THE AGING FELINE: ADVANCES IN NUTRITION AND CARE FOR THE OLDER CAT

Considering Older Cats .................................................................1
Danièlle Gunn-Moore, MRCVS, PhD

Gerardo Pérez-Camargo, MRCVS, PhD

Nutritional Influences on the Immune System in Aging Felines ............11
Korinn E. Saker, DVM, PhD

Effects of Dietary Components on the Development of Hyperthyroidism in Cats .........................................................15
Patricia A. Schenck, DVM, PhD

New Findings in Feline Hypertension ..............................................20
Scott A. Brown, VMD, PhD

Feeding the Aging Cat With Chronic Renal Failure ........................24
Dennis J. Chew, DVM, Patricia A. Schenck, DVM, PhD, and C.A. Tony Buffington, DVM, PhD

NUTRITIONAL ADVANCES IN GASTROINTESTINAL HEALTH AND DISEASE

Probiotics in Health and Disease: Potential for Pets ..........................29
Jalil Benyacoub, PhD, Christoph Cavadini, PhD, and Thierry von der Weid, PhD

An Assessment of Prebiotic Use in Companion Animal Diets ..................34
Kelly S. Swanson, PhD, and George C. Fahey, Jr., PhD

Intestinal Immunity and Oral Tolerance ...........................................43
Nicholas J. Cave, BVSc, MVSc, MACVSc

Feline Inflammatory Bowel Disease: Beyond Qualitative Histopathology .........................................................46
Kenny Simpson, DVM, PhD

Mechanisms and Clinical Applications of Nutrition in Inflammatory Bowel Disease ..........................51
Nicholas J. Cave, BVSc, MVSc, MACVSc, and Stanley L. Marks, BVSc, PhD
RESEARCH ABSTRACTS—ORAL PRESENTATIONS

Long-term food consumption and body weight changes in a controlled population of geriatric cats ..........60
C. Cupp, G. Pérez-Camargo, A. Patil, and W. Kerr

Effect of age on fecal microflora of cats........................................................................................................60
A.R. Patil, L. Rayner, and P.A. Carrión

Immediate appetite stimulation of anorexic cats with midazolam.................................................................61

Older cats with gastrointestinal disease are more likely to be cobalamin deficient.........................................62
D.A. Williams, J.M. Steiner, and C.G. Ruaux

The effects of obesity on the feline immune system .........................................................................................63

Immediate appetite stimulation of anorexic dogs with propofol.....................................................................64

Metabolizable energy requirement for maintenance based on body mass components of diet-restricted and control-fed dogs......................................................................................................................65

Contributions of fatty acids (FA) from diet, de novo synthesis, and adipose FA to milk FA in normal and lipoprotein lipase (LPL)-deficient cats ..........................................................................................66
B.C. Veltri, R.C. Backus, E.J. DePeters, and Q.R. Rogers

Maternal dietary alpha-linolenic acid during gestation and lactation does not increase canine milk docosahexaenoic acid content ........................................................................................................67
J.E. Bauer, K.M. Heinemann, K.E. Bigley, and M.K. Waldron

Screening and selection of pet probiotics ........................................................................................................68
R. Knorr, F. Praplan, J. Benyacoub, and C. Cavadini

Use of herbal antidiarrheal compounds in canines—A novel approach ..........................................................69
V. Deshmukh

Evaluation of two diets in the management of cats with naturally occurring chronic diarrhea ......................70
D.P. Laflamme and G. Long
RESEARCH ABSTRACTS—POSTER PRESENTATIONS

Effect of age and sex on feline skin ................................................................. 71
J. Cline, L. Young, W. Kerr, and S. Nauman

Body composition changes in aging cats ...................................................... 71

Incidence of impaired nutrient digestibility in aging cats ......................... 72
A.R. Patil, C. Cupp, and G. Pérez-Camargo

Effect of diet type on fecal microflora of cats .............................................. 72
A.R. Patil, L. Rayner, and P.A. Carrión

Extrusion effects on in vitro fermentation profiles and viscosity measures of selected fibrous substrates 73

Effect of chicory on fecal quality in dogs and cats .................................... 73
G. Czarnecki-Maulden and A.R. Patil

Effect of aging on blood metabolites in the cat ........................................... 74

Comparison of sodium bisulfate and phosphoric acid as urine acidifiers for cats ......................................................... 75

Influence of fatty acids on glucose clearance, insulin secretion, and lipid metabolism in cats .................. 76
C. Wilkins, M.K. Waldron, D.C. Ferguson, and M. Hoenig

Intra- and extra-myocellular lipid content increased with weight in cats ........ 77
C. Wilkins, R.C. Long Jr., D.C. Ferguson, M.K. Waldron, and M. Hoenig

Evaluation of pyruvate supplementation on body weight and fat loss in overweight dogs ..................... 78
P. Zhang, J.R. Jackson, M. Roos, S. Bhatnagar, and L. Bruns

Influence of lifetime diet restriction on lean and fat body composition of Labrador retriever dogs: Predictive aspects ...................................................... 79
D.F. Lawler, B.T. Larson, E.L. Spitznagel, and R.D. Kealy

Influence of lifetime diet restriction on bone minerals in Labrador retriever dogs ............................................. 80

Use of herbal preparations in dog foods as a therapy in parasitic infestation .................................................. 81
P. Deshpande

Studies on growth promoting and immunostimulation properties of herbs .................................................. 82
A. Deshpande and S. Rajurkar

Maternal diet fatty acids modify canine puppy plasma lipoprotein distribution during the suckling period ............................................................... 83
J.E. Bauer, A.S. Wright, K.E. Bigley, and M.K. Waldron

CONTENTS
There are now more elderly pet cats than ever before. Cats are more popular than dogs as pets, and improvements in nutrition, health care, and management have lead to many cats living to increasingly greater ages. In the United States over the last 10 years, there has been a nearly twofold increase in the percentage of pet cats of over 6 years of age (from 24% to 47%), a 15% increase in cats over 10 years of age, and the proportion of the feline population aged 15 years or older has increased from 5% to 14%. While less data are available for cats in Europe, the average age has increased from 4.7 to 5.3 years, and it is estimated that there are currently about 2.5 million “senior” cats in the United Kingdom. Since this accounts for approximately 30% of the pet cat population, the good management of these individuals is becoming an ever more important consideration for small animal veterinary practitioners and nutritionists.

In order to determine the best ways to care for older cats, we first need to decide at what age a cat becomes “senior” and at what age it becomes “geriatric.” Cats, like humans, do not age consistently, and chronologic age does not always match physiologic age. Some cats show obvious signs of old age after 10 years while others appear almost unchanged until they reach 15 to 16 years of age. That said, it is generally considered that cats become “senior” at about 7 to 8 years of age and progress to “geriatric” by 12 to 15 years of age. Interestingly, some authors recommend that longer-lived breeds, such as Siamese, should be considered “senior” when they reach 11 to 12 years of age, while shorter-lived breeds, such as Persians, may become “senior” by 6 to 7 years of age. Table 1 shows the approximate correlation between cat and human ages.

It is only by understanding how cats change with age that we can try to care for them in ways that best support a long and healthy life. To do this we need to know how their advancing age is affecting their bodies. Some changes are obvious, such as whitening of hair, general decline in body and coat condition, and failing senses (sight and hearing). However, other changes are less obvious and include alterations in the physiology of the digestive tract, immune system, kidneys, liver, brain, and skeleton. Thankfully, there are now an increasing number of studies investigating the effects of aging in cats, so we no longer need to rely on extrapolation from other species.

All aspects of a cat’s life may affect its potential longevity and overall quality of life. However, perhaps the most important concepts to understand involve the complex interplay between concurrent physiologic and pathologic changes and how they affect the older cat’s ability to maintain its body weight, accommodate changes in its environment, fight off infection, and cope with disease. A number of these interacting factors will be discussed below.

Older animals often experience changes in their body weight. It is recommended that owners keep a regular record of their cat’s weight and that it is checked at each clinic visit. This is because significant and/or rapid weight change can have very serious implications, irrespective of the underlying cause.

Until recently, it was assumed that older cats, like dogs and humans, have a significantly reduced energy requirement and therefore a tendency to become obese. Indeed, a slight trend toward a decreased maintenance energy rate (MER) has been shown in cats of up to 10 years of age. However, there is also increasing evidence that there is a much greater tendency for geriatric cats (over 10 years of age) to be underweight. The difference in the risk of midlife obesity between cats and dogs probably results from their differing lifestyles. Dogs tend to be energetic when young and slow down as they age. In contrast, cats are relatively inactive throughout most of their lives. It is probably because of this that they do not show a significant age-related decline in either MER or lean body mass to fat ratio.

Ideally, cats should be fed to maintain their optimal body weight, and probably the single most important aspect to feeding older cats is that their body weight should remain stable. Long-term studies have shown that either obesity or excessive thinness increases mortality. While obesity itself reduces life span, it also increases the risk of many weight-related diseases, including heart disease, diabetes mellitus,
Many older cats experience weight loss. This can result from a number of different, often interacting, factors. These may include physiologic aging changes, the presence of pathologic disease processes, or behavioral alterations. Weight loss is often associated with inappetence, and in older cats this commonly results from reduced senses of smell and taste and/or oral pain associated with periodontal disease. In addition, older cats tend to be less efficient at digesting their food. This probably results from reduced intestinal function, gastric acid production, gastric and intestinal motility, and intestinal blood flow. Older cats may also have reduced pancreatic lipase activity and changes in the composition of bile. While these factors affect the digestion of all dietary components, they particularly affect the digestion and absorption of fats and proteins. Most cats will compensate for this by increasing their daily food intake. However, some individuals may need to increase their intake by as much as 25%. Due to the limitation of their stomach capacity, this means that they need to eat many small meals a day.

Weight loss is likely to result when more frequent meals are not offered or when eating is painful. To compensate for this, many older cats may benefit from being fed a highly palatable, highly digestible, energy-dense food offered frequently in small amounts.

Significant weight changes should always be investigated because weight loss is often the first sign of disease. Interestingly, while many of the diseases seen in older cats are associated with inappetence and a reluctance to eat, this is not always the case. With hyperthyroidism and some of the malassimilation syndromes (e.g., inflammatory bowel disease or early stage gastrointestinal lymphocytic lymphoma), weight loss may be accompanied by a good or even increased appetite. Owners therefore need to know that any alteration in appetite is significant, whether it is an increase or a decrease.

As cats age they have reduced sensitivity to thirst. This results in an increased risk of dehydration, especially when combined with excessive urination. The latter is commonly associated with either concurrent chronic renal insufficiency (CRI) or DM, and both of these conditions occur commonly in older cats. It is often advisable to feed older cats a diet with a high water content. However, if cats are unwilling to eat wet food, it may be helpful to increase their fluid intake using other methods. Drinking can be encouraged by ensuring constant access to free water, using bottled water or pet water fountains, or by giving fishy water or chicken/meat stock (ensure that no onion or onion powder has been added because cats can develop hemolytic anemia if fed too much onion).

Many of the specific nutrient requirements for older cats have yet to be determined. However, a number of studies have been performed and their findings are discussed on page 6 (“Cat Nutrition: What Is New in the Old?” by Dr. Gerardo Pérez-Camargo).

Unfortunately, older cats often cope very poorly with changes in their daily routine. Their response to stress is often to stop eating, hide, and/or alter their toileting habits. Any change within the environment, the family, or even the diet can act as a source of stress. Because diet changes can be stressful, it is important to make changes slowly, gradually introducing the new food in a separate bowl while keeping the old food available. Unfortunately, in some very easily stressed cats, diet changes cannot always be made. Because many older cats experience difficulty coping with alterations in their environment, it is important to consider this when planning changes. When possible, changes should be kept to a minimum, and when they have to be made they should be made slowly and with much reassurance. Some geriatric cats become progressively senile. These cats may benefit from having their area of access reduced while still containing all necessary
Some of these infections are related to the general immune senescence associated with age. However, the major importance of arthritis in older cats is supported by radiographic evidence of degenerative joint disease in 90% of cats over 12 years of age. While the cause of arthritis is usually multifactorial, trauma, diet (obesity), and genetics all play a role. Recognizing and addressing the causes and presence of arthritis can make a considerable difference to the quality of an older cat’s life.

Many older cats develop clinical illness, and the diagnosis and treatment are often complicated by the concurrence of multiple interacting disease processes. Prompt and full investigation is essential if treatment is to be successful. Unfortunately, it is not always easy for owners to recognize the signs of ill health, so it is important that they monitor their older cats for changes in food and water consumption, body weight, production of urine and feces, and behavior. The implementation of senior health care clinics by primary care veterinary practices can be very beneficial. While the clinics need to be tailored to individual cats, in general they should include regular and thorough physical examinations (including assessment of body weight, systolic blood pressure, and retinal examination).

In addition, a blood sample is usually collected for biochemical screening, thyroid level assessment and hematology, and where appropriate, serologic testing for FeLV and/or FIV. A urine sample should undergo routine urinalysis and, where possible, bacterial culture. Initially, most cats will only need to visit a clinic on a yearly basis. However, cats showing significant aging changes may need to visit more frequently for repeated reassessment, monitoring, and treatment.

Once a disease has been diagnosed, it is important to remember that changes in physiology also affect the pharmacokinetics of many drugs. Most drugs need to be metabolized in some way, and most drug metabolism occurs in the liver and/or kidneys. Liver disease, low levels of blood albumin (which binds to many drugs), and CRI all occur frequently in older cats. When coupled with mild dehydration (which is common in older cats), these can result in reduced clearance rates and marked elevations in circulating drug concentrations. When treating geriatric patients, the dose and dosing intervals of some drugs may therefore need to be altered. For example, the dose of metronidazole given for the treatment of suppurative cholangiohepatitis may need to be significantly reduced, while the dosing interval of aspirin given in the management of thrombosis associated with hypertrophic cardiomyopathy may need to be increased. However, it is not only drug overdose that needs to be considered. In humans, adverse drug reactions are two to three times more common.

### TABLE 2

<table>
<thead>
<tr>
<th>Approximate Correlation of Cat Age to Body Condition&lt;sup&gt;6–9&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of cat</td>
<td>% too thin</td>
<td>% too fat</td>
</tr>
<tr>
<td>1–2 years</td>
<td>&lt;10</td>
<td>20</td>
</tr>
<tr>
<td>2–10 years</td>
<td>&lt;10</td>
<td>20–50</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>30–50</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

Facilities. This small area can then be kept safe and constant.

The immune function of all mammals deteriorates with age. While there are only a few studies looking specifically at the effect of aging on the immune system of cats, these studies appear to confirm that this is the case. Older cats have significantly lower numbers of total white blood cells (particularly CD4<sup>+</sup> lymphocytes), while neutrophil counts are raised. These changes are likely to result in a reduced ability to fight infection or to screen for neoplastic cells. This may explain the increased risk of neoplasia in older cats.

