From the Purina Companion Animal Nutrition Summit: *Focus on Gerontology*; Clearwater Beach, FL; March 26-27, 2010.

Dietary Effects on Gastrointestinal Microbiota of Aging Dogs: Potential Tools to Health Improvement

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Abstract

The relationship among nutrition, gut physiology and microbiology, and host immunology were reviewed in the context of the aging dog and cat. The dynamic interrelationship among these three areas opens opportunities to improve gut health and immunological status of aging dogs by diet formulation. Dietary ingredients, processing and nutritional composition, and mainly some special carbohydrates, like prebiotics, can be potential tools to access gut and health changes in geriatric dogs.

Glossary of Abbreviations
FOS: Fructoligosaccharides
GALT: Gut-Associated Lymphoid Tissue
GIT: Gastrointestinal Tract
IgA: Immunoglobulin A
IgG: Immunoglobulin G
MOS: Mannanoligosaccharides
SCFA: Short-Chain Fatty Acids
YCW: Yeast Cell Wall

Introduction

Aging could be defined as the progressive changes that occur after maturity in various organs, leading to a decrease in their functional ability. The aging process is affected by alterations in physiological systems and metabolic processes. Unfortunately, these alterations are not well-defined in pet animals. Although the terms "old," "senior" and "geriatric" often are used interchangeably, they do have distinct definitions. The term "senior" refers to the functionality of an animal. An animal is considered to be senior when it decreases activity, gains or loses weight, and develops other age-related physical and behavioral changes. Conversely, the term "geriatric" refers to the chronological age of the animal. Generally, large- and giant-breed dogs are considered geriatric at 5 years of age, whereas small- or medium-breed dogs and cats are not considered geriatric until 7 or more years of age.[1]

Geriatric dogs and cats are not recognized to have specific nutrient requirements that clearly differentiate them from mature animals in maintenance.[1] Therefore, geriatric nutrition of dogs and cats is more a good sense approach that includes several aspects of the diet such as palatability, physical form, apprehension and chewing, nutrient composition, energy density, digestibility, and especially the use of nutrients and ingredients to promote health and well-being. This nutritional intervention should aim at prolonging the length and quality of life and delaying the onset of geriatric dysfunction and disease states. To accomplish these goals, it is important to understand age-related changes. Defining such mechanisms lays important groundwork for devising interventions that might further prolong the length of life for which an individual may remain clinically healthy (the "health span").[2]

One nutritional approach in geriatric nutrition focuses on the gut. The two main functions of the gastrointestinal tract (GIT) are the digestion and absorption of nutrients and body protection. Beyond the well-known function to provide nutrients to the organism, GIT is a very active immunological organ that has a complex structure and several specialized cell types that play an important role in protecting the body form the external environment.[3,4] As an active organ, GIT has a high nutrient demand, utilizing a significant amount of the energy, protein and amino acids required by the animal.[5,6]

Thus, an adequate nutrient supply to GIT is important to support good organ development and function. The main source of nutrients for the intestinal mucosa is from the gut lumen, with the blood nutrient supply assuming lesser importance. These compounds absorbed form the lumen come from dietary ingredients or are produced and released by intestinal microbiota. It is well-recognized that intestinal microorganisms play an essential role in digestion and gut health.[1] Several microbial byproducts from organic matter utilization, like amines and short-chain fatty acids, have important specific functions in enterocytes and colonocytes. Considering this, the dynamic interrelationship among nutrition, immunology and gut microbiology opens opportunities to improve gut health and the immunological status of aging dogs by diet formulation.

