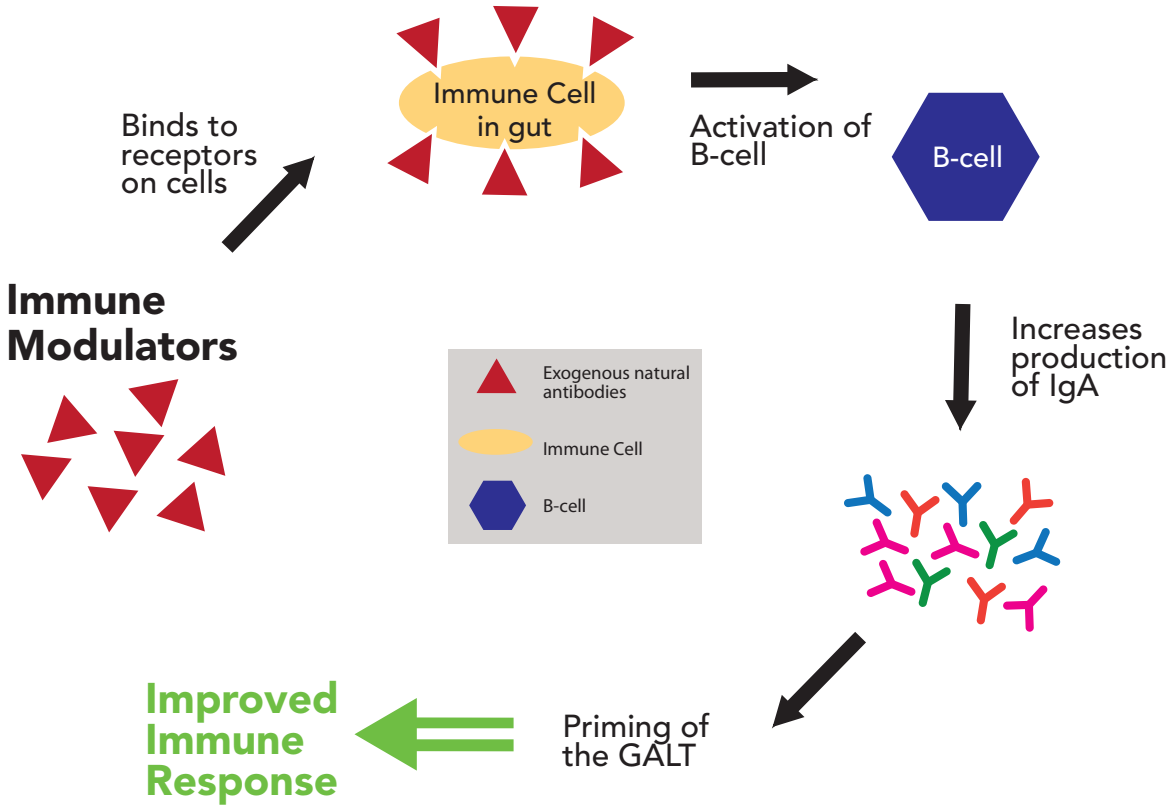


Figure 6. Supplemental ingredients like Hyper-Egg™, Previda®, bioactive whey and probiotics can interact with IgA which "primes" the immune response for future action and increase IgA production which acts as a communicator to the acquired or adaptive immune system.

Hyper-immunized egg has a role in decreasing diarrhea of unspecific origin as well as from identifiable enteric pathogens, stabilizing gut microflora, increasing IgA in the gut of dogs and preserving gut epithelium during NSAID use and decreasing inflammation in the gut and systemically.

Previda®: A Distinctive Prebiotic For Animal Health

Previda® is a new prebiotic shown to improve GI health by increasing beneficial bacteria and improving immune response in dogs.

Prebiotics are selectively fermented ingredients that show specific, beneficial changes in composition and or activity in gut microflora of the host animal (13) (Table 4).

Table 4. Characteristics of prebiotics

Prebiotics Characteristics
Not absorbed by the dog
Pass unchanged through the digestive system until they reach the large intestine
Metabolic selectivity
Food for beneficial bacteria
Not a food for harmful bacteria
Be able to alter the intestinal microflora in favor of a healthier composition
Induce effects that are beneficial to the animal

The main benefits of prebiotics are a positive change in GI environment, and because of the close connection between gut health and immune health, positive changes in immune status (Figure 7). Previda® is a new type of prebiotic with a distinctive blend of carbohydrates that contribute to its biological diversity.

Recent studies showed that Previda® supplementation resulted in increased short-chain fatty acid (SCFA) production nearly double that of commonly found prebiotics like fructooligosaccharides (14).