The age-related risk of infection can perhaps best be demonstrated by looking at the age-related incidence of bacterial cystitis. Clinical signs suggestive of bladder disease include increased frequency of urination, straining to urinate, blood in the urine, or a blocked urinary tract. In cats under 10 years of age, a bacterial cause is found in only 1% to 2% of cases. In the majority of these young cats, no obvious cause can be found (although stress and diet may play a role), and some are found to have bladder stones. However, the situation in older cats with cystitis is very different, with almost 50% of cats over 10 years of age having a bacterial cause. Some of these infections are related to the general immune senescence associated with age. However, the majority is associated with CRI or DM, both of which are diseases that occur commonly in older cats and that are locally and systemically immunosuppressive.

Older animals are susceptible to many diseases, and veterinary surgeons typically list the most common ones as kidney disease, hyperthyroidism, neoplasia, dental disease, DM, and arthritis. Interestingly, owners place arthritis at the top of the list, followed by kidney disease, deafness, blindness, hyperthyroidism, bronchitis, and dental problems. The role of arthritic pain in reducing the quality of life for many older cats has probably been significantly underestimated. Many owners report having to assist their older cats by moving food and water bowls to lower surfaces, adding ramps to allow easier access to favored sleeping areas, and placing low-sided litter boxes within easy reach of the cat. The increasing
CONSIDERING OLDER CATS

in people over 60 years of age. The situation is likely to be similar in cats, so we need to be observant when medicating older cats.

While veterinary medicine can often offer complex therapeutic options and sophisticated prescription diets, it is important to remember that older cats are often poorly tolerant of the stress of hospitalization or excessive physical handling. It is essential that each cat be assessed and treated as an individual. In some cases, investigations and interventions may have to be adapted or even abandoned if they are poorly tolerated for either medical or temperamental reasons. Also, once a patient’s quality of life can no longer be maintained, it is important that euthanasia be discussed and then performed as compassionately as possible.

While it is true that “old age is not a disease,” it is important that we pay particular attention to older cats, feed and care for them appropriately, and observe them closely so we can keep them well for as long as possible.

ACKNOWLEDGMENTS

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REFERENCES

Cat Nutrition:
What Is New in the Old?

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OBJECTIVES AND INTRODUCTION

The main aim of this presentation is to review our current understanding of the life stage physiology of the domestic cat and review data from Nestlé Purina studies that have implications on the nutritional requirements of old cats. We hope to identify facts that might bring improved nutrition and product consistency for old cats and also highlight unclear areas that could be a focus for future research efforts. We welcome your feedback and are hopeful that this may lead to an open discussion and input on new ideas to pursue.

There is a moderate amount of published data on the nutritional requirements of old cats, but there also seem to be a considerable number of misconceptions in this area, particularly concerning protein and energy requirements. As a result, within the global pet food industry, there are inconsistencies among different manufacturers in their approaches to diets for old cats. For example, in comparison with adult life stage diets, some increase protein and others maintain or reduce it. A similar consistency exists for fat and calorie content of diets for old cats versus the adult life stage diets. The only common trend seems to be that most super-premium diets designed for old cats include enriched levels of antioxidants and vitamins. Looking to Association of American Feed Control Officials (AAFCO) for recommendations does not solve the problem because there are currently no AAFCO nutrient profile recommendations specifically for senior cats.

It could be argued that the aged cat will most likely suffer from at least one health condition, suggesting it would make more sense to deal with each old cat individually by providing tailor-made nutrition. However, there are some common trends in the aging process of cats that can help us formulate products that better match the nutrient requirements of the population at different life stages. Aging is a natural process, not a disease.

CHANGES IN BODY WEIGHT OF CATS DURING AGING

Before using body weight (BW) records from a colony to draw conclusions on cat life stages, we must ensure that the colony is a fair representation of the normal pet population. For our studies, we use data from adult cats (>1 year of age) that live in social rooms of 20 cats where they have windows, toys, furniture, interaction with caretakers, and ad libitum access to a variety of foods during the day. The population is evenly distributed between males and females and includes both intact and neutered animals.

The data in Figure 1 (n = 235 cats) show population BW trends with age (1 to 21 years). A nonlinear regression model shows a two-stage segmentation, where cats increase BW steadily between 1 and 9 years following the equation BW (kg) = 3.5 + (0.1 × age in years). After 9 years of age, BW decreases following the equation BW (kg) = 6.6 – (0.2 × age in years). It could be suggested that something changes around age 9, and hence cats over the age of 9 should be considered “old.” If we take this approach, we will have two life stages for grown cats (pre- and post-9 years) with great variability in BW within them. We are trying to identify consistent and co-
herent life stages rather than inflection points.

Using the data from Figure 1, the following age periods were compared: 1 to 7 years (n = 114), 7 to 12 years (n = 39), and >12 years (n = 82). Analysis of variance shows that mean BW (kg) of the three groups are all significantly different: group 1 to 7 years is 3.7 ± 0.8; group 7 to 12 years is 4.4 ± 1.7; and group >12 years is 2.9 ± 1.0. As the mean BW of all the cats, irrespective of age, is close to 4 kg, we looked for the incidence of obesity (>6 kg or 4 kg + 50% BW) and incidence of underweight (<2 kg or 4 kg – 50% BW). Fisher’s exact test showed a higher (P <0.001) incidence of obesity in the group 7 to 12 years and a higher (P <0.001) incidence of underweight cats in the group >12 years.

From these data we can hypothesize that the mature cat undergoes three distinctive life stages: Adulthood, 1 to 7 years, during which most cats show ideal BW but with tendencies to increase with age; Maturity, 7 to 12 years, demonstrating a risk for cats to be overweight or even obese; and Geriatric, >12 years, when BW tends to decrease progressively and become below ideal. We will look next at body composition data to see if these life stages have further rationale.

CHANGES IN BODY COMPOSITION OF CATS DURING AGING

Figure 2 shows data from Dual Energy X-Ray Absorptiometry (DEXA) measurements of the changes in body composition with age of colony cats (n = 256) at 2-year intervals. Bone mineral density ranged from 0.56 to 0.6 g/cm² and was not statistically affected by age, whereas the amount of fat and lean tissues was affected by age.

During the adult life stage (1 to 7 years) cats have a mean of 10% body fat and 87% lean tissue. Toward the end of adulthood and during the mature (7 to 12 years), cats increase their percentage of fat progressively, reaching a mean of approximately 18% by 9 years of age. The incidence of obesity, defined as the percentage of cats with more than 25% body fat, is highest (one-third of cats) during maturity (Figure 4).

The mean percentage of lean tissue in mature cats (7 to 12 years) is lower than in adulthood (Figure 2), but this must be considered in the context that cats actually show higher BW during this life stage, and in absolute values (Figure 3) the lean tissue is slightly increased during maturity.

These changes in body composition during maturity substantiate the belief that old cats tend to become overweight or obese and hence their diet should contain fewer calories and fat. This is valid if the old cats studied are mature (7 to 12 years of age), but it might not be accurate for geriatric cats (>12 years).

Mean percentage body fat values in cats drop progressively after 12 years of age (Figure 2). The percentage of lean tissue seems to increase, but as seen in Figure 1, geriatric cats tend to suffer progressive BW loss, so the values are relative. The lean tissue mean values (Figure 3) drop dramatically after 12 years of age, and by age 15 geriatric cats have a mean lean tissue value under 2 kg, which is one-third less than the mean during adulthood (around 3 kg).

Lean tissue is an indication of muscle mass and is likely to be reflected in the appearance and capacity for activity of the cat. Although our data on cat activity levels at different life stages are not yet analyzed, there are preliminary observations suggesting that the activity levels decrease as cats age and the amount of time spent sleeping increases. Reduced

{fig:2}{fig:3}
THE AGING FELINE

There are reports in the literature concerning reduced nutrient digestibility in old cats. Nestlé Purina has investigated the extent and incidence of this problem in cats across several colonies by feeding the same diets and performing standard AAFCO digestibility protocols. The apparent fat digestibility in the healthy adult cat is typically 90% to 95%. Figure 6 depicts the incidence of low fat digestibility (defined as <80%) in aging cats. The incidence of low fat digestibility in cats increases with age, affecting 10% to 15% of mature cats (7 to 12 years of age) and one-third of geriatric cats (>12 years of age). Findings were consistent across four separate colonies. In some geriatric cats, fat digestibility was found to be as low as 30% with no apparent health problem other than large stools and low BW. The incidence of low fat digestibility does not seem to be affected by diet type, either canned or dry pet foods.

Since fat is the most energy-dense macronutrient, impaired ability to digest fat could contribute, at least in part, to the changes in BW and body composition previously described in geriatric cats. It is likely that the onset of reduced fat digestibility is gradual but over the long term contributes negatively to the energy balance of an important number of geriatric cats.

The incidence of low protein digestibility in cats with increasing age is summarized in Figure 7. Considering that protein digestibility in a healthy adult cat is typically 85% to 90%, we define low protein digestibility as <77%. Low protein digestibility also seems to affect mature and geriatric cats. Although the incidence of low protein digestibility is not as high as low fat digestibility, after the age of 14 years we see that it affects one-fifth of the geriatric cats. Reduced protein digestibility with age seems to occur parallel to the re-
duction of lean tissue (Figure 3) in the geriatric cat, and it might contribute, at least in part, to predisposing cats over >12 years of age to negative nitrogen balance.

Concurrent low fat and protein digestibility occurs in some geriatric cats. In cats >14 years of age, two-thirds have a dry matter digestibility below 77%. There is a significant negative correlation between age and nutrient digestibility in the cat ($P < 0.01$).

**CHANGES IN WATER BALANCE**

Another implication of the progressive loss of lean tissue in geriatric cats (Figure 3) is the reduction in the amount of water in the body. This could make geriatric cats more prone to dehydration or less likely to recover from it.

Nestlé Purina has conducted studies comparing the water balance between adult cats (1 to 7 years) and geriatric cats (>12 years) fed with the same canned diet. All cats used in these studies had healthy renal function (blood urea nitrogen 11.7 to 33.3 mg/dl and creatinine 0.5 to 1.8 mg/dl). In our studies, *water balance* was defined as the relationship between the water entering and the water leaving the body over a period of 3 weeks. *Water intake* includes both the moisture of the food ingested and the amount of drinking water consumed. *Water losses* are represented by the fecal moisture plus urinary volume. The transepidermal, salivation, and respiration water losses could not be accounted for, but it was assumed that there were no differences between adult and geriatric cats because they all shared the same environmental conditions. As shown in Figure 8, there were no significant differences in the water ingested with the food or in drinking water consumed between the two age groups. Likewise, no differences were observed in fecal moisture losses, but the urinary volumes were significantly higher ($P < 0.05$) in the geriatric cats. As a consequence of the urinary volume differences, geriatric cats have higher water losses ($P < 0.05$) than adult cats, possibly due to decreased kidney efficiency to concentrate urine even when no clear signs of renal failure are evident.

Small but continuous water losses could predispose geriatric cats to a negative water balance, and this seems to agree with the changes in lean tissue described in Figure 3. If we consider water as an essential nutrient, it would be advisable to encourage higher intake, particularly in the geriatric cat. Although water intake per se cannot guarantee the maintenance of lean tissue, lack of water availability could be a limiting factor.

**FINAL YEARS**

The BW decline in the last part of the cat’s life, when the causes of death are natural, seems to be a generalized phenomenon. Figure 9 compares historical data on BW losses in cats from a Nestlé Purina colony (n = 258) prior to death. Data were grouped by postmortem diagnosis into cancer (n = 26), chronic renal failure (CRE, n = 50), hyperthyroidism (n = 17), and other causes combined (n = 165). BW records are shown during the 4 years prior to death, plotted quarterly (every 3 months) for a total of 16 quarters. BW from the last quarter prior to death was discarded because it was influenced by parenteral fluid treatment.

The incidence of these diseases in the colony was compared to the general U.S. cat population using data from VMDB at Purdue University (1995–2001). No significant differences were found in the incidence rates of cancer, CRE, and hyperthyroidism between the Nestlé Purina colony and the U.S. cat population.

Two-stage nonlinear regression was used to fit the data in
Figure 3. It was assumed that the cats’ BW was kept constant and then, at the point when BW losses start (inflection point), a quadratic model was fitted to allow for an increasing amount of BW loss as they approach death.

The inflection point in BW for cats that died of cancer, renal failure, and hyperthyroidism was at 10 quarters prior to death (equivalent to 2.5 years). The group of cats dying from other causes had an earlier inflection point at 15 quarters prior to death (equivalent to 3.75 years). The decline in BW in the second year prior to death was over 6% for cancer, CRF, and hyperthyroidism. During the last year of life, the average % BW loss was over 10% for all four groups.

The average age at death did not differ significantly between cancer (13.5 ± 2.3 years), CRF (13.0 ± 3.9 years), and hyperthyroidism (14.3 ± 1.9 years), but the cats from other causes of death died significantly earlier (12.4 ± 3.5 years) than the cats that died from hyperthyroidism. Over the whole 4 years prior to death, cats that died from renal failure lost significantly more BW than the cats in the “other” disease group.

**FACT SUMMARY SHEET**

Although biology is not always an exact science and individual variability has to be considered, the proposed adult (1 to 7 years), mature (7 to 12 years), and geriatric (over 12 years) life stages seem to divide the life of the mature cat into consistent and coherent phases. Table 1 is a summary of the tendencies found during these life stages.

Considering that 7 years of age in a cat is often only about half its total life span, this age seems too early to be defined as “old.” There could be some parallels drawn between the life stages of the cat and humans. According to Lawler and Bebiak, 7 years of age in the cat is equivalent to 45 years in humans, and 12 years in the cat is equivalent to 65 years in humans. The definitions of mature cat as equivalent to 45 to 65 in human years, and geriatric cat defined as equivalent to >65 in human years, could be used to make these life stage concepts clearer to consumers. In general terms, humans have a tendency toward higher BW during maturity (i.e., at 55 years) than they had during early adulthood (i.e., at 25 years), and elderly people (i.e., at 75 years) tend to be thinner or even frail.

The maintenance of ideal BW is vital to maintain lean tissue, which in turn helps maintain activity levels and good quality of life. It is also important to note that BW decline is widespread among most cats >12 years of age and is not driven solely by unhealthy individuals. Even if some cats >12 years of age suffer BW losses due to initial stages of chronic diseases, these losses are not exceptional from the population trend.
GENERAL NUTRITIONAL RECOMMENDATIONS FOR THESE LIFE STAGES

Obesity has been linked to increased risk of hepatic lipoidosis, glucose intolerance, and musculoskeletal problems in cats. It would seem logical to use diets of moderate energy density that could help reduce the risk of weight gain during maturity. Although moderation of calorie intake might be suitable for mature cats, it does not appear to match the needs of geriatric cats. As suggested in Table 2, it would seem more logical to use highly digestible, energy-dense food for geriatric cats in an attempt to slow down the decline in BW and lean body tissue.