Age-Related Changes in the Gastrointestinal Function and Metabolism of Dogs and Cats

Studies on age-related physiological alterations in dogs and cats have become more frequent in the last two decades, perhaps as a result of the recent increase in life expectancy of these animals. Aging brings with it physiological changes. Some changes are obvious, such as whitening of hair, a general decline in body and coat condition, and failing senses (sight and hearing). Other changes are less obvious, however, and these include alterations in the physiology of the digestive tract, immune system, kidneys, and other organs. Pets, like people, do not age consistently, and chronological age does not always match physiological age. Although many pets remain active and youthful well into their teens, some dogs start to slow down and may show signs of aging beginning as early as 5 or 6 years of age.[7,8]

Gut physiology and function are altered during the aging process, which is often accompanied by an increased incidence of gastrointestinal infections. With increasing age, several gastrointestinal dysfunctions can manifest in human beings, including slowing of intestinal transit times, decreasing organ reserves, alterations in enzyme activity, impaired circulation, and reduced bile and pancreatic secretions.[9,10] It is not certain if these alterations occur in dogs or cats. In old cats, alterations in GIT function are mainly related to a decrease in digestibility of nutrients such as protein, fat and starch.[11,12] On the other hand, advanced age generally does not reduce apparent nutrient digestibility in dogs.[13] Other age-related changes in cats and dogs include an increased incidence of periodontal disease, difficulty in apprehension and chewing, and increases in diarrhea, vomiting and regurgitation.[14]

Reduced digestibility in old cats does not seem to be related to the duration of gastric emptying nor to intestinal transit time, as no differences were found between the passage of ingested foods through the intestinal tract of aging cats compared to younger cats.[15] Morphological changes in the intestine do not seem to be the cause of decreased nutrient digestibility in humans.[16] However, this does not appear to have been studied in cats; therefore, this hypothesis cannot be rejected as a cause of reduced digestibility in this specie. The consequences of reduced digestibility in aging cats are not precisely known. The reduced digestibility may contribute to decreased fat mass, lean mass and body weight of aging felines.[17] This effect is further reinforced by a lack of change in voluntary eating behavior with aging.[18,19] Thus, the maintenance of food ingestion habits, associated with a reduced utilization of ingested food, would result in lower uptake of bioavailable nutrients.

The concept of "gut health" is complex and broadly defined. According to Conway,[20] three major components of "gut health" can be considered: diet, intestinal mucosa and intestinal microbiota. Intestinal mucosa morphology changes according to nutrition, stress, aging, and/or disease. These changes may affect the physiology of the intestine, influencing nutrient absorption and metabolism. Studying the age and diet effects, Kuzmuk et al.[21] observed morphological differences in young (1.2 years) and old (12.1 years) dogs fed either a plant- or animal product-based diet. Jejunal villus height increased in young dogs consuming the plant product-based diet compared with both young dogs consuming an animal product-based diet and old dogs consuming either a plant product- or animal product-based diet. Colonic crypt depth also was greater in old dogs as compared with young ones.

Fermentable carbohydrates may be considered an important part of "gut nutrition" in old age. They include some types of fiber, resistant starch, non-starch polysaccharides such as mannanoligosaccharides, fructooligosaccharides, stachyose and raffinose, and nonabsorbed sugars that reach the colon and are suitable of bacterial fermentation. They allow an adequate organic matter supply for the large intestine.[22] Bacterial fermentation of these compounds results in short-chain fatty acid (SCFA) production and pH reduction, which could modify the composition and metabolic activity of the intestinal bacteria,[23] which in turn could reduce the quantity of nitrogenous waste materials entering the bloodstream. SCFAs, especially butyric acid, are important energy sources for the colonocytes,[1,24] lead to suitable ion absorption, and act in intestinal blood flow and peristalsis. In a study with dogs, fermentable fiber promoted better development of colon mucosa, greater relationship between colon volume and surface, and improved the histological mucosa structure.[25] Thus, whereas non-fermentable fiber acts as bulk, fermentable fiber plays other important physiological roles.