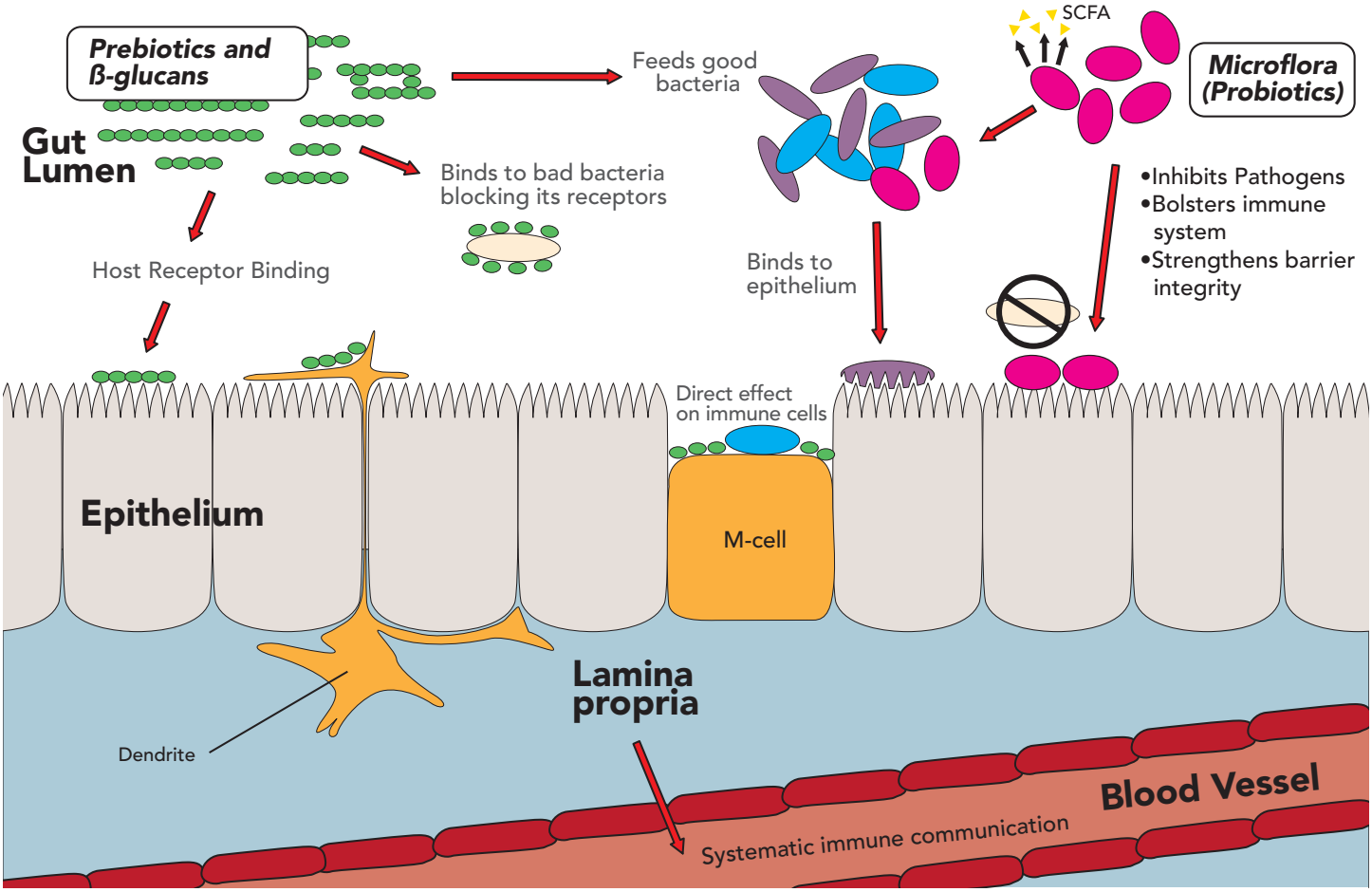


Figure 7. Proposed function of prebiotics, ß-glucans and probiotics. Prebiotics provide substrate for probiotic proliferation and can prevent adhesion of some pathogenic bacteria as well as interacting directly with the immune system via M-cells or dendrites. Glucans interact with immune cells in the gut as well as interacting with potential pathogens to prevent colonization. Probiotics prevent attachment of bacteria to the gut epithelium, secrete short-chain fatty acids, excrete metabolites which act directly on pathogens and improve gut barrier integrity to prevent toxin entry.

The presence of SCFA in the colon is thought to prevent diarrhea by facilitating ion absorption. The absorption of sodium is enhanced along with the absorption of SCFAs (15). SCFAs also help create a stronger barrier in the GI tract, a component of overall immune health. The mucus layer forms a physical-chemical barrier on the epithelial layer separating the gut lumen from the lamina propria, thus having an important function in scavenging dietary and microbial antigens. In vitro studies show that increasing SCFA in the intestine stimulates myofibroblasts (the cell layer under epithelial cells) which in turn stimulate epithelial cells to produce mucin which contributes to the protective mucus layer (16) thereby providing an additional deterrent to pathogens.

Previda® supplementation resulted in a very high butyrate production in dog fecal inoculum when compared to the prebiotics fructooligosaccharide (FOS) and yeast cell wall extract (Safmannan) (14) (Table 5). Butyrate, one of the SCFAs found in the gut has gained much attention as it acts as the primary fuel source for colonic cells, promotes mucosal restitution, induces cell differentiation, inhibits inflammatory response, inhibits tumor growth and was also shown to alter the ratio of anti-inflammatory prostaglandins (PGE1)to pro-inflammatory prostaglandins (PGE2) (16) (17) (18). Butyrate most effectively stimulated PGE2 and appears to modulate the expression of COX-1 and SCFAs can also down regulate the production of COX-2 from myofibroblasts (16).

*Table 5. pH change and short chain fatty acid (SCFA) production following 12 hours of in vitro fermentation with dog fecal inoculum. Adapted from Faber et al (14). * p<0.05 different from the others. + different from FOS only.*

Item	FOS	Safmannan	Previda®
pH change from initial	-1.22	-0.29	-0.86
SCFA			
Acetate	86.1	23.1	192.5*
Proprionate	76.1	25.3	119.2*
Butyrate	0.0	15.8	16.2+
Total	162.2	64.1	327.9*

The inclusion of Previda® resulted in a significant increase in beneficial bacteria (Lactobacillus spp. and Bifidobacterium spp.) and a decrease in potentially harmful bacteria in vitro studies (Figure 8) and an increase in Bifidobacterium spp. in vivo (19). Increasing Bifidobacterium spp. is beneficial to dogs because it may suppress pathogenic bacteria and improve macronutrient uptake which is critical in animals that may be stressed or immune-compromised.