ACKNOWLEDGMENTS

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REFERENCES


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THE AGING FELINE

Nutritional Influences on the Immune System in Aging Felines

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EXPECTATIONS OF AGING

The aging process is a continuum of stages beginning at conception and ending with death. Although this implies that the process will inevitably proceed, the rate of onset and duration of subsequent stages can possibly be influenced over time. Of the multiple factors likely to slow or accelerate the aging process, it is well documented that nutrition can alter the health and longevity of the elderly human population.1–3 Other studies, albeit limited in number, indicate a similar scenario for our population of companion animals. Various theories on aging have been proposed. Genetic control involving aspects of codon restriction, somatic cell mutation, and gene regulation is one such theory. Others include gradual deconstruction of physiologic systems associated with immunity, neuroendocrine, musculoskeletal, cardiac, and renal functions. Age-related physiologic changes have also been linked to consequences of oxidative stress resulting in tissue, cell, and DNA damage from unbalanced free radical production.4,5 The multiplicity of factors influencing the physiologic alterations of aging often parallel behavior changes (i.e., incontinence, dementia, aggression, despondence, anorexia, dehydration, diarrhea, exercise intolerance, etc.) reported in both human and veterinary literature.6,7 Management strategies for geriatric patients would appear to be most beneficial if approached from a “wholistic” perspective. Diet, exercise, and preventive health care should be integrated to optimize quality and longevity of life.

NUTRIENTS, IMMUNITY, AND AGING

Diet, or more specifically, utilization of nutrients, has been shown to influence multiple aspects of the aging process. For example, aging may, in part, be a result of nutrient-associated dysregulation of the immune system. The interaction between nutrition and the immune system has received much attention, and nutrients such as Vitamin E, β-carotene, polyunsaturated fatty acids (PUFA), protein, and fiber appear to modify the compromised immunocompetence concurrent with the aging process. Likewise, antioxidant nutrients can temper the degenerative changes to cell function that result from accumulation of free radicals during aging. Understanding that a relationship exists between nutrition, immune health, and aging will allow development of individualized as well as general nutritional support protocols to optimize health in aging patients.

Physiologic changes associated with altered immunocompetence have been reported in the aged canine,8–10 but few reports support this conjecture in the feline. This has resulted in very generalized feeding recommendations for older cats that vary little from adult maintenance feeding guidelines. Cats 7 to 8 years of age are considered “senior” or “geriatric” beginning at 10 to 12 years of age.11 This is the time when prevalence of disease increases in conjunction with gradual onset of behavior changes as well as physical and metabolic alterations. Older cats are less active, have a decreased lean body mass, diminished digestive function, immune response, renal function, and glucose tolerance.12 The aging patient also exhibits an altered perception of taste and smell, which suggests that diet palatability may be a consideration to optimize intake. Aging and physiologic changes occur at variable rates for any one animal; therefore, individualization may be a key consideration in nutritional management. Tables 1 and 2 summarize physiologic system changes with subsequent clinical manifestations that occur with aging and the general goals of nutritional management for senior or geriatric felines.

Accurate patient assessment is critical to optimizing nutrient support. Blood, tissue, and cellular indices obtained from the complete blood count, biochemistry profile, urinalysis,
moderately lean body condition through modification of calorie intake can slow the onset and progression of numerous age-related physiologic changes and associated disease states and perhaps influence response to healthy vaccine protocols in older patients.

### CLINICAL SIGNIFICANCE OF NUTRIENT–IMMUNE INTERACTIONS IN THE FELINE

In the early “senior” years, cats often develop an overweight–obese body condition due to decreased activity and altered nutrient metabolism with no modification of calorie intake. Further along the aging continuum, loss of sensory perception associated with eating, poor dentition, compromised organ function, and periods of inappetence result in reduction of lean body mass, making the maintenance of body weight and condition a challenge. Calorie reduction in these scenarios can have profound effects on physiologic parameters, especially immune system function and incidence of disease.

Studies indicate that total nutrient deprivation (with the exception of daily fluids) to 25% of maintenance calorie requirements for 7 days profoundly influenced specific immune parameters and biochemical parameters in healthy, aged felines. Decreases were observed in leukocyte number (P < 0.05), lymphocyte number (P < 0.05), and CD4+ subset (P < 0.05). A simultaneous increase in CD8+ subset cells and cellular calcium flux was reported, resulting in a lowered CD4/CD8 ratio during short-term nutrient restriction. Lymphocyte proliferative capacity (P = 0.07) and delayed type hypersensitivity (DTH) reaction (P < 0.05) in aged felines (>8 years of age) and biopsy can help identify appropriate nutrient profiles for individual patients. Indices regarding organ and immune cell function, antioxidant status, and body weight changes should be evaluated to accurately alter minimum or maximum nutrient allowances to support physiologic and immunologic changes and reduce risk of diseases in older cats.
THE AGING FELINE

age) was negatively impacted within 4 days of severe nutrient restriction. \(^{24}\) Recovery to baseline took three times longer in old versus young feline subjects. Monocyte cell function (phagocytic activity and major histocompatibility complex [MHC] class II expression, \(P < 0.05\)) was diminished and acute phase protein (fibronectin) concentration was altered \(\left(P < 0.05\right)\) in old versus young, healthy felines during short-term nutrient deprivation. \(^{25}\) The reversal of diminished immune cell function and body weight loss with re-feeding at maintenance calorie levels occurred faster in young as compared to older cats (2.3 versus 7.4 days).

Cancer is another prevalent disease of older cats. Dietary intervention in companion animal oncology patients has been limited to one commercially formulated canine cancer management diet and piecemeal manipulations in dietary fat, specific amino acids, and antioxidants. Feline cancer patients may or may not benefit from canine model-based nutrition oncology studies. Recently, feline-specific studies identified a useful cancer biomarker (mitogen-activated protein kinase, or MAPK) that is influenced by age and dietary omega-6:omega–3 PUFA ratio. \(^{28,29}\) Leukocyte MAPK activity was monitored in a group of cats (9 to 16 years of age) diagnosed with renal, gastrointestinal, or mammary neoplasia based on surgical biopsy. Activity of this biomarker increased linearly over a 2- to 3-month time-course of disease. Manipulation of dietary PUFA to increase omega-3 levels resulted in a decrease of active MAPK and slowing of tumor growth and improved quality of life (based on appetite, activity, alertness, and voiding behavior) in approximately 92% of the feline patients evaluated.

The controversy over whether or not to vaccinate older cats appears to be ongoing. Vaccines are designed to protect from foreign viral and bacterial agents by stimulating a defensive immune response in the patient. Immune cell function is dependent upon adequate nutriture and a competent intracellular communication system. Since aging as well as inadequate nutrition alter the competency of all branches of the immune system, individual patient assessment regarding these parameters, along with current clinical state and disease risk potential, should help dictate the vaccination plan for “senior” pets. Overstimulation of the immune system can be detrimental with production of a pro-oxidative state and has been shown to occur with excessive or inappropriately timed nutrient and/or dietary antioxidant supplementation.\(^{4}\) Immunosuppression can likewise result from specific nutrient manipulations of diets for senior pets. Timing of specific nutrient alteration with stress and desired immune response is critical.

Vaccine challenge studies in a group of 28 aged felines demonstrated the relationship between nutrients and im-

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**TABLE 2**

**Overall Goals for the Nutritional Management of Senior and Geriatric Cats**

1. Maintenance of optimal nutrition
   - Adequate (not excessive) intake of a balanced diet
   - Ensure adequate hydration
   - Ideal body weight and body condition

2. Disease management
   - Optimize immunocompetence
   - Appropriate vaccination protocol
   - Manage environment and diet to:
     - minimize common diseases
     - slow onset and progression of chronic diseases

3. Improve quality of life
   - Maximize immunohealth through diet
   - Feed to enhance organ function, attitude, and activity level during aging

munity. Cats fed diets enriched in omega-6 or omega-3 fatty acids exhibited an enhanced innate immune cell and T-helper cell response to a bacterial immunomodulator but either failed to elicit a substantial cell-mediated response or exhibited a diminished response to a viral-directed vaccine challenge. This suggests that diets enriched in PUFA can both stimulate and suppress immune cell function. Clinical implications include consideration of diet manipulation in light of goals for immunohalt (vaccine protocols) in aged felines.

**IMPLICATIONS**

A solid understanding by practitioners of the complex nature of the immune system will increase the clinician’s ability to formulate feeding plans for “senior” cats to optimize their defenses against disease challenge and enhance their immune function in acute and chronic disease states. Commercial diets providing an appropriate fat source, supplemental antioxidants including vitamin E, selenium, zinc, and immune-enhancing amino acids can prove beneficial in tempering the adverse metabolic and physical changes that occur with the diseases of aging including diabetes, renal and cardiac failure, and cancer. Weight management to prevent obesity during the “early senior years” can promote longevity and immune health. Assuring adequate calorie intake, specifically focusing on fat and protein sources and dietary levels along with appropriate use of antioxidant supplements and additives, can improve quality of life and slow the onset of end-stage disease in the aging feline.

**REFERENCES**


THE AGING FELINE

Effects of Dietary Components on the Development of Hyperthyroidism in Cats

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Hyperthyroidism was first reported in cats in 1979. Since that time, it has become the most common endocrinopathy in cats. Morphology of affected thyroid glands has been well characterized, but unfortunately the pathogenesis still remains a mystery. A number of factors have been postulated to play a role in pathogenesis, including heredity, genetics, breed, environment, and diet. These factors could affect any step in thyroid gland metabolism.

REVIEW OF THYROID HORMONE SYNTHESIS

Thyroid hormone synthesis is comprised of a number of coordinated and controlled steps. The first step involves the active uptake of iodide by the thyroid gland. The Na+/I- symporter (NIS) is an efficient system that ensures that most ingested dietary iodine is accumulated in the thyroid gland. Because of NIS, the thyroid gland can concentrate iodine by 20- to 40-fold over the concentration seen in blood. Approximately 90% of the iodine found in the body is concentrated in the thyroid gland. NIS mediates the first key step by supplying iodine to the thyroid against its electrochemical gradient.

The next two steps involve iodination of tyrosyl residues of thyroglobulin (Tg), and the coupling of moniodotyrosine (MIT) and diiodotyrosine (DIT) within Tg to form triiodothyronine (T3) and thyroxine (T4). These steps are catalyzed by thyroid peroxidase (TPO), which requires H2O2 for activity. Next there is proteolysis of Tg with release of free iodotyrosine and iodothyronines, and secretion of iodothyronines into the blood. Deiodination of iodothyronines occurs within the thyroid gland so that the liberated iodide can be reused. Lastly, there is deiodination of T4 by type I and type II deiodinases to produce the biologically active hormone T3. Iodothyronine deiodinase type I is a selenoenzyme requiring selenocysteine at its active site.

DEVELOPMENT OF NODULAR GOITER

Feline hyperthyroidism has a strong clinical and pathologic resemblance to toxic nodular goiter in humans; however, little is known regarding the etiology. Goiter is characterized by thyroid cells that have the capacity to grow and produce T4 and T3 autonomously, in the absence of thyroid stimulating hormone (TSH). Thyroid follicular cells are a heterogeneous population and differ in functional characteristics. Goiter evolves gradually, starting with a small hyperfunctioning adenoma. This small adenoma is slightly more active than the rest of the thyroid gland and contributes little to thyroid secretion. As the adenoma grows, its contribution to secreted hormones increases, resulting in a decrease in TSH release. The decrease in TSH results in decreased function of other thyroidal tissue. With further growth, thyroid secretion becomes supernormal, TSH concentration remains low, and the adenoma displays autonomous function (toxic nodular goiter or thyrotoxicosis). This growth rate appears to be slow in humans. More than 75% of human patients are euthyroid when adenoma is first detectable, with thyrotoxicosis developing in about 4% per year thereafter. Studies in Germany have shown that nearly 50% of deceased cats exhibited nodular adenomas in their thyroid glands in the absence of hyperthyroidism. Approximately 99% of hyperthyroid cats have functional thyroid adenomas, suggesting the slow progression of a nontoxic goiter to thyrotoxicosis in cats as well.

In the cat, immunoglobulins that stimulate thyroid membrane adenyl cyclase were not found and cannot account for thyroid hyperfunction. However, there is evidence for a specific immunoglobulin population that mediates thyroid cell growth (but not function) in patients with nodular goiter. These growth immunoglobulins may act via the TSH receptor and inhibit TSH binding to thyroid membranes. Since thyroid cells are naturally heterogeneous in their growth and
functional potential, the presence of a weak stimulator such as growth immunoglobulin may stimulate only those cells with higher intrinsic growth activity. This would account for the nodular characteristics of human and feline nodular goiter.

**POTENTIAL ENVIRONMENTAL CAUSES OF HYPERTHYROIDISM**

Several groups have tried to identify environmental or nutritional links to the incidence of feline hyperthyroidism. In a study of 56 hyperthyroid cats, a development of hyperthyroidism was significantly associated with exposure to flea powders and sprays, fertilizers, herbicides, an indoor lifestyle, and the consumption of canned cat food. In a subsequent study of 56 hyperthyroid cats, a development of hyperthyroidism was significantly associated with exposure to flea powders and sprays, fertilizers, herbicides, an indoor lifestyle, and the consumption of canned cat food. In a subsequent study, owners of 379 hyperthyroid cats and 351 control cats were questioned. In this study, cats consuming predominantly canned cat foods were at twice the risk for developing hyperthyroidism. Cats using cat litter and those in which topical parasite preparations were used were also at increased risk.

Certain breeds (Siamese and Himalayan) showed a decreased risk of hyperthyroidism, indicating a potential genetic component to the development of hyperthyroidism. Interestingly, the only other variable associated with a decreased risk of hyperthyroidism was the regular use of beef and poultry as a dietary supplement. Unfortunately, no further details were given regarding the supplemental beef or poultry, such as quantity, frequency of use, or parts fed (muscle meat, liver, etc.). Factors not associated with hyperthyroidism included exposure to smoke, sex or neutering status, number of cats in the household, frequency of vaccination, other dietary supplements (hairball products, urinary acidifiers, vitamins, yeast, baby food), brand of cat litter (types of cat litter were not specified), fertilizer, environmental insecticides, or dry cat food consumption. Unfortunately, water source or quality was not assessed in this study.

**IODINE**

A deficiency of iodine has long been recognized as a cause of nodular goiter in humans. With severe iodine deficiency, there is a depletion of thyroidal iodine followed by thyroidal enlargement. Concentration of T₄ declines and TSH increases. The degree of iodination of thyroglobulin decreases, resulting in an increased MIT/DIT ratio and increase in thyroidal T₃/T₄ ratio.