Although not the focus of this review, it is important to consider that GIT has a high metabolic rate and represents a high demand for nutrients delivered by dietary ingredients. The intestinal mucosa has the highest rates of proliferation and cell renewal throughout the body, and this process can utilize from 10 to 20% of the energy and up to 50% of the daily protein requirement.[26] Protein, arginine, glutamate, glutamine, glutathione, glycine, histidine, vitamin A, zinc, and fatty acids are among nutrients that are key for the intestinal mucosa[27] and that must be adequately supplied by diet to ensure the intestine develops adequately its functions of digestion and protection.

Alterations in nutrient profile and dietary fermentation patterns as related to old age have not been well studied in companion animals. It is possible that by manipulating nutrient composition, and gut microbial composition, activity and fermentation byproduct formation, the gastrointestinal health of old companion animals could be enhanced, ameliorating some of the consequences of old age and promoting health and well-being.

Gut Microbiota

The GIT of dogs and cats presents a microbial colonization pattern similar to that of other mammals. At birth, the intestines

are sterile but are rapidly colonized by environmental bacteria. The colonization process follows successive populations' changes affected by age, health status, diet, and environment.[28,29] The normal intestinal microbiota plays an important role in host digestion and metabolism, and provides a natural defense mechanism against invading pathogens.[1]

Although the microbiota in adults has been studied, less is known about the changes that occur with aging.[3,30] These may have important consequences in senior pets, especially in those receiving antibiotic therapy and that are most susceptible to intestinal dysbiosis. Intestinal microbiota of young adult dogs seems to comprise large numbers of *bacteroides, bifidobacteria, lactobacilli*, and *anaerobic cocci*, while older animals harbor greater numbers of *clostridia* and *enterococci*.[32] However, some studies have found conflicting results, such as increasing *lactobacilli* and *bacteroides* in old dogs.[33]

The causes of changes in the microbiota with age are still unclear. A number of physiological and immunological changes occur in the body with advancing age. Hopkins et al.[32] suggested that some bacterial strains could take advantage of new ecological niches, thereby inducing a shift in the composition of the gut microbiota. It has been proposed that reduced adhesion to the mucosa may be a factor involved in the decreasing colonization of old subjects by certain species of *bifidobacteria*.[34] These bacterial community shifts in the large bowel may have great effects on host physiology and metabolism, aspects that need further studies.

Dogs and Cats Immunosenescence and Inflammageing

Immunosenescence may be defined as the multifactorial complex of changes that occur in the immune system of elderly individuals that predispose them to increased morbidity and mortality due to infection and age-related diseases.[2] Changes in immune status are considered major contributing factors toward morbidity and mortality in aging humans. In many other species, this age-related remodeling of the immune system has also been recognized and commonly involves the deterioration of some aspects of immunity accompanied by the enhancement of others. Such changes may leave aged individuals susceptible to infection and are possibly related to the increased incidents of cancer observed in the elderly of all species.[35]

Inflammageing is a term that refers to changes in the balance of pro-inflammatory and anti-inflammatory agents produced in the elderly.[36] During a lifetime of constant antigenic challenges, the adaptations of the animal to produce efficient inflammatory responses can confer high resistance to infectious diseases, but also an increased susceptibility to inflammation-based diseases later in life. On the other side, low inflammatory responses, while rendering the subject more susceptible to infectious diseases, can confer a survival advantage in old age. A large part of the aging phenotype, including immunosenescence, is explained by an imbalance between inflammatory and anti-inflammatory networks, which results in the low-grade chronic pro-inflammatory status called inflammageing.[36] For successful longevity, an individual must therefore find the means of reducing the impact of pro-inflammatory factors while still maintaining the essential aspects of protective immunity and preventing the emergence of deleterious (e.g., auto reactive) immunity.

Mounting evidence for prolongation of the canine and feline health span through nutritional intervention is becoming available.[37,38,39] Pet food manufacturers have been active in the investigation of immunosenescence and inflammageing in the dog and cat, with a view to formulating supplemented specialized diets that may slow these processes.