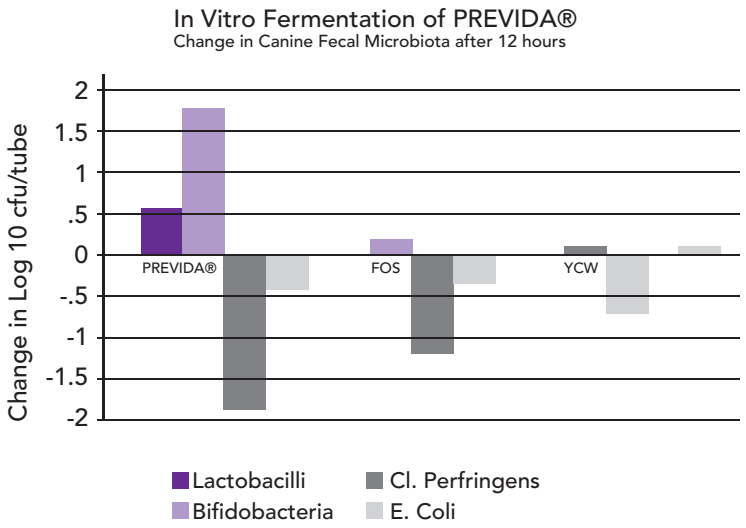


Figure 8. Previda® increases beneficial bacteria and decrease potentially harmful bacteria more effectively than fructooligosaccharides (FOS) or yeast cell walls (YCW).

Based on in vitro studies, Previda® prevents the adhesion of some species of pathogenic bacteria by binding to the carbohydrate receptors on the bacteria, preventing them from attaching to the epithelial cells of the GI tract. (Figure 9). Thus, Previda® has a direct effect on the immune system, unlike other prebiotics which act primarily by providing substrate for residential bacteria.

Previda® is a new class of prebiotic for dogs proven to provide substrate for beneficial bacteria; help decrease the proliferation of potentially harmful bacteria; and to significantly increase SCFA production. In addition to that, Previda® alters pathogenic bacteria's ability to adhere to the GI tract.

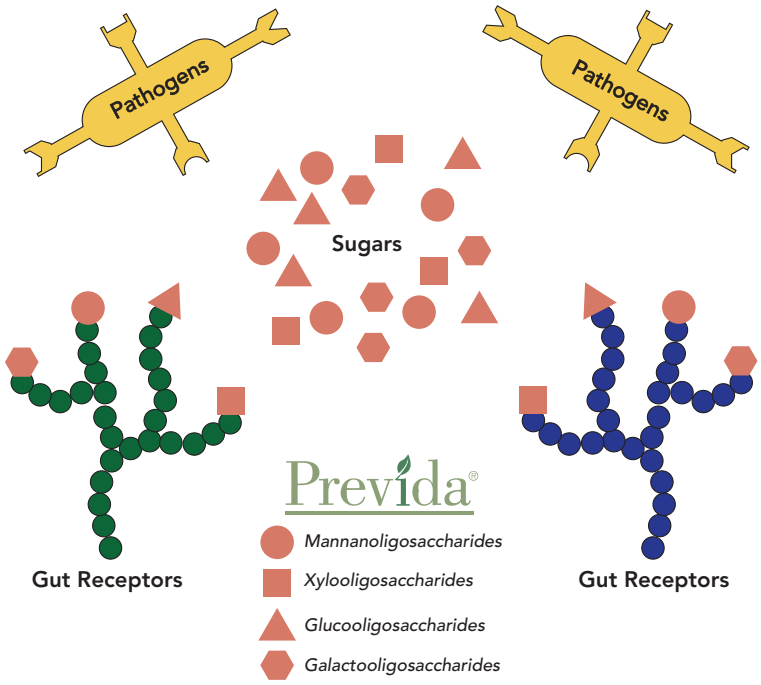


Figure 9. Previda appears to bind to the carbohydrate receptors of bacteria preventing adhesion to the gut epithelium.

Probiotic Blend: Directly And Indirectly Improve Immune Health

Probiotics are live microorganisms that, when consumed in sufficient quantities provide a variety of healthy benefits to animals and people. The most common probiotic organisms are lactic acid bacteria,including lactobacillus,bifidobacterium and Enterococcus spp. (20) (21). Reported health benefits of probiotics range from increasing the population of beneficial bacteria in the GI tract and decreasing potentially harmful bacteria to direct effects on the immune system. Unlike the resident microflora, probiotics do not colonize the bowel so they do not become permanent members of the resident GI microflora. Thus, they must be consumed on an ongoing basis or as needed to provide health benefits. The characteristics of probiotics are listed in Table 6.

Table 6. Characteristics of probiotics

Probiotics Characteristics
Survive the GI tract
Adhere to intestinal cells or transiently localize
Exclude or reduce pathogenic adherence (sometimes called pathogen exclusion)
Produce acids, hydrogen peroxide and/or bacteriocins that are antagonistic to pathogen growth
Coaggregate to help achieve normal balanced microflora populations
Be safe, noninvasive, noncarcinogenic and nonpathogenic to the animal

Bacteria found in the gut can be divided into three categories. One group includes potentially pathogenic bacteria, such as clostridia, staphylococci and salmonella. These bacteria have the potential to invade the GI mucosa, produce toxins that can cause diarrhea and activate carcinogens in the gut (22). Other bacteria, such as lactobacillus, bifidobacteria and some enterococcus, fall into a group of "health-giving" bacteria (22). These bacteria are thought to provide protection against infection. In adults, they may be the principal species responsible for GI barrier function and for stimulating healthy immune function (22). A third category of bacteria includes the hundreds of other species and strains that neither harm nor provide known health benefits (Figure 10).