Both severe and moderate iodine deficiency can produce thyroid enlargement. In mildly iodine-deficient areas, intrathyroidal iodine decreases, but thyroid enlargement occurs without an elevation of TSH. Decreased intrathyroidal iodine precedes elevated TSH in goitrogenesis, and goiter development correlates better with low thyroidal iodine than with elevated TSH levels. In Europe, the frequency of toxic adenoma was inversely related to iodine intake, and in Switzerland, the frequency of toxic nodular goiter decreased by 73% after the iodine content of salt was increased. In southern Italy, where iodine intake is low, the prevalence of goiter, thyroid nodularity, and functional autonomy increased with age (up to 60% of adults), suggesting the slow course of progression.

Iodine supplementation may induce hyperthyroidism, and this phenomenon has been reported in all iodine-supplementing programs. Iodine-induced hyperthyroidism (IIH) is due to the sudden introduction of iodized salt in populations that have had a chronic iodine deficiency. Iodine deficiency increases thyroid cell proliferation and mutation with the development of autonomous nodules in the thyroid. Thus IIH is technically an iodine deficiency disorder. IIH appears to be a transient problem in humans with a decline in cases after about 3 years of an iodine-replete diet. IIH is not prevented even if only a physiologic dose of supplemental iodine is used. In Switzerland, the incidence of hyperthyroidism increased 27% in iodine-deficient humans in the first year after increasing iodine intake to a normal level. After another year, however, the incidence of hyperthyroidism decreased.

Iodine deficiency may result in thyroid hyperplasia in the cat. In an early report (1961), thyroid glands from kittens fed a severely iodine-deficient diet for 8 weeks were larger and heavier than from those fed a control diet. However, there are no detailed studies in cats to determine what level of daily iodine intake is consistent with normal thyroid function over a long period of time. In a 6-week-long study where iodine intake was increased every 2 weeks, fecal iodine excretion remained constant and was independent of iodine intake. En-dogenous fecal loss was 13 ± 4 µg/kg body weight (BW)/24 hours. Urinary excretion of iodine was significantly correlated to iodine intake, but extrapolation of iodine intake to zero results in an endogenous renal iodine loss of 6 µg iodine/kg BW/24 hours. Thus an intake of approximately 20 µg iodine/kg BW/24 hours may be sufficient for iodine balance. Unfortunately, the bioavailability of iodine in cat foods is unknown. Diets high in iodine in the pigment erythrosine may have poor bioavailability since the digestibility of this pigment is low.

Until Fall 2003, Current National Research Council
iodine requirements for kittens was 350 µg/kg diet on a dry matter (DM) basis. This is based on a diet providing 5.0 kcal/g DM metabolizable energy (ME) and was equivalent to 7 µg iodine/100 kcal ME. In the literature, recommended iodine intake for cats ranges from 1.4 to 4.0 mg iodine/kg DM (28 to 80 µg iodine/100 kcal ME) up to 50 µg iodine/kg BW/24 hours (95 µg iodine/100 kcal ME). Comparing previous NRC requirements for iodine (350 µg iodine/kg DM or 7 µg iodine/100 kcal ME) to endogenous daily losses of iodine (20 µg iodine/kg BW/24 hours or 38 µg iodine/100 kcal ME), it would appear that this minimum requirement did not meet the endogenous daily loss of iodine. Thus if cat foods only met the minimum requirement for iodine, cats may have received an insufficient quantity of iodine per day. To meet 38 µg iodine/100 kcal ME endogenous loss, the minimum quantity of dietary iodine would need to be raised to approximately 2 mg iodine/kg DM.

Cat foods can vary widely in iodine content. In a survey of 13 cat foods from New York in 1983, iodine contents ranged from 1.0 to 36.8 mg iodine/kg DM (25 to 920 µg iodine/100 kcal ME). All of these diets met then current NRC requirements for iodine, but four did not meet calculated endogenous iodine losses. In France, 19 dry and 19 wet cat foods were evaluated for iodine content. Foods ranged from 3.8 to 791 µg iodine/100 kcal ME, with only 50% of foods meeting endogenous iodine losses. A study of 28 commercial cat foods (23 canned, 5 dry) in New Zealand showed a wide variation in iodine content. Canned cat foods had a more dramatic variation in iodine content (nondetectable to 401 µg iodine/100 kcal ME), but only 2 of 23 canned foods had iodine concentrations greater than the level of endogenous loss. None of the dry foods met endogenous iodine loss; however, iodine content overall was higher in the dry foods. In Germany, 92 commercial cat foods were analyzed (74 canned, 18 dry) for iodine content. Iodine content ranged from 473 to 3,181 µg iodine/kg DM (12 to 68 µg iodine/100 kcal ME) in dry foods and from 218 to 6,356 µg iodine/kg DM (5 to 122 µg iodine/100 kcal ME) in canned foods. Again, the widest variation was seen in canned cat foods. Overall, only 47% of the foods had sufficient iodine to meet endogenous loss calculations. Thus there is great evidence that many cat foods around the world may not contain sufficient iodine to meet daily endogenous losses.

A few studies have attempted to correlate iodine intake to thyroid hormone concentration in cats. In cats fed varying iodine content diets for 2-week periods, serum-free T4 concentrations were acutely affected. However, when cats were fed varying iodine content diets for 5 months, there were no significant differences in free T4 levels. Cats were able to adapt to varying iodine concentrations to maintain a fairly constant level of free T4.

Abrupt changes in iodine intake can result in hyperthyroidism in iodine-deficient humans. Variability in iodine intake may also result in iodine-induced hyperthyroidism (Jodbasedow syndrome). It may be that continually switching between diets that are high or low in iodine content overwhelms the adaptive mechanisms in the cat, eventually leading to thyroid dysfunction.

In the fall of 2003, the NRC iodine requirement was increased to 550 mg iodine/1000 kcal ME for adult cats. This requirement exceeds the 380 mg/1000 kcal ME minimum iodine content required to meet endogenous iodine loss. In an adult cat diet providing 4000 kcal/kg, the iodine content must be 2200 mg iodine/kg diet to meet the new NRC requirement. In a kitten diet providing 5000 kcal/kg, the iodine content must be 2750 mg iodine/kg diet to meet the new NRC requirement. These levels are considerably higher than the previous requirement of 350 mg iodine/kg diet. If many cats are currently iodine deficient, it will be interesting to note whether a transient increase in the incidence of hyperthyroidism occurs as a result of iodine supplementation as has been seen in human supplementation programs.

**IODINE AND METHIMAZOLE TREATMENT**

Methimazole acts by inactivating TPO, and this inactivation of TPO can be prevented by increasing the dietary iodine content. The ratio of iodide to methimazole is important, as the ratio favors irreversible inhibition. Since cat foods can vary greatly in iodine content, it is possible that the concurrent ingestion of high iodine-containing cat foods may in part be responsible for the apparent “poor response” to methimazole in some hyperthyroid cats.

**THYROID IODOLIPIDS**

Iodolipids are a class of iodinated compounds also present in the thyroid glands. These iodolipids are metabolites of arachidonic acid and docosahexaenoic acid (DHA). The most important iodolipids are 6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid Δ-lactone (Δ-IL) from arachidonic acid, and 5-iodo-7,10,13,16,19-docosapentaenoic-γ-lactone (γ-IL).
from DHA. These compounds are important in autoregulation and help inhibit thyroid cell proliferation. In rats, Δ-IL prevented goiter formation and produced involution of previously produced goiter. Any impairment in autoregulatory mechanisms can lead to abnormal growth. Goitrogenesis may be related to a relative iodolactone deficiency, and availability of arachidonic acid may be a limiting factor. This may be important in the cat because of its requirement for arachidonic acid in the diet. Variation in essential fatty acid (EFA) ingestion may influence the iodolactones synthesized within the thyroid gland. The utilization of iodolactones or certain fatty acids in the prevention or treatment of goiter may be an area for future investigation.

**SELENIUM**

Selenium is typically present in high concentrations in the normal thyroid gland. Selenium is present in glutathione peroxidase (GPX) and superoxide dismutase, which are enzymes responsible for detoxification of toxic derivatives of oxygen such as H₂O₂. It is also present in type I iodothyronine 5′-deiodinase responsible for conversion of T₄ to T₃. If selenium is deficient, less T₄ is converted to T₃ due to decreased activity of the deiodinase. If iodine deficiency is also present, the resulting increase in TSH leads to an increased production of H₂O₂ in thyroid cells and the decreased activity of GPX allows H₂O₂ to accumulate, causing thyroid cell destruction. There is no evidence in the cat that excesses of selenium are toxic; however, there is the potential that excess selenium could result in increased activity of GPX, making H₂O₂ less available for TPO, with a decrease in iodination and coupling as a result.

**OTHER GOITROGENS**

There are a number of agents present in the environment that may affect thyroid gland morphology and function. In humans, iodine deficiency is well recognized as a cause of goiter formation; however, hyperthyroidism with goiter has occurred in the presence of a sufficient quantity of iodine. Thus other thyroid stimulatory factors may exist in the pathogenesis of goiter.

Environmental goitrogens are agents that cause thyroid enlargement.¹³ They may act directly on thyroid cells or indirectly by interfering with steps in thyroid hormone synthesis and metabolism. Natural goitrogens in foods were first discovered in *Brassica* (cabbage family) due to the presence of thioglucosides, which release thiocyanate and isothiocyanate. Goitrin, a specific thioglucoside, is also found in many *Cruciferae* (mustard family). Cyanoglucosides are found in cassava, bamboo shoots, turnips, sweet potatoes, and lima beans. Disulfides present in onions and garlic exert antithyroid activity. Flavonoids are found in high concentration in millet, sorghum, and soybeans, and possess antithyroid effects. Flavonoids inhibit TPO and also inhibit peripheral metabolism of thyroid hormones. While most of these ingredients are not used in pet foods (except for sorghum and soybeans), goitrogens not yet identified may exist in more common ingredients. Other compounds, such as ascorbic acid and copper sulfate, inhibit TPO-catalyzed iodination and may also act as goitrogens.

Goitrogens may also be present in drinking water. High concentrations of lithium may be goitrogenic, and incidence of goiter is increased in coal and shale-rich areas. Coal is a source of many antithyroid compounds including phenol, resorcinol, dihydroxybenzenes, thiocyanate, disulfides, phthalic acids, pyridines, and polycyclic aromatic hydrocarbons. Contamination with *Escherichia coli* or *Yersinia enterocolitica* may play a role in goiter pathogenesis because antibodies to these bacteria exert a growth-promoting effect on thyroid cells. Goitrogens in water may be important in areas with well water, chemically treated water, or in animals routinely drinking ground water. Goitrogens in water may also be introduced into cat foods during the manufacturing process. Goitrogenic compounds may be metabolized by hepatic glucuronidation, which is a limiting pathway in cats. This slow degradation of goitrogens may contribute to the development of hyperthyroidism.

**NUTRITIONAL STATUS**

Overall nutrition may be important in the development of goiter, as poor nutrition increases goiter development in humans. In rats, a low-protein diet impairs iodine transport by the thyroid and leads to thyroid gland enlargement. Protein calorie malnutrition alters thyroid morphology and function, and enhances the effects of goitrogens. Early reports (1958) also suggest that diets high in protein may increase the requirement for dietary iodine. Thyroid hyperplasia was more pronounced in rats receiving a 30% protein diet versus a 20% protein diet that was supplemented with the same amount of iodine. However, this could also be due to a relative iodine deficiency since less of the high-protein diet would have been consumed to meet caloric needs, and therefore less iodine was consumed as well. Thus the cat's unique requirements for high protein may potentially play a role in the development of goiter and hyperthyroidism.
In conclusion, many factors, including species characteristics, genetics, nutrition, and environment, undoubtedly are involved in the pathogenesis of feline hyperthyroidism. Considerable research is needed for the elucidation of these mechanisms.

REFERENCES
NEW FINDINGS IN FELINE HYPERTENSION

New Findings in Feline Hypertension

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SYSTEMIC HYPERTENSION

High systemic arterial blood pressure is commonly observed in cats with chronic kidney disease (CKD) and in a variety of other metabolic and endocrinologic abnormalities.1–5 In veterinary medicine, systemic hypertension has been associated with ocular pathology, chronic progressive kidney damage, neurologic complications, and cardiovascular changes. Unfortunately, progress in this area has been hindered by the difficulties veterinarians encounter in measuring blood pressure in cats and by the lack of clear treatment guidelines.

ADVERSE EFFECTS OF SYSTEMIC HYPERTENSION

There is a clear association between ocular injury and marked systemic hypertension in cats (Table 1).2,6–8 Findings associated with hypertensive injury to the eye include hemorrhage within the choroid, retina, vitreous, and anterior chamber; retinal detachment and atrophy; retinal edema; perivasculitis; retinal vessel tortuosity; and glaucoma.

Hypertensive injury to the brain may be observed as an acutely developing neurologic syndrome.9,10 The genesis of this syndrome is not completely understood, though cats seem particularly sensitive to the development of cerebral edema whenever blood pressure rises >180 mm Hg, particularly if the increase in blood pressure occurs rapidly (<48 hours). This syndrome has been observed following renal transplantation9,10 and in models of hypertensive CKD.11 The mechanism of the sudden rise in blood pressure in these settings is unclear but most likely involves the renin–angiotensin–aldosterone and/or sympathetic nervous systems. Unless the hypertensive encephalopathy syndrome progresses to brain herniation, clinical signs abate rapidly (<12 hours) with effective antihypertensive therapy. A stroke (cerebrovascular accident, or CVA) may also occur in hypertensive cats. In contrast to early hypertensive encephalopathy, clinical signs attributable to a stroke are generally not rapidly reversible with antihypertensive therapy.

In a hypertensive cat, the heart is working against an increased arterial pressure (i.e., afterload); left ventricular hypertrophy and secondary valvular insufficiency thus may be observed.12 Tachycardia is not a common finding with hypertension, although some primary diseases that lead to secondary hypertension, such as hyperthyroidism, may also lead to an elevated heart rate. Left ventricular hypertrophy may regress with antihypertensive treatment.12

The kidney is susceptible to hypertensive injury, particularly the glomerular capillary bed.13 However, preglomerular arterioles usually constrict whenever blood pressure is elevated, serving to protect the renal glomerulus from hypertensive injury. In dogs with renal insufficiency, these preglomerular arterioles are dilated and poorly responsive to changes in blood pressure,14 and the same is likely to be true in cats. Thus it is likely that elevated systemic arterial blood pressure is transmitted directly to the glomerular capillary bed in cats with CKD. This would cause an increase in glomerular capillary pressure, referred to as glomerular hypertension, which may produce glomerular damage and a progressive fall in renal function.13

DIAGNOSIS OF SYSTEMIC HYPERTENSION

A diagnosis of systemic hypertension is based upon the determination of systemic arterial blood pressure. Further, the indiscriminate use of antihypertensive therapy in the absence of reliable values for systemic arterial blood pressure is inappropriate.