The effects of the aging process on dogs' and cats' immune system were recently reviewed by Day.[2] Briefly, these include an age-related decrease in the proliferative response of blood mononuclear cells to mitogens; a decline in the numbers of peripheral blood lymphocytes, B-cells, T-cells, and relative percentage of CD4+ T-cells (helper); and an increase in the relative percentage of CD8+ T-cells (natural killer) resulting in a decreased ratio CD4+:CD8+ cells. Phenotypic alteration is accompanied by functional changes, such as reduced ability to respond to stimulation by nonspecific mitogens, relative changes in the balance of Th1 versus Th2 CD4+ T-cell activity, and reduced delayed type hypersensitivity response to mitogens. Although there is general agreement on age-related changes to immune status, contradictory results have been produced by different research groups that can be attributed to some extent to the use of different techniques or different subject selection criteria.[35]

These changes in peripheral blood lymphocytes seem to occur also within the intestinal lamina propria of the aging dog with reduced T cell numbers and lower proliferative activity of intestinal cell populations.[2,40] The GIT is the largest lymphatic cell-bearing organ, playing a major role in both local and systemic immunity, including blocking pathogens, modulation of immune response and oral tolerance.[40] As a result, these immunological consequences of aging are likely to have a health impact in these animals. The mucosal immune system of GIT in dogs consists of organized lymphoid structures, including Peyer's patches, mesenteric lymph nodes, and the intestinal lamina propria.[41] The latter is populated by cell types including T and B lymphocytes, macrophages, mast cells, dendritic cells, neutrophils, and eosinophils.[42] The gut-associated lymphoid tissue (GALT) performs the following activities: a) capture, processing and presentation of ingested antigens; b) local antibody production, especially immunoglobulin A (IgA); and c) activation of immune-mediated responses, particularly those mediated by cytotoxic T cells CD8+ or NK (natural killer cells) and macrophages.

It is speculated that these gut-associated immunological

changes may be caused by alterations in intestinal microbiota that are reported in aging dogs.[2] The increased numbers of aerobic and anaerobic bacteria in fecal samples, especially of *clostridia* from old dogs compared with young dogs, could be one of the causative factors of these immune changes. The possible use of fermentable carbohydrates, prebiotics and probiotics to induce a favorable shift in gut microbial population and bacterial end products formation in old animals is an interesting avenue of research, with interesting prospects of alleviating the adverse effect of immunosenescence in companion animals.

Potential Tools to Health Improvement Through Exchange in Gut Microbiota

Companion animal health is linked to many factors, not least being the maintenance of gut function and environment. The last decade has provided useful research into this area, allowing nutritionists to use commercial products to help establish and maintain the gut environment.

A key factor in animal health is the status of *eubiosis*, i.e., the establishment and maintenance of a stable and healthy microbiota in the digestive tract. *Eubiosis* can be broken down into several areas: digestion of nutrients, vitamin synthesis, stimulation of the immune system, protection/strengthening of mucosa as a barrier to invasion, and antagonistic effects against pathogenic microorganisms.[43] At any life stage those would be key points to maintain health, but those considerations should be mostly important during the time that animals may be more susceptible to disbiosis and other manifestations of GIT dysfunction — as in old age.

The mechanisms by which the normal microbiota helps the host animal's health include lowering the colonic pH and producing SCFAs. Low pH is inhibitory to the growth of many pathogenic bacteria and may reduce the intestinal absorption of potentially toxic compounds, such as ammonia. The SCFAs produced are rapidly absorbed from the intestinal lumen, with 95 to 99% being absorbed before reaching the distal colon. Besides being the primary energy source for colonocytes, SCFAs promote local mucosal health and integrity by stimulating proliferation, maturation and differentiation of colonocytes in the crypts, and facilitating the normal secretory and absorptive functions of the colon. They also stimulate protein synthesis and mucin production, assuring the integrity and effectiveness of the physical barrier.[1,44] Moreover, butyrate may also inhibit the development of malignant colonic cells, but this effect is still controversial and seems to depend on the joint presence of both butyrate and fish oil.[45]