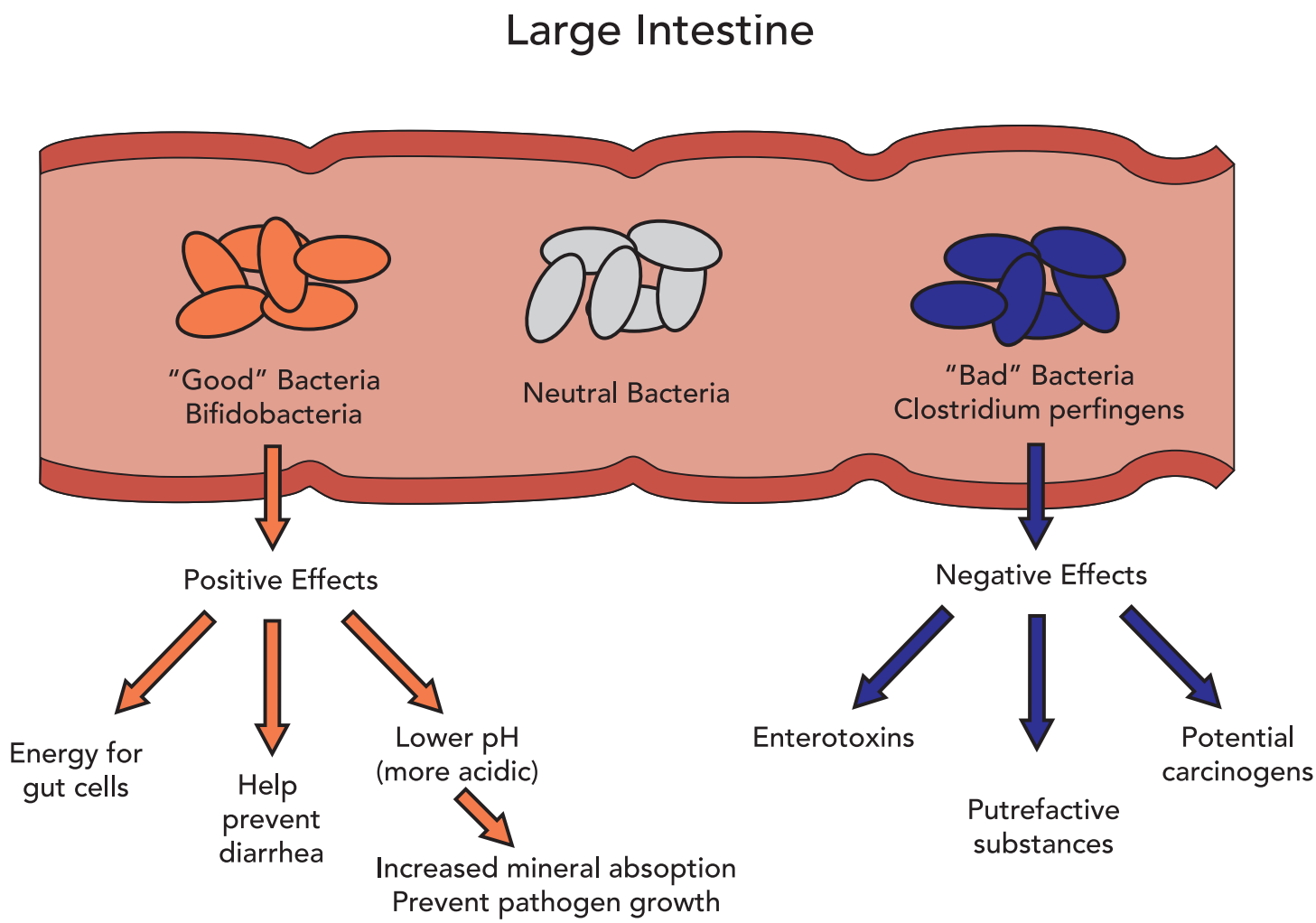


Figure 10. Bacteria in the GI tract can be loosely catagories into three groups: beneficial bacteria which produce metabolites that improve function of the gut and body; potentially pathogenic bacteria produce metabolites which have negative effects on the GI tract; and neutral bacteria which have no known function.

The GI microflora interacts directly with the host animal immune system in a number of ways. The microflora provides a primary stimulus for the development and maturation of lymphoid cells in the Peyer's patches, as well as IgA producing lymphocytes. Secretory IgA is critically important in protecting the intestines against bacteria and viruses. The GI microflora modulates the specific immune responses and allows the induction of oral tolerance (versus allergy) to food antigens (23). Certain species and strains of GI bacteria, including enterococcus, lactobacillus and others, can alter systemic immune responses, such as serum antibody concentrations, as well as promote local IgA production. In addition to their beneficial effects on the host animal's immune system, the microflora can have additional direct and indirect protective effects (Figure 7.).

The GI microflora influences the mucus layer that overlays the epithelial cells of the intestines (24). As such, the defensive capacity of the mucus, which partly lies in its ability to entrap pathogenic bacteria, can be enhanced or compromised. For example probiotic strains of lactobacillus induced increased expression of the MUC2 and MUC3 genes that promote mucus secretion in the colon and inhibited the attachment of pathogenic E. coli (24). When the natural flora of the intestine is eliminated or its composition dramatically changed, such as from the use of antibiotics, then an overgrowth of potentially pathogenic bacteria can occur, leading to diarrhea or severe colitis (2).

One of the best-documented benefits of probiotic use is in the management of diarrhea. In humans, there is a long tradition of treating diarrhea with bacteria to address disruptions in the normal GI

microflora (25). This tradition has been complemented by a number of well-designed animal and human clinical studies over the past 20 years, resulting in a significant basis of evidence in support of the use of probiotics (26) (25) (27). Probiotics are now well-recognized to be of benefit in conditions such as traveler's diarrhea, antibiotic-associated diarrhea, clostridial enteritis, viral diarrhea, lactose intolerance, and others (Table 7) (25) (27).