While it is possible to measure blood pressure in all clinical patients, currently there is not sufficient rationale to do so in cats. On the other hand, if the veterinarian only measures blood pressure in those animals suspected of having complications secondary to hypertensive injury, the opportunity for early identification of hypertension and intervention is lost. Thus patients with no evidence of hypertensive injury but known to be at risk for the development of systemic hyper-
The aging feline is midway between two available sizes, the larger cuff should be used since it will theoretically produce the least error. The cuff may be placed around the brachial, median, cranial tibial, or medial coccygeal arteries. For the Doppler technique, the cuff may be placed over the median artery, and the transducer is placed between the carpal and metacarpal pad. Clipping of hair and application of acoustic gel at the site of transducer placement may enhance the signal. For the oscillometric technique, the median or coccygeal artery is commonly used. The cuff should be placed at the level of the aortic valve. If not, compensation can be made for gravitational effect with a 1.0 mm Hg rise in blood pressure expected for each 1.3 cm of vertical distance between the level of the cuff and the level of the aortic valve. Generally at least three, and preferably five, consistent measurements made at a single site are considered a pressure measuring session, and at least two sessions, separated temporally by >30 minutes, should be relied upon to establish a diagnosis (Table 2). If a high value is obtained for blood pressure (i.e., systolic >140 mm Hg and/or diastolic >90 mm Hg), the patient's blood pressure should be evaluated at a different cuff site (e.g., contralateral limb) during that same measurement session.

In light of the uncertainty and difficulties associated with blood pressure measurement in cats, only those animals with tension also should be assessed. In addition to the routine screening of patients with CKD, hyperthyroidism, and advancing age, conditions in which blood pressure measurements are indicated include obesity, hyperadrenocorticism (endogenous or exogenous), mineralocorticoid-secreting tumor, and pheochromocytoma. Elderly cats, particularly those with a low body condition score, history of polyuria/polydipsia, and/or reduced appetite, are also candidates for blood pressure screening.

Blood pressure may be measured by either direct or indirect methods. Direct blood pressure measurement is the “gold standard” and usually involves placement of a needle or indwelling catheter into a peripheral artery. The indirect techniques are more applicable to a clinical setting, since they require less restraint and are technically easier to perform. Indirect methods of blood pressure measurement include the auscultatory, ultrasonic Doppler, oscillometric, and photoplethysmographic methods.

A standard protocol should be followed in determining blood pressure in a cat. All of these indirect techniques employ an inflatable cuff wrapped around an extremity. The pressure in the cuff is measured with the aid of a manometer or a pressure transducer. A squeeze bulb is utilized to inflate the cuff to a pressure in excess of systolic blood pressure, thereby occluding the underlying artery. As the cuff is gradually deflated, changes in arterial flow are detected by one of several means; the value for cuff pressure at various levels of deflation is then correlated with systolic, diastolic, and/or mean blood pressure. This detection method varies between different indirect methods. An oversized cuff may give erroneously low recordings; an undersized cuff may give a falsely high reading. In cats, indirect blood pressure measurement studies should employ a cuff width that measures 30% to 40% of the circumference of the limb. If the ideal cuff width is midway between two available sizes, the larger cuff should be used since it will theoretically produce the least error. The cuff may be placed around the brachial, median, cranial tibial, or medial coccygeal arteries. For the Doppler technique, the cuff may be placed over the median artery, and the transducer is placed between the carpal and metacarpal pad. Clipping of hair and application of acoustic gel at the site of transducer placement may enhance the signal. For the oscillometric technique, the median or coccygeal artery is commonly used. The cuff should be placed at the level of the aortic valve. If not, compensation can be made for gravitational effect with a 1.0 mm Hg rise in blood pressure expected for each 1.3 cm of vertical distance between the level of the cuff and the level of the aortic valve. Generally at least three, and preferably five, consistent measurements made at a single site are considered a pressure measuring session, and at least two sessions, separated temporally by >30 minutes, should be relied upon to establish a diagnosis (Table 2). If a high value is obtained for blood pressure (i.e., systolic ≥140 mm Hg and/or diastolic ≥90 mm Hg), the patient’s blood pressure should be evaluated at a different cuff site (e.g., contralateral limb) during that same measurement session.

In light of the uncertainty and difficulties associated with blood pressure measurement in cats, only those animals with

<table>
<thead>
<tr>
<th>Organ</th>
<th>Adverse effect(s)</th>
<th>Generally observed at systolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>Hypertensive retinopathy (hemorrhage, vessel tortuosity, edema, hemorrhage, and/or detachment)</td>
<td>≥180</td>
</tr>
<tr>
<td>Brain</td>
<td>Hypertensive encephalopathy (edema leading to progressive loss of consciousness, altered mentation, and/or seizures) and/or stroke (seizures)</td>
<td>≥180</td>
</tr>
<tr>
<td>Heart and blood vessels</td>
<td>Left ventricular hypertrophy</td>
<td>≥160</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Progressive renal damage</td>
<td>≥160</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Blood pressure classification</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Diastolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;140</td>
<td>and</td>
</tr>
<tr>
<td>Prehypertensive</td>
<td>140–159</td>
<td>or</td>
</tr>
<tr>
<td>Stage I hypertension</td>
<td>160–179</td>
<td>or</td>
</tr>
<tr>
<td>Stage II hypertension</td>
<td>≥180</td>
<td>or</td>
</tr>
</tbody>
</table>
NEW FINDINGS IN FELINE HYPERTENSION

apparent elevations of indirectly measured blood pressure and/or clinical abnormalities directly attributable to hypertensive injury should be considered candidates for treatment. While systolic blood pressure is generally a more important indicator of the potential for end-organ damage, both values are useful and each should be evaluated independently. Accordingly, a classification system parallel to that recommended for humans is herein proposed (Table 3). Because of the association of marked systemic hypertension with ocular injury, the author considers antihypertensive treatment to be indicated in any dog or cat with a sustained systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg (stage II hypertension), regardless of other clinical findings. Initial therapy in cats in this stage should be a calcium channel antagonist (e.g., 0.25 mg amlodipine besylate/kg body weight PO once daily) because of the usually rapid antihypertensive efficacy of this class of agent.11,15,16 A cat with a systolic/diastolic blood pressure that consistently exceeds 160/100 mm Hg (stage I hypertension) should receive antihypertensive treatment if clinical evaluation has identified abnormalities (e.g., retinal lesions or CKD) that could be caused or exacerbated by systemic hypertension. In animals with stage I hypertension in which no clinical abnormalities related to systemic hypertension are identified, the rationale for therapy is less clear. Currently, some clinicians recommend treatment for animals in this range, while others do not. Initial therapy with an inhibitor of the renin-angiotensin–aldosterone axis is prudent (e.g., angiotensin converting enzyme inhibitor such as 0.5 mg benazepril hydrochloride/kg body weight PO once daily, angiotensin receptor antagonist, and/or aldosterone antagonist). These agents may be less effective at lowering systemic arterial blood pressure, but based on a variety of published studies, they have strategic advantages in preserving end-organ structure and function. Cats in the prehypertension stage should be carefully monitored; however, on the basis of our present knowledge, it seems reasonable to conclude that these cats are unlikely to experience adverse effects unless blood pressure rises further and they are not generally candidates for antihypertensive therapy.

It is usually not possible to restore blood pressure to normal values when treating a hypertensive animal. It should be the veterinarian’s goal to lower the blood pressure to within 25 mm Hg of the normal ranges for blood pressure, thus reducing pressure to the prehypertension stage or lower (i.e., systolic <160 mm Hg and diastolic <100 mm Hg).

IS THERE A ROLE FOR DIETARY THERAPY IN HYPERTENSIVE CATS?

Though poorly studied, the usual recommendation is to initially institute a low-sodium diet. This recommendation was based on the idea that such a dietary approach would provide further blood pressure-lowering benefits as per the Guyton hypothesis of the role of salt and volume in blood pressure regulation.17 Unfortunately, recent studies suggest that dietary sodium chloride restriction may not lower blood pressure in cats but instead reduce glomerular filtration rate and enhance potassium excretion18 (Table 4). These effects would at least partly be attributable to activation of the renin–angiotensin–aldosterone axis by this dietary change. Activation of this system may have other long-term adverse effects by promoting glomerular hypertension and renal fibrosis.

Theoretically, supplementation of diet with omega-3 polyunsaturated fatty acids could lower intraglomerular pressure (based on studies in dogs19) and systemic arterial blood pressure (based on studies in humans20,21). Unfortunately, there is a paucity of data in cats in this area.

Obesity can elevate systemic arterial pressure in humans and

<table>
<thead>
<tr>
<th>Blood pressure classification</th>
<th>Diagnostic and antihypertensive treatment recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>Prehypertensive</td>
<td>Repeat blood pressure measurements every 3–6 months, more frequently if evidence of end-organ damage is present. Generally, no antihypertensive therapy indicated.</td>
</tr>
<tr>
<td>Stage I hypertension</td>
<td>Repeat blood pressure measurements every 1–3 months. Treat with inhibitor of renin-angiotensin–aldosterone axis; add calcium channel antagonist if needed.</td>
</tr>
<tr>
<td>Stage II hypertension</td>
<td>Repeat blood pressure measurements every 1–3 months. Treat with inhibitor of renin-angiotensin–aldosterone axis; add angiotensin converting enzyme inhibitor once stable.</td>
</tr>
</tbody>
</table>
dogs\textsuperscript{22} and, perhaps, in cats. Consequently, weight loss is desirable in obese, hypertensive animals. However, the effect of obesity on blood pressure is relatively modest by itself. It is likely that weight loss will be of some benefit to obese, hypertensive cats and should be a long-term goal of medium priority.

REFERENCES


\begin{table}[h]
\centering
\caption{Potential Effects of Dietary Sodium Chloride Restriction in Cats}
\begin{tabular}{|l|l|}
\hline
\textbf{Parameter} & \textbf{Potential effect of dietary sodium chloride restriction} \\
\hline
Blood pressure & None \\
Glomerular filtration rate & Reduction accompanied by efferent arteriolar vasoconstriction \\
Potassium homeostasis & Kaliuresis and potassium depletion \\
Renin–angiotensin–aldosterone axis & Activation with increased intraglomerular hypertension, kaliuresis, and progressive renal fibrosis \\
\hline
\end{tabular}
\end{table}
INTRODUCTION AND GENERAL GOALS OF TREATMENT

Chronic renal failure (CRF) most commonly occurs in cats >10 years of age (31% are between 10 and 15 years, and 32% are >15 years of age).1 Cats at any age can be diagnosed with CRF. It appears that old cats develop CRF more frequently than do dogs of similar age. In a study involving primary care practices, renal disease in all ages of cats had a prevalence of 2.2% compared to 0.8% in dogs.2 Chronic renal failure was noted to be the second leading cause of nonaccidental death in cats in a recent survey of primary care practices.3 Increasing age and decreasing body condition score increased the likelihood for diagnosis of renal disease. Himalayan, Persian, mixed breed, and Siamese cats are at increased risk for renal disease or renal failure. CRF is a common cause of death in aging cats.

The goals in treating CRF are to minimize the clinical signs of uremia, retard the progressive loss of renal functions, and preserve or enhance the nutritional status of the patient. A major endpoint for all of our treatments is adequate nutrient intake to allow for a good quality of life with reasonable body condition. Food intake and body condition commonly deteriorate during CRF and may result from physical changes (oral and stomach ulcers, tongue necrosis), an altered sense of smell and taste, metabolic changes that suppress appetite (anemia, hypokalemia, metabolic acidosis, azotemia, hyperparathyroidism), and dietary interventions that adversely affect food intake.

PRACTICAL CONSIDERATIONS FOR FEEDING CATS WITH CRF

Maintaining or Increasing Dietary Intake

Medical treatments may be helpful in maintaining or increasing food intake. Full hydration maintained by periodic infusion of SC fluids enhances excretion of uremic waste products. As a result, the cat feels better and there is increased gastric blood flow, lessening uremic gastropathy. Treatment with H2-receptor antagonists (famotidine, ranitidine) may lessen the degree of gastric ulceration and increase food intake. We routinely use H2 blockers during the transition to a new diet, even in cats that are still eating and not vomiting. In some cats, the use of H2 blockers is temporary, while in others this treatment will be lifelong. Continued use of H2 blockers is maintained if food intake decreases or vomiting increases when H2 blockers are initially discontinued. Some cats also increase their food intake when systemic hypertension is controlled.

What Foods Should Be Fed to Cats With CRF?

An ideal kidney diet (“renal diet”) for cats with CRF should generate little or no nitrogenous waste for renal excretion and help maintain normal hydration. Commercial renal diets usually have mild to moderate protein restriction, phosphate and sodium restriction, potassium and alkali precursor supplementation, and a decreased ratio of N6 to N3 polyunsaturated fatty acids compared to maintenance diets. Unfortunately, renal diets appear to be less palatable than maintenance foods due to decreased protein and salt content. Palatability of food is important for maintenance of
food intake in sick cats. However, approximately 60% to 80% of cats will accept a renal diet if special efforts to make the transition are employed.

Canned renal diets are preferred to dry foods for three reasons. Canned foods provide substantially more water than dry foods, which helps maintain the patient’s hydration. Additionally, canned foods are usually less acidifying and provide less phosphorus than dry foods. However, dry foods contain considerably more potassium than canned foods in an attempt to decrease kalliopenic nephropathy.

Some cats that have exclusively eaten dry foods may have great difficulty transitioning to canned foods. Providing both foods in separate bowls next to each other may encourage transition to the new food. The amount of canned veterinary food is gradually increased while the amount of maintenance food is reduced. Increasing the palatability of the canned veterinary food by adding flavor enhancers (tuna juice, clam juice, or meat drippings) may encourage intake. If the cat fails to transition to a canned veterinary food, it is best to let the cat eat some of its previous maintenance diet. Though eating the previous maintenance diet is not optimal, consuming an inadequate amount of veterinary food will only further contribute to protein and calorie malnutrition because catabolism of body tissues increases to provide energy. This catabolism contributes further nitrogenous solutes for renal excretion, as well as acid by-products.

Restriction of Dietary Protein Intake

The protein requirement in cats is higher than that in dogs. Nitrogen catabolic enzymes in the cat’s liver are unable to adapt to changes in dietary protein intake. These enzymes function at a high rate of activity independent of level of protein fed, and thus a large amount of protein is catabolized after every meal regardless of the quantity of protein ingested. Cats also have two unique amino acid requirements, requiring higher levels of arginine and taurine. Cats synthesize only small quantities of taurine and cannot convert to the use of glycine for bile acid conjugation if taurine is restricted. Thus taurine from animal sources must be continually provided in the diet to replace taurine losses. For these reasons it is easier for the cat to develop protein calorie malnutrition as compared to the dog.