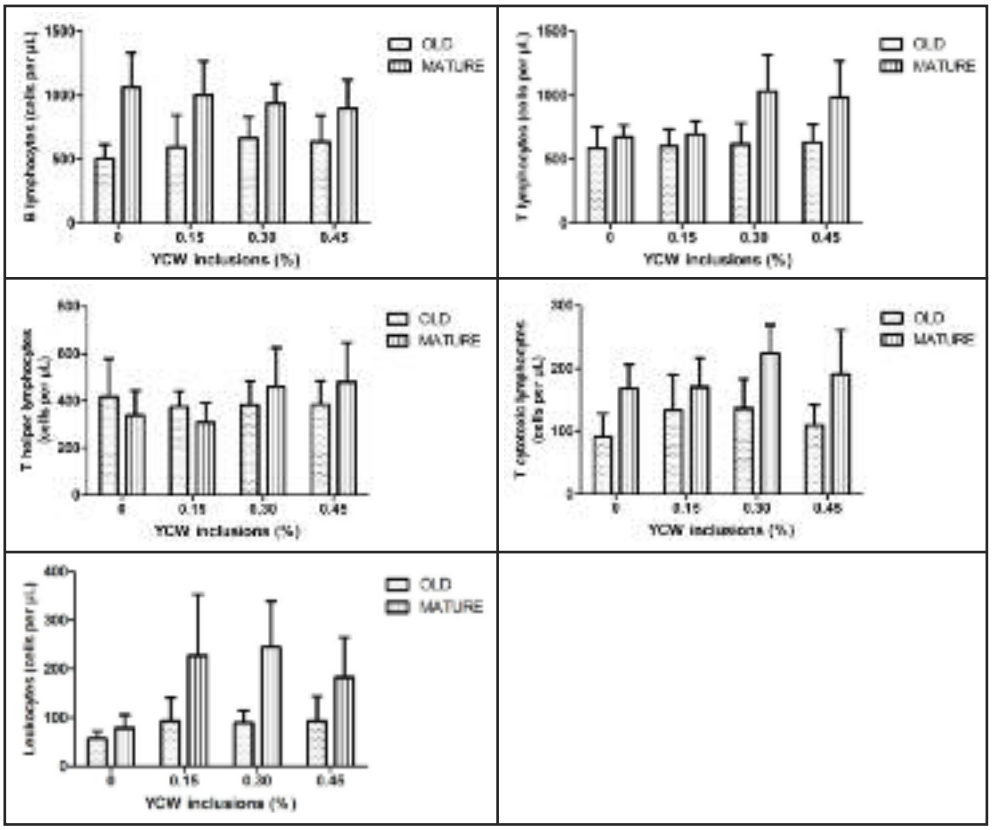
In vitro and *in vivo* studies show that end products of fermentation produced by colonic bacteria depend largely on the chemical composition of the digesta reaching the large bowel, especially protein and undigested carbohydrates. The amount produced and the ratio of individual SCFAs (e.g., acetate, propionate and butyrate) and lactic acid vary, depending on substrate and colonic microbial populations. Microbial fermentation of undigested amino acids results in the production of several putrefactive compounds. These include ammonia, which results from the deamination of amino acids, phenols, indoles (products of aromatic amine decarboxylation), branched-chain fatty acids (derived from branched-chain amino acid catabolism), and several biogenic amines, such as putrescine, cadaverine, histamine, pheniletilamine, and others. These protein catabolites not only result in fecal odor, but also can be toxic at high concentrations.[21]