Table 7. Causes of diarrhea that may benefit from probiotics. Adapted from Laflamme (23)

Causes	
Coronavirus diarrhea	Boarding
Rotovirus diarrhea	Environmental changes
Clostridium perfringens or C. difficile	Psychological or physical stress
Giardia spp	Helicobacter gastritis
Salmonella spp	Lactose intolerance
Antibiotic induced diarrhea	Small intestinal bacterial overgrowth
Stress induced diarrhea	Irritable bowel colitis
Weaning	Inflammatory bowel disease

A number of different probiotics, including Lactobacillus rhamnosus GG, L. casei, Entero -coccus faecium SF68 and others, have shown efficacy in infectious diarrhea in humans. Specific benefits included decreased frequency of infections and enhanced local or systemic immune response (25) (28). Prophylactic treatment with lactobacilli decreased the incidence, severity and duration of viral diarrheas in children (26) (29). The protective effect appears to be mediated through an increase in secretory IgA (27). Several studies have shown a reduction in recurrence of clostridial diarrhea in patients treated with probiotics (29) (30). Probiotics, especially *Lactobacillus GG* and *Saccharomyces boulardii*, also have demonstrated efficacy against traveler's diarrhea in many studies (31). Antibiotic treatments create a disruption in the normal GI microflora, which can result in clinical signs including diarrhea. A placebo-controlled trial in children showed a reduction in incidence of antibiotic-associated diarrhea (AAD) from 16% in the placebo group to 5% in the probiotic treated group (32). A meta-analysis of AAD predominantly in adult patients treated with *Lactobacillus GG* or *Saccharomyces boulardii* showed an overall reduction in prevalence of diarrhea from 22% to 10% (33).

The health benefits of probiotics have been studied in many species. Probiotics appear to provide their benefits through interaction with the hosts' immune and defense mechanisms (Table 8) (34) (35) (20) (26).

Table 8. Protective effects of probiotics. Adapted from Laflamme (23)

General	Humoral Immune System	Cellular Immune System
Produces growth and coagulation factors	Stimulates IgA production	Stimulates macrophage function
Activates mucosal or gastrointestinal lymphoid tissue (GALT)	Inhibits IgE production	Stimulates natural killer cell activity
Modulates anti-inflammatory/ proinflammatory immune response	Stimulates nitric oxide production	Promotes growth and regeneration of intestinal cells
Promotes antioxidant actions	Modulate cytokine responses	Promotes apoptosis
Controls potentially pathogenic microorganisms		
Reduces production of endotoxins		
Reduces mutagenicity		

Probiotic bacteria help regulate the balance between appropriate and inappropriate immune responses in part by enhancing the response of T-helper cell-1 (TH1), a specialized lymphocyte and inducing immunoregulatory cytokines. In addition, probiotics protect the intestines by competing with pathogens for bacterial attachment sites, strengthening enteric tight junctions and enhancing the mucosal immune response (IgA) to pathogens (36). Specifically, *Saccharmyces boulardii* seals the tight junctions in the GI tract, preventing dietary debris and potential pathogens from crossing the gut wall (36). Bifidobacterium spp. have a variety of function in the gut including: increasing macrophage and natural killer cell activity; decreasing luminal pH, competitively binding to receptors used by pathogenic E. *coli* and increasing phagocytic activity (37) (38). Lactobacillus spp. acidify the colonic environment, reduce adhesion by Clostridium perfringens in dogs, decrease pathogen shedding in cats and increase system IgG production (39) (40) (41).

While most of the clinical data on the use of probiotics has been generated in humans, research shows similar benefits and/or mechanisms occurring in dogs, cats and other animals. A lactobacillus probiotic positively altered fecal microflora, including a reduction in clostridia, when given to healthy adult cats (42). These same cats showed a decrease in plasma endotoxin during probiotic administration, suggesting either an enhanced GI barrier or reduced luminal endotoxin production. Puppies fed *Enterococcus faecium* had an improved response to canine distemper vaccine, confirming the close connection between local GI immune enhancement and systemic immune enhancement (43).

The majority of research on probiotics has been on specific microorganisms to elucidate the effects of htat particular species. However, many researchers believe that a cocktail of probiotics is more effective at improving gut health and promoting a strong immune response because of their combined broad spectrum effect (44). A combination of *S. boulardii*, L. rhamnosus and other probiotics decreased antibiotic induced diarrhea more effectively that each alone (45). A study in dogs evaluated the efficacy of probiotics for irritable bowel syndrome. When used alone, the probiotics strains had no significant effect on cytokine concentrations of the effected dogs; however the combination of 3 Lactobacillus spp. led to changes in the proportions of regulatory and pro-inflammatory cytokines in ex vivo cultured biopsy specimens of the effected dogs (46). In addition, dogs with dietary sensitivity responded positively to a cocktail of probiotics (47).

Probiotics are a powerful, natural way to directly and indirectly effect the immune system of animals locally in the GI tract and systemically. The benefits of probiotics are many, particularly when combinations of probiotics are used (Table 9). Prophylactic supplementation with probiotics can prevent or alleviate pathogen uptake in the gut.