Two studies have examined the effects of diet on experimentally created CRF in cats. Whether or not restricting protein intake is beneficial is unclear. In one study, healthy cats with 5/6 nephrectomy fed a high-protein diet had more severe glomerular and tubulo-interstitial lesions than cats fed a low-protein diet. However, there was no difference in renal function in these two groups over the course of 1 year. Cats fed the low-protein diet weighed less, had decreased muscle mass, poor hair growth, and consumed fewer calories as compared to cats receiving the higher-protein diet. The lower protein diet-fed group also exhibited lower serum albumin and hematocrit concentrations. Further, as originally fed in the study, the high-protein diet was deficient in potassium, which may have contributed to adverse signs in this group of cats. A second study suggested that dietary protein restriction may not be necessary for cats with CRF. In this study, cats with subtotal nephrectomy did not develop glomerular lesions regardless of protein intake. A second study suggested that dietary protein restriction may not be necessary for cats with CRF. In this study, cats with subtotal nephrectomy did not develop glomerular lesions regardless of protein intake. Renal function remained stable for a year independent of protein or calorie intake. There was no difference in severity of kidney lesions or glomerular filtration rate (GFR) in cats with experimental CRF fed 9 g protein/kg body weight (BW)/day compared to those fed 5.2 g protein/kg BW/day. Hypokalemia developed in 4 of 7 cats with CRF consuming more protein, whereas CRF or control cats fed a low-protein diet did not develop hypokalemia. Hypokalemia developed despite higher caloric and potassium intake in cats fed the high-protein diet, which is consistent with the fact that potassium needs are directionally proportional to protein intake.

Restriction of protein, phosphate, and sodium intake has also been studied in clinical cases of CRF. A veterinary renal diet slowed the progressive loss of renal function in cats with CRF as compared to those eating a maintenance diet. This veterinary diet supplied 6.45 g dietary protein/100 kcal and was sufficient to maintain the nutritional status of cats with CRF while reducing signs of uremia and azotemia. Subjective criteria for quality of life and physical condition as rated by owners and clinicians were better for those cats receiving the veterinary diet.

Depletion of protein reserves can occur if too little protein is fed or consumed. Lower amounts of protein can be fed initially to allow severely azotemic cats to feel better, but higher amounts of protein content should be fed chronically. Providing protein intake as a fixed percentage of calories allows adequate dietary protein only if the cat is consuming its full caloric needs. It is important to consider the amount of protein as a specific dose. We recommend an absolute dose of 3 g high-quality protein/kg BW/day for cats with CRF to avoid protein depletion. The percentage of protein in the diet will need to be increased for those eating less than full caloric
needs for maintenance. Caloric intake is recommended at 20 to 40 kcal/kg based on level of activity, as most cats with CRF have reduced activity. In a 5-kg cat with CRF eating 200 kcal/day, the minimum protein requirement is 15 g of protein/day; to provide 15 g protein in 200 kcal, the diet must contain 7.5 g/100 kcal. If this cat consumes only 100 kcal/day, it would be impossible to meet minimal protein needs because there are no products available that provide 15 g protein/100 kcal. Thus, the lower the daily energy intake, the higher the percentage of dietary protein needed to maintain body reserves while still avoiding excessive protein intake. This concept of dosing can also be used for other nutrients, such as sodium and phosphorus.

We send samples of dry and canned veterinary foods home with the client. This allows the cat to select the diet it prefers when it is feeling better and is in a familiar environment. We have been unable to identify any consistent preference among diets, but most patients will consume adequate amounts of at least one of the diets offered. If clients cannot switch their cat with CRF totally to a veterinary food, consumption of the veterinary food as even part of the daily food intake will be beneficial. The risk of protein depletion is balanced by the risk of increased blood urea nitrogen (BUN) and associated deterioration in those that consume excess dietary protein.

CONTROL OF HYPERPHOSPHATEMIA AND RENAL SECONDARY HYPERPARATHYROIDISM

Renal secondary hyperparathyroidism eventually develops in all patients with progressive loss of nephron mass and renal failure. Inadequate renal production of calcitriol releases inhibition of parathyroid hormone (PTH) synthesis resulting in increased PTH concentrations. Increased phosphorus retention also decreases calcitriol synthesis by decreasing activity of the 1-α-hydroxylase enzyme system. Decreased ionized calcium also contributes to increased PTH synthesis and secretion, but only when serum phosphorus levels are increased above 8 to 10 mg/dL. PTH is toxic to renal tissues, and control of PTH levels to less toxic levels is important.

Restriction of dietary phosphate may be more important than restriction of protein. Cats with 5/6 experimental nephrectomy receiving a normal phosphate diet had higher PTH and serum phosphorus concentrations compared to cats receiving a phosphate-restricted diet. Renal mineralization was absent in cats eating the phosphate-restricted diet; however, BUN and creatinine did not differ between treatment groups. The percent change in GFR was not different between diets, but GFR was not measured in three cats that died in the non-phosphate-restricted diet group.

Control of serum phosphorus and PTH levels may be pivotal for increased survival of cats with clinical CRF. In an unblinded, nonrandom study, cats with CRF were offered a veterinary renal diet; those that did not accept the veterinary diet were assigned a maintenance diet. Survival time for cats eating the veterinary diet was twice that for cats eating the maintenance diet. Although food intake was not recorded in this study, serum phosphorus and PTH concentrations were lower in cats receiving the veterinary renal diet, and diet alone was able to normalize PTH in 86% of the cats early in the course of CRF. As CRF advanced, diet alone was unable to control PTH production, and treatment with intestinal phosphate binders became necessary to achieve control of PTH.

Serum phosphorus is not an adequate measure of the cellular phosphorus burden. The first step in reducing phosphorus burden is to decrease phosphorus intake, but currently there are no diets available that are restricted in phosphate alone. Restriction of dietary phosphate is typically accomplished by restricting animal-source proteins. However, beneficial effects of phosphorus restriction are independent of protein restriction. Phosphorus restriction may reduce renal mineralization by reducing the calcium X phosphorus concentration product. Additional phosphate restriction may be needed if serum phosphorus does not return to normal through dietary restriction. The use of intestinal phosphate binders may be needed in these patients. Phosphate binders work best when given either with food or near the time of ingestion. They irreversibly bind phosphate within the intestinal lumen, thereby lessening absorption and increasing fecal excretion of phosphate. Return of serum phosphorus to normal does not guarantee that serum PTH levels will return to normal, since phosphorus restriction alone only works in patients with sufficient remaining calcitriol synthetic capacity. In advanced renal failure, it may be impossible to achieve adequate control of serum phosphorus due to uncontrolled metabolic acidosis and secondary hyperparathyroidism. These conditions contribute to hyperphosphatemia by accelerating bone dissolution. Failure to control serum phosphorus adequately is a poor prognostic factor.

In patients with early kidney failure, intestinal phosphate binders (aluminum hydroxide, calcium carbonate, calcium acetate) may provide phosphorus restriction without the necessity to change the diet. It has been conventional to administer intestinal phosphate binders only when the serum
phosphorus is elevated, but there may be reasons to administer them to patients with CRF before the serum phosphorus concentration rises. Phosphorus intake should be slowly reduced to approximately 60 mg/kg/day as GFR decreases with disease progression.

**CORRECTION OF HYPOKALEMIA**

Hypokalemia occurs more commonly in cats than in dogs with CRF. Veterinary foods designed for renal failure often contain additional potassium supplementation in the form of potassium citrate. Appropriate potassium supplementation protocols for cats with chronic renal failure and normal serum potassium concentration remain controversial. A study at The Ohio State University could not find a benefit for potassium gluconate over sodium gluconate supplementation in cats with CRF and normal serum potassium.

Muscle content of potassium in cats with CRF and normal serum potassium was lower than that of normal cats. Muscle potassium increased to a similar degree with either sodium or potassium gluconate supplementation. GFR did not differ by gluconate salt.

**CORRECTION OF METABOLIC ACIDOSIS**

Metabolic acidosis often accompanies CRF and may cause anorexia, nausea, vomiting, and weight loss. Muscle weakness, lethargy, hypokalemia, skeletal demineralization, hyperphosphatemia, and hypercalciuria may be exacerbated by chronic metabolic acidosis. Accelerated progression of chronic renal failure attributed to increased tubular ammoniagenesis during chronic metabolic acidosis has been suggested in some but not other rodent models of CRF; but this effect has not been studied in cats or dog with CRF. Diet influences the degree of acid end products required for excretion. Egg protein has traditionally been assumed to be the most biologically utilizable protein, but studies in dogs with CRF revealed that egg protein-containing diets, which are high in sulfur-containing amino acids (methionine and cysteine/cystine), might be more acidifying than vegetable protein-based diets. Lower-protein diets can result in less acid for excretion, especially if they reduce sulfur-containing amino acid intake. Veterinary foods designed for the treatment of renal failure are usually designed to be mildly alkalinizing by the addition of salts that are metabolized to bicarbonate (e.g., potassium citrate). Since many commercial foods for healthy cats in the United States have been formulated to be acidifying, these diets should be discontinued in favor of a veterinary food, if possible, particularly if the alkalinizing potential of phosphate binders cannot provide adequate control of the acidosis. Acid-base balance should be reevaluated after dietary modification to see if supplemental alkali is needed. Sodium bicarbonate, potassium citrate, calcium carbonate, and calcium acetate are sources of alkali.

**FOLLOWING THE NUTRITIONAL STATUS OF CATS WITH CRF**

Cats are susceptible to the development of protein calorie malnutrition during CRF with or without dietary intervention. Cats on a restricted protein intake need to be carefully monitored. Body condition score (BCS) and muscle condition score (MCS) should be serially recorded and evaluated. Depending on the cat’s overall condition, recheck visits should be scheduled at 1, 3, and 6 months after the start of treatment. More frequent recheck evaluations will be required for those cats that are not doing well and those with major metabolic instability or systemic hypertension.

Serum chemistry should include BUN, creatinine, and phosphorus for monitoring renal function, though BUN is a less useful monitor of renal function while on a restricted-protein intake. Serum total protein, albumin, cholesterol, and hematocrit should be assessed to measure potential effects of protein restriction on nutritional status. Serum potassium and TCO$_2$ should be monitored to determine adequacy of potassium and alkali supplementation. Handheld devices to measure blood gases periodically may be useful. Inadequate protein in the diet can mimic progressive CRF (decreasing BCS and MCS, increasing creatinine, decreasing hematocrit, decreasing HCO$_3$). In those cats where protein malnutrition is suspected, dietary protein intake should be increased for the next month and the patient’s status re-assessed.

**ALTERNATIVE METHODS FOR FEEDING**

Nasogastric (NG) tube feeding can be useful during acute rescue from a uremic crisis during decompensated CRF. Prior hydration with IV fluids and H$_2$ blockers will facilitate successful NG feeding. Most cats tolerate NG tube feeding well. Nutrition can be pulse-dosed every few hours or given constantly by gravity or with an infusion pump. Metoclopramide may be useful if there is severe salivation during feeding or if there is gastrointestinal ileus. Early feeding may limit the duration of intensive care days prior to release from the hospital. Veterinary and human liquid diets are available for infusion and
provide about 1 kcal/mL with 20% to 30% of calories from protein. Liquid diets designed for human use do not provide enough taurine and arginine and must be supplemented with these if used. Veterinary renal foods cannot be used through the NG tube because they cannot be blenderized fully to allow passage through small-diameter NG tubes. Feeding for 3 to 5 days may be adequate to allow recompensation.

Esophagostomy or percutaneous endoscopic gastrostomy (PEG) tubes can be considered for chronic feeding of cats that do not maintain adequate intake and body condition. Blenderized veterinary renal diets will pass through these tubes. Esophagostomy tubes may be preferred for initial chronic treatment because short-term anesthesia is used and no special equipment is required. PEG tube placement requires longer anesthesia and endoscopy to confirm proper placement, though there are techniques for nonendoscopic placement. PEG tubes are recommended for long-term feeding. Many cats return to normal body condition score with these types of chronic feedings and have an improved quality of life despite CRF.

In summary, adequate caloric and protein intake is the single most important consideration for the long-term well-being of cats with CRF. Restriction of phosphate intake is the dietary maneuver that is most likely to increase life span and preserve renal function of cats with CRF. Restriction of dietary protein intake is not as important in the management of CRF in cats as was previously thought, though it can be helpful in reducing clinical signs in cats with severe azotemia. Monitoring of serum potassium concentrations is important because some cats with CRF develop hypokalemia that is best managed with potassium salt supplementation. Some cats with CRF develop metabolic acidosis that will require additional alkali supplementation.

REFERENCES

SUGGESTED READING
INTRODUCTION

Probiotics are microorganisms that can be added to food with the purpose of exerting beneficial effects on the host. According to Fuller,1 a probiotic is “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” The most often-used probiotic genera in humans and farm animals are enterococci, lactobacilli, and bifidobacteria,2 which are normal inhabitants of their colonic flora.3–5 Probiotics can be consumed in the form of both fermented and nonfermented foods such as yogurt, vegetables, meat, cheese, and milk-based drinks. When introduced into the animal, the probiotics may become a transient constituent of the gut microflora capable of exerting their biologic effects, thus giving a rationale for their use as a component of functional foods. Weaning, stress, dietary changes, use of antibiotics, and intestinal infections are all conditions that affect the natural balance of the intestinal microflora of pets and for which the application of probiotics might be beneficial. This is the reason why there is an increasing interest to include probiotics in canine and feline nutrition.6–8

The intestinal microflora plays a crucial role in host defense as demonstrated by its ability to modulate both innate and acquired immunity at the local as well as systemic levels.9,10 The ability of probiotics to modulate the constituents of the intestinal flora is one mechanism by which they affect the immune system. However, a direct interaction with the mucosal immune system to activate the production of immunoregulatory cytokines has also been postulated.10

This review will discuss how the administration of probiotics to pets could modulate biologic and physiologic processes that would ultimately provide beneficial health effects.

SELECTION OF PROBIOTICS AS FUNCTIONAL INGREDIENTS

Multiple criteria have been described for selecting specific strains as candidates with potential health benefits.11 These include resistance to conditions prevailing in the gastrointestinal (GI) tract (low pH, bile), adherence to intestinal mucosa, colonization and survival in the target host species, strong activity against pathogenic microorganisms, and modulation of the immune system. Adaptations to intestinal conditions (e.g., the ability to survive and become metabolically active as well as the ability to adhere to intestinal epithelial cells or mucus) are important factors that may provide the probiotic with a competitive advantage in the small bowel, where no true stable microflora exists. To date, several probiotic bacteria have been described as adherent to GI lining cells in vitro and in vivo in dogs and cats.6

Antagonism to enteropathogens is also an important property that needs to be demonstrated systematically (in vitro and in vivo) for any new probiotic claiming functionality. Several lactic acid bacteria have been shown to inhibit the growth of a wide range of enteropathogens.13–19 Competition for essential nutrients, aggregation with pathogenic microorganisms,20 competition for receptor sites,21 modification of the metabolic activity of the gut microflora, and the direct antagonism through the action of antimicrobial metabolites22–24 have all been postulated to play a role. Immune modulation (i.e., the increase of host reactivity toward the challenge of pathogenic microorganisms or the decrease of host reactivity toward innocuous antigens such as food antigens) is another important feature of probiotic activity.10,25,26 The in vitro analysis of cytokine profiles produced by peripheral blood mononuclear cells upon interaction with probiotics is very helpful for screening candidate strains for specific antagonistic responses against canine and feline intestinal pathologies. Therefore, consideration of both ecologic and immunomodulatory properties of
candidate probiotic strains is fundamental in designing functional foods with most promising efficacy.