Of special interest for the formulation of pet food and specific veterinary diets is the use of special ingredients that can influence the composition and metabolic activity of the intestinal microbiota, promoting the maintenance of the eubiosis status.[46] Noteworthy among these compounds are the prebiotics. A prebiotic is "a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health."[47] Because of their chemical structure, these compounds are not absorbed in the upper part of the GIT or hydrolyzed by digestive enzymes,[48] reaching relatively intact into the colon. Modification by prebiotics of the composition of the colonic microbiota leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, *lactobacilli* and *bifidobacteria*.[47] Basically, two mechanisms of microbiota alteration by prebiotic use are proposed: nutrient supply for desirable bacteria and competitive exclusion. Prebiotics can also modify the metabolic activity of the colonic microbiota, reducing the concentration of undesirable compounds like ammonia, biogenic amines, indoles, fenoles,[30] even without changes in fecal bacterial counts.[50]

Prebiotic use in companion animal nutrition was recently reviewed.[51] In comparison to other species, little information is available in dogs and especially in cats. There are still controversies about the effectiveness of these compounds,[52] with conflicting results among studies. One important consideration about prebiotic action is the strong influence of basal diet; diet type, diet nutritional composition and ingredient quality also influence the composition of colon microbiota and bacterial end product formation,[53] pointing, perhaps, to the need for prebiotic application in select dietary situations.

Among the prebiotics, fructoligosaccharides (FOS) are the most studied in dogs and cats. They can be used to alleviate small intestinal bacterial overgrowth,[54] to promote a reduction in *clostridia*, an increase in *bifdobacteria* and *lactobacilli* populations,[55] and to reduce the concentrations of protein catabolites produced in the colon. FOS is a readily available energy source for gut microbiota, reducing the bacterial fermentation of protein to supply energy and increasing the incorporation of N-containing substances into bacterial protein. High doses, however, can result in soft feces production and reduced nutrient digestibility.

Figure 1: Lymphocytes subset consentrations in peripheral blood of mature (4 years old) and old (10 years old) Beagle dogs, after consumption of diets with different inclusions of yeast cell wall (YCW).



Another prebiotic evaluated in dogs is the mannanoligosaccharide (MOS). MOS can be isolated from yeast cell wall (YCW), from which the mannan fraction can be as much as 31%, making YCW a potential prebiotic source for pet food. The consulted literature assigns to YCW the ability to change beneficially the intestinal microbial counts and/or their metabolic activity.[46] Moreover, there are reports of immunomodulatory effects for this prebiotic, including increased concentrations of IgA, immunoglobulin G (IgG) and plasma lymphocytes.[51] Mucus secretion by intestinal goblet cells also appears to increase in diets supplemented with YCW, strengthening the defense barrier in the gut. MOS has the ability to agglutinate *Escherichia coli* and *Salmonella* strains that possess mannose-specific fimbriae (Type-1 adhesins), reducing the intestinal binding and colonization of these bacteria.[56] However, MOS has other mechanisms for beneficial change of enteric microbiota, since its addition to diet can also reduce *Clostridium perfringens* counts in dog feces,[52] and *clostridia* species do not possess mannose-specific fimbriae.

In vitro studies suggest that MOS is moderately fermentable by canine and feline microbiota,[57] being a source of energy to lactate-producing bacteria. This explains the reduced fecal pH and fecal ammonia excretion verified in dogs, improving indices of colonic health.[46] MOS is a surface carbohydrate of yeasts, being detected by the animal immune system through mannan-binding lectin receptors in macrophages. These receptors recognize cell wall compounds of pathogens, including many bacteria and some viruses, and can result in opsonization and activation of the complement cascade.[58]

In an study conducted by Gomes et al. (unpublished data)[59] to evaluate the prebiotic potential of the YCW for dogs, four isonutrient kibble diets were used with inclusions of 0%, 0.15%, 0.30% and 0.45% of YCW. Eight Beagle dogs were used, divided in two groups; four mature animals (4 years old), and four old dogs (10 years old). The experiment followed a two 4x4 Latin square design, one Latin square for mature and the other for the old dogs. In each period an adaptation phase of 15 days preceded a five-day total feces collection for digestibility trial and one-day collection of fresh fecal samples for bacterial enumeration, pH measurement and determination of short-chain fatty acids and bioactive amines concentration. On day 21, blood samples were collected for immunophenotypic quantification of lymphocyte subsets through flow cytometry. The data were evaluated using Proc GLM of SAS; means were compared by Tukey test with polynomial and orthogonal contrasts ($p<0.1$).