Table 9. Health benefits of probiotics in multiple species

Improved Immune Function	Protection vs Pathogens
Prolonged vaccine response in dogs	Improve overall immune function
Increased secretory IgA in dogs	Block pathogen adhesion to epithelia
"Prime" the immune systems to respond quickly	Fermentation products create an undesirable luminal environment for pathogens
Increase phagocytosis: direct destruction of invading pathogens	Stronger barrier integrity inhibits toxin absorption
Some probiotics are anti-inflammatory	
Effect vs Specific Disease	**Other Benefits**
Chronic diarrhea	More consistent fecal quality
Inflammatory bowel disease	Prevent diarrhea after/during antibiotic therapy
Colitis	Prevent atopic dermatitis
Traveler's diarrhea	Anti-allergenic
Urinary tract infections	

Synbiotic Effects: Probiotics, Prebiotics And Minerals Combinations

Synbiotic is a recently coined term to describe the administration of prebiotics and probiotics together to improve the action of both supplements. Multiple researchers have reported the efficacy of synbiotics in dogs (48) (49) (50). Galactooligosaccharides, similar to those found in Previda® and B.*bifidum* in combination increased bifidobacterium spp. in dogs and decreased Clostridium spp. (49). Symbiotic preparations seem to promote microbiota diversity, lessen morbidity symptoms like diarrhea and increase SCFA production compared to probiotics alone (49) (50). One study compared the combination of hyperimmunized egg, prebiotics and probiotics in preruminant calves and found a 50% reduction in enteric morbidity vs. control calves (51). Limited studies also indicate that the addition of minerals to a probiotic supplement potentiates the effects of the probiotics. The addition magnesium increased L.*casei* and L.*plantarum* counts in vivo and zinc in combination with probiotics has been used successfully in the treatment of E. *coli* diarrhea in piglets (52) (53).

Minerals: Boost Immune Response

Zinc, manganese and copper are important cofactors for enzyme pathways in dogs including those contributing to antioxidant status like superoxide dismutase (SOD). Manganese is required for the optimal uptake of certain vitamins and minerals. All three minerals are important for macronutrient absorption. Table 10 highlights the influence of zinc, copper and manganese have on the immune system function (54) (55).

Table 10. The role of zinc, copper and manganese on the immune system.

Mineral	Innate Immune	Adaptive Immune	Antioxidant Status	Other
Zinc	Influences natural killer cells and neutrophil proliferation	Influences immunoglobulin (Ig) and cytokine production	Cofactor for many antioxidant enzymes including SOD	Contributes to membrane stability and has antiviral effects
Copper	Influences neutrophil and macrophage production	Influences lymphoid tissue proliferation and cytokine production	Cofactor for many antioxidant enzymes including SOD	Direct antimicrobial effects
Manganese	—	—	Contributes to antioxidant status and SOD production	Important for the uptake of other vitamins and minerals

In addition, metal ions like copper and zinc inhibit the growth of certain bacteria through a variety of mechanisms (Figure 11) (56). Zinc is a component of specialized metalloproteins which prevent RNA translation of viruses and, therefore, proliferation (57).

The addition of mineral chelates bolster immune function by improving antioxidant status, directly influencing immune cell proliferation and suppressing bacterial and viral growth.

Sodium Copper Chlorophyllin: A Plant Derived Antioxidant And Anti-Mutagenic

Sodium copper chlorphyllin is a natural extract from green plants which acts as an antioxidant and anti-inflammatory agent as well as a potent anti-mutagenic agent. This extract is the water soluble portion of chlorophyll and is ten times more potent as an antioxidant than chlorophyll (58). Chlorphyllin has been shown to significantly inhibit mutagenic activity of a variety of carcinogens by mechanisms likely to include: direct antioxidant activity; formation of complexes with mutagens/carcinogens facilitating the excretion of these substances. Clinical trials indicate that individuals exposed to aflatoxin had reduced aflatoxin-DNA adducts after oral ingestion of chlorphyllin (59). Chlorophyllin is one of the chemical compounds found in green leafy vegetables which contribute to the anticancer properties of these vegetables.

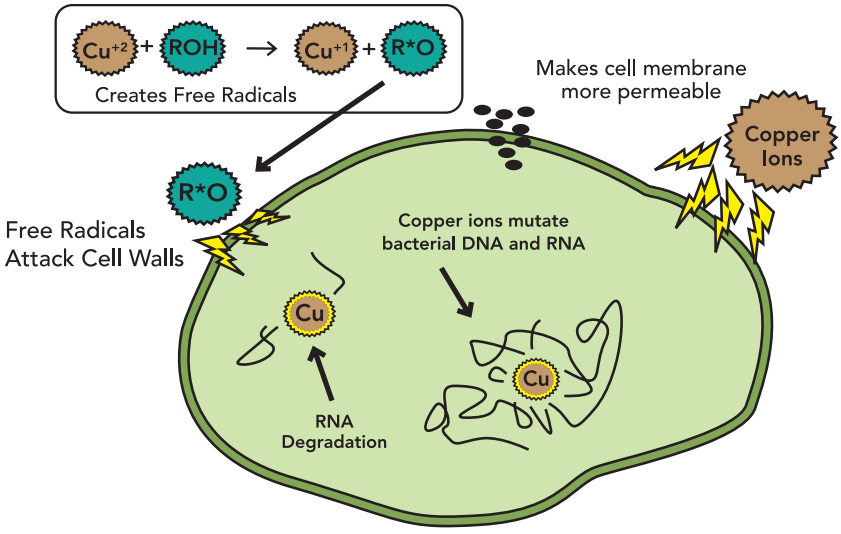


Figure 11. Metal ions like cooper and zinc can directly inhibit bacterial proliferation in three ways: produce free radicals to disrupt the bacterial cell wall; alter the permeability of bacterial cell walls resulting in effusion; altering DNA and RNA replication and transcription.

Sodium cooper chlorophyllin in addition to being an antioxidant, anti-inflammatory and anti-mutagenic agent also increases the bioavailability of the copper to which it is bound.

Bioactive Whey: A Multifaceted Immune Modulator

Whey proteins are the primary component of bioactive whey and are known to modulate health in four primary ways: as a prebiotic; an antioxidant; an antibacterial and antiviral and anticancer agent; and as an immune-modulator. Table 11 lists the main proteins found in bioactive whey (60).

Table 11. Bioactive proteins in whey. Adapted from Beaulieu.