Finally, since probiotics are living bacteria that are often administered at high concentrations to the host, it is imperative that a history of safe long-term use is documented. Although this is the best proof of no detrimental health effect, new, additional safety aspects need to be continuously assessed. For the time being, traditional toxicity tests have been performed with the current probiotic bacteria. However, new strains require new evaluation since there is no possible generalization on the safety or potential pathogenicity factors among strains. In pets, the probiotic strain that has demonstrated the greatest efficacy in vivo is the Enterococcus faecium SF68 (NCIMB 10415). This bacterium has been reported to be clear of currently recognized virulence factors. This strain is vancomycin sensitive and does not contain transferable antibiotic resistance factors. E. faecium SF68 has been used for a long time in the treatment of antibiotic-associated as well as acute diarrhea in humans. Furthermore, during the last two decades, E. faecium SF68 has been prescribed by veterinarians in Austria and Switzerland for the stabilization of the intestinal flora in animals showing disturbances caused by a change in diet, stress, or antibiotic treatments. To date, no adverse effects have been reported. Based on the results of the molecular analysis of E. faecium SF68 and its long history in veterinary practice at high concentrations, this probiotic can be considered safe for use in pets. Furthermore, in a recent study examining the immune-enhancing properties of E. faecium SF68 in pets over a 1-year period of feeding, we did not observe any deleterious effects on the indigenous bacterial flora or on immune markers implicated in immune-related diseases such as allergy, autoimmunity, and inflammation.

PROBIOTIC BENEFITS ACROSS DISTINCT GASTROINTESTINAL REGIONS

Among the great diversity of functional health claims, it is now widely accepted that certain probiotics are efficient for preventing intestinal infections. The main motive for many studies has been the need to find alternatives to classical antibiotic treatments whose widespread use has led to an increase in antibiotic resistance. The use of probiotics in a preventive fashion could diminish the need for antibiotic treatments in humans or house animals at risk of infections, such as elderly and immunocompromised hosts, and be helpful for fast recovery as well as limitation of negative side effects often observed during antibiotic therapy. There is certainly increased interest by the scientific community to provide probiotics, which reinforce the immune system in highly susceptible populations. To this end, we have recently shown that feeding young puppies with a dry dog food supplemented with E. faecium SF68 probiotic strain improves immune response to canine distemper vaccine.

Further health benefits of probiotics have been recently reported in animal models and human patients suffering from immune-related inflammatory disorders, such as allergy and idiopathic inflammatory bowel disease (IBD). The goals have been to decrease the massive use of antiinflammatory drugs and antibiotics in the treatment of these states and prevent the high relapse rate that is characteristic of these chronic conditions.

Stomach

The extremely low pH of normal gastric secretions creates a hostile environment for bacterial development and provides an efficient nonspecific mechanism of defense against infections. Gastric acidity represents the first barrier of the GI tract against bacterial passage. Probiotic microorganisms orally administered to healthy people or pets have to overcome this acid stress. Since the benefits conferred by probiotics may depend, at least in part, on their metabolic activity and subsequent interaction with the indigenous microflora, the capacity of probiotics to survive in such an environment is crucial for their protective role.

Helicobacter pylori is involved in gastritis, gastric and duodenal ulcers, and some gastric cancers in humans. Although H. pylori and numerous other strains of Helicobacter have been shown to infect companion animals worldwide, the impact of the infection on feline or canine gastric health is still unclear.

However, some lactic acid bacteria have been shown to inhibit H. pylori through the bactericidal effect of their lactic acid in humans and animal models. Recently, a whey-based medium fermented with Lactobacillus johnsonii strain LA1 (NCC 533) has been reported to inhibit H. pylori gastric colonization in humans. This effect may be due to the production of bacterial metabolites such as lactic acid and/or other bacteriostatic compounds.

Small Bowel

The establishment of the bacterial flora of dogs is a gradual process that begins immediately after birth. The instability of the resident bacteria during early development may represent a
rationale for preferential application of probiotics in young dogs. Moreover, nutritional supplementation with probiotics early in life could help neonatal immune development.

Compared to the highly colonized large bowel, the small intestine harbors a relatively poor resident microflora whose barrier effect against pathogens is rather limited. This might be the reason why most bacterial and viral intestinal infections target the small bowel. To effectively antagonize small-bowel infectious agents, probiotics probably need to be continuously administered and need to become physiologically active in the target intestinal environment, including the production of antimicrobial metabolites, competition for essential nutrients and/or adhesion receptors, and target immune stimulation. In addition to competition for adhesion receptors, probiotics can elicit a direct antagonistic activity against small bowel pathogens, as shown in humans and farm animals.

The majority of the gut-associated lymphoid tissue is located in the small bowel and immune reactions take place preferentially, if not exclusively, in the specific constituent of the mucosa called Peyer’s patches. A large number of studies indicate that probiotic administration has a profound impact on the immune activity of the host, thus providing an interesting approach to fight intestinal infections. For example, several strains of lactobacilli have been shown to increase phagocytic activity in rodents and in humans. In addition, L. johnsonii (NCC 533, LA1) was shown to boost the specific IgA production in response to an attenuated Salmonella typhi vaccine in humans.

A number of viruses also infect the mammalian host via the small bowel. One of the most prevalent viral infections in infants is rotavirus, a leading causative agent of chronic diarrhea. This and other viruses are also found in stools of young dogs with diarrhea, suggesting that rotavirus may also be etiologically implicated in diarrhea observed in pets. Studies with Bifidobacterium bifidum have demonstrated that probiotic supplementation can decrease virus shedding and diarrhea in infected infants, but it is not known whether this protection results from a direct antagonism of the virus or an immunoadjuvant effect of the probiotic bacteria. In our study on young dogs, we have observed that administration of E. faecium SF68 triggers the mucosal immune system underlying the small bowel, induces a self-specific immune response, and stimulates the production of polyclonal secretory IgA. This last observation was recently confirmed in adult dogs fed E. faecium SF68.

Colon

Probably the strongest clinical evidence supporting a role for the colonic microflora in intestinal inflammation comes from studies that reported side effects following the use of antibiotics that disrupt the composition of the colonic flora. Antibiotic-associated diarrhea (AAD) is a frequent complication of antibacterial treatments in hospitalized human patients. The compromised barrier function provided by the colonic microflora during antibiotic treatment promotes overgrowth of opportunistic pathogens such as toxigenic Clostridium difficile and Bacteroides fragilis. However, the probiotics E. faecium SF68, Saccharomyces boulardii, and L. rhamnosus GG have shown promising results in the prevention and treatment of AAD in humans. Furthermore, a number of studies in animal models of colitis have demonstrated that several strains of the genus Lactobacillus effectively prevent the onset of disease. Fecal bacterial content of the colon remains the only noninvasive and ethically accepted technique that can be...
used to monitor bacterial ecology in feeding studies and clinical trials. Our experiments have shown that *E. faecium* SF68 was able to colonize the canine and feline intestine during feeding, with values ranging from $10^7$ to $10^8$ CFU/g of feces. It was thereafter rapidly cleared when the feeding was stopped, indicating that the probiotic was able to colonize the gut only transiently. Moreover, feeding *E. faecium* SF68 to dogs also resulted in a trend toward a decrease of *Clostridium perfringens* concentration in the feces. This finding is often considered a positive indicator of colon health. However, it seems that this effect is dependent on the concentration of proteins in the diet.

The term IBD is used in canine medicine to describe a spectrum of diseases that are generally defined by the dominant histopathologic features of gut biopsies (e.g., lymphoplasmacytic enteritis, eosinophilic gastroenteritis). These disorders have similar clinical and histopathologic manifestations and underlying pathogenic mechanisms. Thus, some similarities probably exist between idiopathic IBD, dietary hypersensitivity, and the poorly understood disorder “small intestinal overgrowth” (SIBO). These enteropathies are of major clinical significance in veterinary medicine, and some of the most popular breeds of dogs are particularly affected. Moreover, these intestinal disorders are chronic in nature, and the majority of cases evolve with frequent relapses of clinical disease throughout life. If administration of probiotics can be shown to reduce the incidence of clinical relapse in dogs, this will have a major impact on pet health and well-being.

**CONCLUSION**

There is increasing experimental and clinical evidence supporting an important role for probiotics in the maintenance of a healthy GI tract, particularly in the prevention of intestinal infections and inflammatory conditions. Several studies also suggest that probiotics may be used as therapeutic adjuvants in the treatment of certain pathologies. In addition to the large numbers of studies reporting the efficacy of probiotics in vitro, in rodent models, farm animals, and humans, efficacy of the probiotic *E. faecium* SF68 has now been demonstrated in pets. Due to the similarities of GI tract physiology and microflora among these species, it seems reasonable to assume that probiotic functionalities reported in humans, farm animals, and rodents might also be confirmed in pets. However, the effects need to be carefully investigated and demonstrated in the target species before any beneficial health claims can be made.

**REFERENCES**


An Assessment of Prebiotic Use in Companion Animal Diets

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INTRODUCTION

The complex mixture of intestinal microbes serves an important role in the health of the gastrointestinal (GI) tract and of the host animal. Depending on what species are most prevalent, gut microbial inhabitation can have a beneficial or detrimental effect on the host. In addition to their role in the development of the GI immune system, commensal microbial populations are critically important in resisting the colonization of pathogenic species and producing short-chain fatty acids (SCFA), the major energy source of colonocytes.1 The composition of the colonic microbiota has recently been shown to modulate the expression of genes involved with several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation.2 Although a symbiotic relationship appears to exist between host and microbes, their presence in the gut is not always beneficial. Microbial inhabitation of the gut increases energy expenditure by the host, primarily due to increased turnover of mucus and epithelial cells and constant production of inflammatory and immune cell populations.

Over time, mammals have adapted and become dependent on microbes and their activities in the GI tract. Proper microbial balance in the gut is key to maintaining host health. In addition to host genotype and environmental factors, the diet plays an important role in determining the composition of the gut microflora. Both the amount and type of substrate available to colonic microbes largely impacts gut microbial ecology. While microbial fermentation of carbohydrates primarily produces SCFA, degradation of nitrogenous compounds results in the production of numerous putrefactive catabolites (e.g., ammonia, biogenic amines, phenols) that are implicated as the major odor components of feces.3,4 More importantly, many of these protein catabolites may have negative influences on gut health. The dietary inclusion of prebiotics, nondigestible ingredients that selectively stimulate the growth and/or activity of a limited number of bacteria in the colon, is a popular approach used to attain and maintain proper microbial balance.5

Although the most common prebiotics studied are fructans, the beneficial effects of mannans, lactosucrose, lactulose, and others have been reported. Because prebiotics manipulate colonic microbial populations, they may have an effect on several indices associated with gut health including fecal characteristics, fermentative end-product concentrations, and immune function. In addition to selectively stimulating the activity and/or growth of beneficial bacterial populations (e.g., bifidobacteria, lactobacilli) in the colon, prebiotic supplementation has been shown to produce several other beneficial effects on gut and host health. For example, fructans have been shown to effectively relieve the symptoms of constipation in humans,6 improve mineral absorption in rats,7 and decrease concentrations of protein catabolites produced in the colon of dogs.8,9 Mannans aid in pathogenic resistance,10 influence GI immune status,9,11,12 and possess antimutagenic and antioxidative activity.13,14

CANINE PREBIOTIC EXPERIMENTS

Although a large body of literature exists regarding the effects of prebiotics on human health, little information exists regarding dogs. The effects of several prebiotics have been tested in dogs, including chicory (a natural source of long-chain fructan), inulin (a long-chain fructan), lactosucrose, lactulose, mannanoligosaccharides (MOS), oligofructose (OF; fructan chains with 8 to 10 units), short-chain fructooligosaccharides (scFOS; fructan chains with 3 to 5 units), transgalactosylated oligosaccharides (TOS), and xylooligosaccharides (XOS). Because most of the prebiotics have been tested in...
only a limited number of experiments and primary outcomes confined to nutrient digestibility, stool quality, or microbial concentrations, much remains unknown regarding their function. All published canine prebiotic experiments known to us are reviewed below.

To our knowledge, the first prebiotic experiment in dogs tested the effects of lactosucrose (a trisaccharide produced from lactose and sucrose) on microbial populations and fecal metabolites. Adult dogs were given 1.5 g lactosucrose/day for 2 weeks. Fecal and microbial characteristics were measured before lactosucrose administration, on day 7 and day 14 of lactosucrose administration, and 7 days after administration had ceased. Bifidobacteria populations were increased \( P < 0.05 \) from 8.9 colony-forming units (CFU) \( \log_{10}/g \) (baseline) to 9.4 and 9.4 CFU \( \log_{10}/g \) after 7 and 14 days of lactosucrose feeding, respectively. Bifidobacteria populations decreased to baseline levels (8.8 CFU \( \log_{10}/g \)) 7 days after feeding had stopped. Lecithinase-positive clostridia populations, which include \textit{C. perfringens}, decreased after 7 days \( P < 0.05; \) 4.4 versus 6.0 CFU \( \log_{10}/g \) and 14 days \( P < 0.01; \) 3.1 versus 6.0 CFU \( \log_{10}/g \) of lactosucrose feeding. Lecithinase-negative clostridia concentrations, however, were unaffected. Lactosucrose feeding also increased \( P < 0.05 \) fecal water content (74.3% at baseline versus 77.8% and 76.9% after 7 and 14 days of supplementation, respectively) and decreased fecal concentrations of ammonia (172.5 versus 427.4 \( \mu g/g \) wet feces), phenol (25.9 versus 49.2 \( \mu g/g \) wet feces), ethylphenol (0.0 versus 8.5 \( \mu g/g \) wet feces), indole (18.9 versus 34.4 \( \mu g/g \) wet feces), and skatole (1.3 versus 3.0 \( \mu g/g \) wet feces) after 14 days of supplementation. The shift in microbial populations (increased bifidobacteria, decreased clostridia) and decrease in putrefactive compound concentrations suggest enhanced colonic health as a result of lactosucrose supplementation.