Nutrient digestibility and metabolizable energy did not vary between diets, showing no effects of YCW ($p>0.05$) and also no effect of age ($p>0.05$). Yeast cell wall supplementation did not result in differences in fecal counts (log of CFU per g of feces DM) of total aerobes, total anaerobes, *E.coli, Clostridium spp, Lactobacillus spp*, and *bifdobacterium spp* ($p>0.10$). An age effect on fecal bacteria counts was not verified; only a tendency for total aerobe increases in old dogs ($p=0.15$) was found.

Some indices of bacterial metabolic activity, on the other hand, changed. The inclusion of YCW resulted in a linear increase in fecal concentration of butyrate (mMol/kg DM; $p=0.055$), and in reductions of fecal concentrations of some bioactive amines (tyramine, histamine, phenylethylamine, and tryptamine). These alterations suggest that YCW may improve

gut health, reducing the formation of toxic compounds delivered during protein fermentation and increasing butyrate supply to colonic mucosa. An age effect on microbial degradation products was also verified. Older dogs presented lower fecal concentrations of butyrate (p=0.01), histamine (p=0.04), agmatine (p<0.01), and spermine (p=0.01), and higher fecal pH (p=0.03). These findings suggest alteration in bacterial metabolic activity and end product formation, with a decrease in colonic fermentation with aging.

Dogs showed a linear increase in T lymphocyte subset concentration (cells/ L; p=0.1) and a higher number of B lymphocytes (p=0.05) with YCW addition, evidencing immune stimulation of the animals. Compared to younger adult dogs, older dogs showed a decrease of T lymphocytes (p=0.01), T-cytotoxic lymphocytes (p<0.01), and B lymphocytes (p<0.01) concentrations. Although the evaluation of the two groups together demonstrated an YCW effect, adult dogs exhibited a more exuberant change in peripheral lymphocyte subsets than the old dogs to this prebiotic (Figure 1). Unfortunately, the small number of dogs in each group (only four) does not allow an adequate statistical comparison of these findings, but one possible implication is that higher doses of oligosaccharides are required for senior animals due to the reduced fermentation activity in their large bowel.

Conclusion

The importance of nutrition in old animals lies with the aim of preventing or slowing the progression of the metabolic changes that follow the aging process. Research into the desirable dietary characteristics of the foods for old dogs and cats is needed, opening opportunities to improve health and well-being. The comprehension of the underling mechanisms that result in gut microbial population and/or metabolic activity alterations in old age, their consequences to host immune status, and how to use the diet to favorably manipulate gut microbiota of old dogs and cats might allow the development of dietary tools for health promotion in these animals.

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Q&A Discussion

Q: Dr. Avi Patil, Nestlé Purina Research: In your study you included beat pulp, which is a soluble fiber, in your diets. Do you think that if you did not have beet pulp in the diet, you would have had a stronger response in your study?

A: Dr. Carciofi: Yes. What we provided was a minimum, about 2%, fiber in the diets. Now, we are working on adding soluble and nonfermentable fibers derived from sugar cane. Certainly the base diet will influence the outcomes. This is why it is difficult to compare results across studies. For example, we had a study that included 15% soybean meal as a protein source in the diet. Soybean meal contains various nondigestible sugars, stachyose and raffinose. We did not find any benefit from the addition of prebiotics to this base diet because there already were plenty of fermentable carbohydrates.

Q: Dr. Richard Hill, University of Florida: What was the water content of the food?

A: Dr. Carciofi: I did not show the results, but the water did not have an effect.