Protein	Biological Activity
ß-lactoglobulin	Retinol carrier Binding of fatty acids Antioxidant
α-lactalbumin	Increase cytokine production by macrophages Immunomodulation Anticarcinogenic
Immunoglobulins	Increase GSH, an antioxidant Activate complement system Increase phagocytosis
Glycomacropeptide	Bifidobacteria growth Immunomodulation Antiviral
Lactoferrin	Antimicrobial, antiviral, wound healing Antioxidant Anticarcinogenic Antitoxin Anti-inflammatory Antithrombotic Immunomodulation Fe absorption Promote immune cell differentation
Lactoperoxidase	Antimicrobial, wound healing
Lysozyme	Antimicrobial, wound healing Synergistic effect with lactoferrin Synergistic effect with immunoglobulins

Lactoferrin and glycomacropeptide support Bifidobacterium spp. Growth and exhibit other properties attributed to prebiotics (61). In addition, Lactoferrin is an antioxidant glycoprotein with iron-binding properties, and appears to be the best immune-modulator. Whey proteins prevent lipid oxidation and decrease oxidant burden (62). The proteins from whey are rich in cysteine, a critical component of the glutathione (GSH) antioxidant system which is the principle mechanism that protects cells against oxidative stress (63). In comparison to other commercially available protein sources, whey protein optimizes the immune response via enhance production of GSH. Interesting, a recent study showed that whey protein concentrate renders tumor cells more vulnerable to chemotherapy by depleting GSH (64).

Antimicrobial peptides represent an important component of the innate immunity. They can be generated through the proteolytic digestion of whey proteins like γ-lactalbumin, β-lactoglobulin and lactoferrin. The iron-binding capacity of lactoferrin may also contribute to its antimicrobial potential (65). Lactoferrin also acts as anti-viral. Immunoglobulins have been shown to bind the toxin produced by Clostridium difficile, thereby reducing the deleterious effects of infection (66).

There are several reports that whey protein supplementation enhances the immune system. It has been show to significantly improve vaccine response in mice and dogs. In mice, oral whey protein improved response to influenza virus, tetanus toxin and diphtheria (67). In a research study conducted with adult dogs, the immune enhancing benefits of colostrum (which contains 80% whey) were evaluated. The supplementation of colostrum significantly enhanced adult dog immune status and response to canine distemper vaccine as well as increasing the level of GALT activity and stabilizing gut microflora (68). Stimulating immune cells in the gut leads to a cascade of immune cell activation resulting in the secretion of IgA and cytokines may reach the rest of the immune cells via circulation resulting in the improved immune response (Figure 6).

Lactoferrin is immune-modularly in that it exerts both immunoreactive and immunosuppressive actions. Immune effects occur as a result of interaction with the GALT where lactoferrin may bind to epithelial cells or interact with M-cells in the Peyer's patches. IgG is the major Ig found in whey followed by IgM and IgA. Ingested Ig's are known to retain their immunological activities in the human ilium and presume to be equally conserved in dog GI tract (69). They are responsible for a variety of actions which can bolster both the innate immune system provide protection against potential pathogens.

Finally, whey protein is rich in amino acids, particularly glutamate which is a key fuel for immune cells and gut epithelium as they cannot manufacture glutamate independently. Whey protein also contains a variety of GI growth factors which promote GI tract repair after injury, cell proliferation and differentiation and increase mucin production.

Bioactive whey, with whey proteins provide a plethora of bioactive compounds which contribute to immune modulation through a variety of mechanisms resulting in improved vaccine response, antibacterial and antiviral activity and antioxidant activity. The whey proteins interacted with the immune system through GALT leading to systemic effects which benefit dogs.

β1,3-glucans: An indigestible carbohydrate with powerful immune boosting effects

β1,3-glucans modulate both the innate and adaptive immune system through binding with specific receptors and through interaction with immune cells (Figure 12).

The most documented effect of β 1,3-glucans s is its ability to bind phagocytic cells, specifically macrophages through receptors which mediates downstream signaling pathways (70). In addition glucans activate the antimicrobial activity of mononuclear cells and neutrophils in vivo (71) and it can adsorb a variety of toxins including some of those which inadvertently occur in human and pet food sources from time to time (72).

β 1,3-glucans supplementation has been documented to decrease infections in livestock species (73)and dogs and cats against various immune. Two clinical studies conducted in Norway evaluated 80 dogs and 8 cats (74) (75). All age groups of dogs were studied and each dog served as its own control. The following health conditions for each dog were monitored by the veterinarian before and after feeding with β-1,3/1,6 glucan: skin disorders, joint disorders,

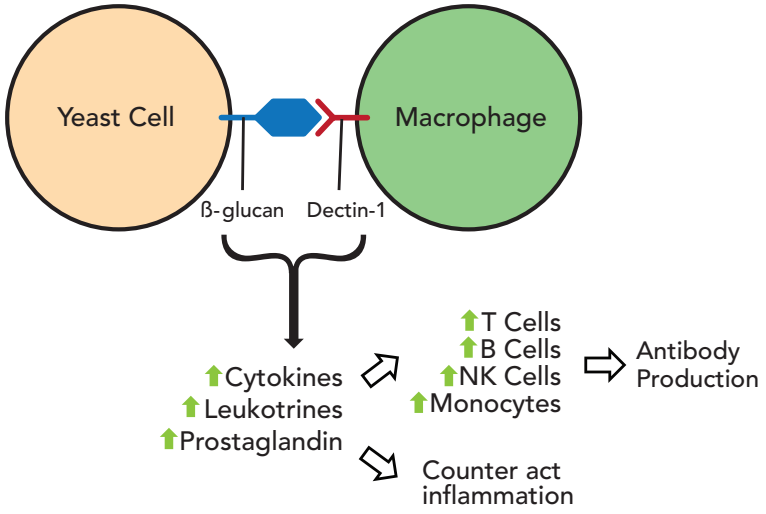


Figure 12. β1,3 glucans bind specific receptors on macrophages which result in cytokine production, inflammatory modulation and antibody production.

mouth disorders, airway disorders, aging symptoms and immune related disorders. After treatment with β-glucan, chronic health conditions were reduced in 70% of the dogs. In a similar cat study, the following health conditions were monitored by the veterinarian before and after feeding with β-1,3/1,6 glucan: mouth disorders, bacterial infections (acute) and aging symptoms (chronic). Each cat served as its own control. After treatment with beta-glucan health conditions were improved in 6 out of 8 cats. Another study used beagles that were fed a control diet or a diet with 500 ppm β-1,3/1,6 glucan from baker's yeast. The beagles fed the β-1,3/1,6 glucan showed increased IgA, and IgM but not IgG after initial and booster vaccinations (76). These results suggest that glucans reach not only Peyer's patches in the GALT but also mucosa-draining lymph nodes as they represent the transition between GALT and the systemic immune system. In another study, healthy adult Labradors had improved vaccine response when fed a diet containing β 1,3-glucans s or brewer's yeast (77).

β 1,3-glucans s have an active and well documented role in immune modulation of both healthy and sick animals.

Enzymes: Bolster Nutrient Absorption And Immune Function

In animals with compromised gut integrity, for example with chronic gut inflammation or irritation, the addition of enzymes may help improve nutrient absorption ad may decrease the likelihood of dietary debris crossing the gut barrier and engaging the immune system. Additionally, proteolytic enzymes have been shown to have anti-inflammatory properties (78).

When the gut is compromised by inflammation or altered transit times for whatever reason, there is usually concomitant malabsorption. Exogenous enzymes, particularly those with a wide range of pH sensitivity, can degrade macronutrients into molecules which are more easily assimilated by an upset gut. The additional advantage of extra digestion of macronutrients is to help prevent dietary debris like large proteins or stress-induced peptides from crossing the gut barrier and increasing the possibility of dietary hypersensitivity or increasing the white blood cell count (79).

Certain bacteria produce carbohydrate metabolites used for messaging and receptor binding receptivity. Enzymes like cellulose, amylase and alpha-galatosidase that digest these bacterial carbohydrates interrupt proliferation of potentially harmful bacteria (80). Lipases can degrade the lipid biofilm of some potentially pathogenic bacteria, again disrupting proliferation of bacteria (79). Oral hydrolytic enzymes are used widely in Europe for human and animals and are reported to be fibrinolytic, anti-edematous, anti-inflammatory and analgesic. The anti-inflammatory effect of oral hydrolytic or proteolytic enzymes appears to be quite complex and involve several different mechanisms including the cleavage of proteins in the complement protein cascade.

Enzymes can improve nutrient absorption, potentially decrease inflammation and discourage pathogenic bacterial proliferation.

Gamma Oryzanol: A Potent Antioxidant

Gamma oryzanol is a rice bran oil derivative with purported antioxidant and anti-stress capabilities presumably from the phenolic acid and sterol components. Gamma oryzanol has traditionally been used as an antioxidant in human foods and as a dietary supplement in Eastern diets. However, new research shows gamma oryzanol to be beneficial for inflammatory conditions and stress (81) (82).

In vitro studies indicate that the components of gamma oryzanol are widely distributed throughout the body and have systemic antioxidant effects (83). Recently, studies in murine models indicate that oral gamma oryzanol may have anti-aging effects on skin, most likely linked to the potent protection from oxidation that it confers. Further murine studies show that oral gamma oryzanol upregulates antioxidant genes and down regulates oxidative stress genes after exercise stress (81). Gamma ory-

zanol inhibits transcription factors for pro-inflammatory genes in macrophages (84).

Gamma oryzanol has long been used for digestive upset and ulcers in humans and the mechanism seems to be two-fold. The first mechanism is related to its ability to normalize the autonomic nervous system response, specifically the vagus nerve system by altering brain neurotransmitter levels (83). The end result of gamma oryzanol supplementation is a decrease in stress related metabolism. The second mechanism appears to be that gamma oryzanol has a direct effect on inflammation in the GI tract. Sever colitis was induced in mice and a series of indices were measured. Animals fed gamma oryzanol has less weight loss and better stool consistency and decreased internal bleeding when compared to controls. Gamma oryzanol decreased mucosal damage and inhibited the transcription of pro-inflammatory cytokines and decreased COX-2 expression (82).

Gamma oryzanol is a potent antioxidant with significant anti-inflammatory properties which can help down regulate inflammation and oxidative stress in the gut and systemically.

Deglycyrrhized Licorice: A Natural Antimicrobial And Mucin Booster

Deglycyrrhized licorice (DGL) is an extract of licorice root reported to increase mucin production in the GI tract and have antimicrobial activity.

Studies in human and murine models indicate DGL confer significant protection against mucosal damage caused by aspirin and other non-steroidal anti-inflammatory drugs by protecting the mucosa. The mechanism behind gastro-protection and antiulcer activity involves the accelerated production of mucin through increased synthesis of glycoprotein sand prolonged life of epithelial cells (85). In a clinical study of chronic duodenal ulcers, DGL was as effective as current antacids or drug therapy. In addition, because of its modulation of prostaglandin and leukotriene production, DGL has anti-inflammatory effects (86). DGL also contains flavonoids that have antimicrobial activity (87).

Deglycyrrhized licorice is a natural herb which has positive effects on mucosa function and mucin production, and because of its chemical composition, has anti-inflammatory and antimicrobial benefits.

Summary

The GI tract is the gateway to the immune system and one of the most effective ways to improve the immune system is through supplementation of natural ingredients which have direct effect on the GI tract. Dietary supplements like Hyper Egg® and Previda® along with a synergistic combination including probiotics, bioactive whey and β 1,3 glucans can significantly improve the immune and overall health of dogs which may be in stressful situations, like traveling or recovering from antibiotic therapy.

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