Small intestinal bacterial overgrowth (SIBO) is a serious problem in dogs with IgA deficiency. Therefore, Willard et al supplemented scFOS to IgA-deficient German shepherds to decrease small intestinal microbial density and ameliorate the clinical signs associated with SIBO. All dogs \( n = 16 \) were fed a control diet for 3 months and then were randomized into two groups: no scFOS (control) or 1% scFOS. Intestinal fluid and tissue were collected at, before, and 46 to 51 days after scFOS administration. It was reported that scFOS supplementation was beneficial; scFOS-supplemented dogs had decreased \( P < 0.05 \) aerobic and anaerobic bacteria in tissue samples and aerobic bacteria in the duodenal/jejunal fluid samples. In control dogs, aerobic/facultative anaerobic microbes increased from baseline in intestinal fluid (5,713,600 versus 618,875 cells) and tissue (99,475 versus 11,875 cells) samples. In control dogs, anaerobic microbes also increased in intestinal fluid (378,150 versus 87,000 cells) and tissue (41,350 versus 33,500 cells) samples. However, in dogs fed scFOS, aerobic/facultative anaerobic microbes decreased in intestinal fluid (1,388,750 versus 2,171,275 cells) but increased in tissue (16,450 versus 6,250 cells) samples. In addition, scFOS-supplemented dogs had decreased anaerobic populations in tissue samples (2,600 versus 15,000 cells) but not in intestinal fluid samples. Although the results of this experiment suggest that scFOS supplementation may be helpful in the prevention or treatment of SIBO in dogs, more research is needed in this area.

Diez et al evaluated the effects of a blend of scFOS and sugar beet fiber \( 4:1 \) on nutrient digestibility and plasma metabolite concentrations in healthy beagles. A control diet was compared with diets containing either 5% or 10% of the fiber blend. Wet feces excreted increased \( P < 0.05 \) linearly with increasing amounts of fiber in the diet (139, 180, and 222 g wet feces/day in dogs fed the basal diet, the diet containing 5% fermentable fiber, and the diet containing 10% fermentable fiber, respectively). No differences were observed among treatments in dry weight of feces excreted/day. However, % dry matter (DM) of feces decreased \( P < 0.05 \) linearly with increasing amount of fiber in the diet. These results can be explained by the capability of fermentable fibers to bind water and increase wet fecal weight without influencing dry fecal weight. Apparent crude protein (CP) digestibility decreased \( P < 0.05 \) with increasing amounts of the fiber blend (87.8%, 86.3%, and 83.8% digestibility for dogs fed the basal diet, the diet containing 5% fermentable fiber, and the diet containing 10% fermentable fiber, respectively). No differences were observed among treatments in apparent DM, organic matter (OM), fat, or ash digestibilities were observed in this experiment.

Yeast cell wall is a rich source of MOS. However, the specific oligosaccharide fraction has yet to be isolated and tested. It is important to note, then, that when MOS is referred to, what is actually being tested is yeast cell wall. Analysis of several MOS sources in our laboratory has shown that its chemical composition is highly variable depending on the source: DM 91% to 96%; OM 82% to 98% (dry matter basis [DMB]); CP 34% to 43% (DMB); TDF 21% to 41% (DMB); fat 6% to 12% (DMB); glucose 188 to 346 mg/g (DMB); mannose 59 to 144 mg/g (DMB); galactose 0 to 36 mg/g (DMB).
O’Carra\textsuperscript{18} performed two experiments examining MOS and its effects on immune function in dogs. In the first experiment, adult beagles were randomized into four treatment groups. A control diet was compared with diets containing 1, 2, or 4 g MOS/kg. Blood samples were collected at baseline (pretreatment) and on days 15 and 31 of treatment for plasma protein and IgG measurements. No changes were observed among groups. In the second experiment, Border collie pups were assigned to a control diet (no MOS) or a diet containing 2 g MOS/kg. After a 7-day adaptation period, a vaccination protocol was initiated. All dogs were vaccinated against parvovirus, leptospirosis, adenovirus, and distemper. Vaccine boosters were applied on day 21 for leptospirosis and day 35 for parvovirus. Blood characteristics were measured over a 9-week period. No changes were observed in weight gain, lysozyme activity, plasma protein concentration, or plasma IgG concentration. Neutrophil activity was numerically increased in pups fed the diet containing MOS after vaccination (approximately 18 versus 14 nitroblue tetrazolium [NBT]+ cells/slide). However, due to low animal numbers (n = 3/group), statistical significance was not reached. Given the results of these studies, it is unknown whether MOS supplementation is beneficial to companion animals.

Three experiments by Russell\textsuperscript{19} examined the effects of soy, chicory as a natural source of inulin, and scFOS on bifidobacteria and clostridia populations, fecal pH, fecal moisture, fecal SCFA concentrations, and total tract nutrient digestibility. In the first experiment, three diets were used: 1) 12% soy (control); 2) 12% soy + 1% scFOS; and 3) 12% soy + 3% chicory. Chichory or scFOS did not influence apparent digestibility. Dogs fed scFOS and chicory had decreased (P <0.05) clostridia and increased (P <0.05) bifidobacteria populations compared to dogs fed the control diet. Clostridia concentrations were lower in dogs fed 1% scFOS (1.3 × 10\textsuperscript{10} CFU/g) and 3% chicory (4.2 × 10\textsuperscript{10} CFU/g) compared to those consuming 12% soy (1.6 × 10\textsuperscript{11} CFU/g). In contrast, bifidobacteria concentrations increased in dogs fed 1% scFOS (8.2 × 10\textsuperscript{10} CFU/g) and 3% chicory (4.5 × 10\textsuperscript{10} CFU/g) compared with those fed 12% soy (1 × 10\textsuperscript{9} CFU/g). The inclusion of 3% chicory significantly increased fecal volume (data were not shown in the paper), indicating that a lower concentration should be examined. The second experiment compared a control diet versus diets containing 1.5%, 3.0%, or 5.0% chicory. All concentrations of chicory tested increased (P <0.05) bifidobacteria (1.27 × 10\textsuperscript{8} CFU/g in dogs fed control versus 7.88 × 10\textsuperscript{7}, 1.23 × 10\textsuperscript{8}, and 4.83 × 10\textsuperscript{7} CFU/g in dogs fed 1.5%, 3.0%, and 5.0% chicory, respectively), but failed to change clostridia concentrations. No differences were observed in apparent DM, nitrogen, or gross energy (GE) digestibility. In the third experiment, ten diets containing different concentrations of chicory and soy were examined. Chicory was used at 0%, 0.5%, 2.0%, or 4.0% concentrations in combination with three soy levels (0%, 6.0%, or 12.0%). Chicory again increased (P <0.05) bifidobacteria populations (approximately 4.98, 8.53, 7.89, and 8.01 CFU log\textsubscript{10}/g with 0%, 0.5%, 2.0%, and 4.0% chicory, respectively), independent of soy concentration. Inclusion rates >2% chicory reduced digestibility (data were not shown) and increased fecal volume (data were not shown). Although chicory supplementation had beneficial effects on microbial populations (e.g., increased bifidobacteria, decreased clostridia), the author suggested a maximal inclusion rate of 2% chicory to attain a desirable fecal consistency and avoid decreases in digestibility and increases in fecal volume.

Howard et al\textsuperscript{20} evaluated the effects of different fiber sources on epithelial cell proliferation, intestinal weight, and colonic blood flow in dogs. Twenty-eight adult dogs surgically fitted with ultrasonic blood flow probes were randomly assigned to one of four treatments: 1) beet pulp (6% of diet); 2) scFOS (1.5% of diet); 3) cellulose (6% of diet); and 4) fiber blend (composed of beet pulp [6% of diet], gum talha [2% of diet], and scFOS [1.5% of diet]). A transient increase (P <0.05; increased blood flow at 0600, but not at 1200, 1630, or 2100 h) in colonic blood flow was observed with scFOS supplementation, which the authors suggested was likely due to SCFA absorption resulting from rapid fermentation of scFOS. In addition, scFOS consumption reduced cell proliferation (smaller proliferation zone [P <0.01] and shorter leading edge [P <0.1]) in the proximal colon, suggesting that more crypt cells underwent differentiation. Although this result was unexpected, enhanced rates of differentiation may protect against cancer by decreasing the number of proliferating colonocytes exposed to carcinogenic compounds present in colonic digesta. These results complement the observations in rats where a probiotic and FOS reduced the presence of aberrant crypts and foci.\textsuperscript{21}

Using the same treatments as in the previous experiment, Howard et al\textsuperscript{22} evaluated the effects of fiber source on nitrogen and energy metabolism and microflora populations in dogs. Apparent DM digestibility was greater (P <0.05) for dogs fed the scFOS diet than for those fed the diet containing cellulose. However, these data were confounded by the fact that
DM intake (expressed as a percent of BW) was reduced with diets containing fermentable fiber sources, especially with the scFOS diet. The decreased food intake by dogs fed scFOS may be in response to SCFA, which are potent stimulators of peptide YY.23 Because peptide YY has been shown to slow gastric emptying and intestinal transit,24 it may have resulted in the increased satiety and nutrient digestibility observed in this experiment. Total coliform populations from the duodenum, ileum, proximal colon, and distal colon were not different among treatments. Dogs supplemented with scFOS had greater ($P < 0.10$) numbers of aerobic species in the distal colon, but not in the duodenum, ileum, or proximal colon, compared to dogs consuming the other treatments.

Using adult ileal cannulated dogs in a $4 \times 7$ incomplete Latin square design, Strickling et al.25 tested the effects of various oligosaccharides (0.5% of diet; OF, MOS, or XOS) on ileal and total tract nutrient digestibilities, microbial populations, ileal pH, ammonia and SCFA concentrations, blood glucose, and fecal consistency. Besides minor changes in ileal SCFA concentrations, the only significant finding was a decrease ($P = 0.07$) in fecal C. perfringens populations in dogs fed MOS (4.48 CFU log$_{10}$/g) versus dogs fed XOS (5.16 CFU log$_{10}$/g) or OF (4.74 CFU log$_{10}$/g). Because clostridia species do not possess mannose-specific fimbriae, MOS appeared to be acting through a mechanism other than that of a fimbrial analog. The general lack of significant findings may be due to the low dose of prebiotics consumed (only ~1.3 g/d) or the use of a control diet containing 15% soybean meal, which contains significant amounts of naturally occurring oligosaccharides.

The effects of OF on fecal bacterial populations, fecal nitrogen excretion, and mineral absorption in dogs was tested by Beynen et al.26 Five healthy adult dogs were used in a crossover experiment to test a control diet versus a diet containing 1% OF. Each period consisted of a 16-day adaptation phase followed by a 5-day collection phase of feces and urine. On the final day of the collection phase, a fresh fecal sample was collected for identification and enumeration of fecal bacterial populations. Although OF consumption did not affect fecal or urine production, several fecal bacterial populations were affected. OF supplementation increased total anaerobes ($P < 0.05$), total aerobes ($P < 0.05$), lactobacilli ($P = 0.08$), streptococci ($P < 0.05$), clostridia ($P < 0.05$), and bifidobacteria ($P < 0.05$). Although nitrogen balance was unaffected by OF supplementation, it resulted in increased ($P < 0.05$) Ca and Mg absorption.

Swanson et al.8 performed two experiments using 40 adult dogs (20 dogs/experiment) to determine whether scFOS and/or the probiotic (live microbial food supplement) Lactobacillus acidophilus (LAC) affected concentrations of gut microbial populations, fermentative end-products, and nutrient digestibilities in healthy adult dogs. In general, dogs in experiment 1 (mean BW = 23.0 kg; mean age = 6.3 years) were older and weighed more than dogs in experiment 2 (mean BW = 21.2 kg; mean age = 2.2 years). Dogs in each experiment were randomly assigned to one of the treatments, which were dosed orally via gelatin capsule twice daily: 1) 2 g sucrose + 80 mg cellulose; 2) 2 g FOS + 80 mg cellulose; 3) 2 g sucrose + 1 × 10$^9$ CFU LAC; or 4) 2 g FOS + 1 × 10$^9$ CFU LAC. In experiment 1, LAC resulted in lower ($P = 0.08$) C. perfringens and greater fecal butyrate ($P = 0.06$) and lactate ($P < 0.05$) concentrations. In experiment 2, FOS supplementation increased ($P < 0.05$) bifidobacteria, increased lactobacilli ($P = 0.08$), increased fecal lactate ($P = 0.06$) and butyrate ($P < 0.05$), and decreased ($P < 0.05$) fecal ammonia, isobutyrate, isovalerate, and total branched-chain fatty acid concentrations. Dogs fed LAC had the highest fecal concentrations of hydrogen sulfide and methanethiol in experiment 1 and dimethyl sulfide in experiment 2, while dogs fed FOS had the lowest concentrations of these compounds. Overall, FOS appeared to enhance indices of gut health by positively altering gut microbial ecology and fecal protein catabolites, while LAC was more effective when fed in combination with FOS rather than fed alone.

A $4 \times 4$ Latin square design with 14-day periods was used by Swanson et al.9 to examine the effects of scFOS and (or) MOS on indices of gut health in ileal cannulated dogs. Dogs were dosed with one of the following treatments twice daily: 1) control (no scFOS or MOS); 2) 1 g scFOS; 3) 1 g MOS; or 4) 1 g scFOS + 1 g MOS. Blood, ileal, and fecal samples were collected during the last 4 days of each period to measure protein catabolite concentrations, microbial populations, immune characteristics, and nutrient digestibilities. Dogs supplemented with MOS had lower ($P = 0.05$) fecal total aerobes and tended ($P = 0.13$) to have greater fecal Lactobacillus concentrations compared to control. Dogs fed MOS also had greater ($P < 0.05$) plasma lymphocytes (% of total white blood cells) and tended ($P = 0.13$) to have greater serum IgA concentrations versus control. Ileal IgA concentrations were greater ($P = 0.05$) in dogs supplemented with scFOS + MOS versus control. Dogs fed scFOS and scFOS + MOS had lower ($P < 0.05$) fecal total indole and phenol concentrations. Finally, dogs fed MOS tended to have higher fecal pH ($P = 0.09$) and lower ileal DM ($P = 0.15$) and OM ($P = 0.15$) digestibili-
ties versus control. scFOS supplementation did not influence fecal microbial, SCFA, or ammonia concentrations in this experiment.

To follow up on results of the previous experiment, Swan-son et al12 performed another study to evaluate the effects of MOS + scFOS on immune function and ileal and fecal microbial populations in dogs. This experiment used a crossover design to evaluate the combination of MOS + scFOS (1 g MOS + 2 g scFOS twice daily) versus a placebo (1 g sucrose twice daily). In this experiment, supplementation of MOS + scFOS increased (P <0.05) fecal total aerobes, Bifidobacterium, and Lactobacillus concentrations. Interestingly, supplementation of MOS + scFOS also increased (P <0.05) ileal Lactoba-cillus concentrations compared to placebo. Similar to Swan-son et al,3 dogs supplemented with MOS + scFOS tended to have a shift in plasma immune cells, having lower (P = 0.08) blood neutrophils (% of total white blood cells) and greater (P = 0.06) blood lymphocytes (% of total white blood cells) compared to dogs given the placebo. In contrast to the previous experiment, ileal IgA concentrations were not different among treatments.

Zentek et al27 used four dogs in a 4 × 4 Latin square design to determine the effects of MOS, TOS, lactose, and lactulose on fecal characteristics, total tract digestibility, and concentrations of microbial end products in feces and urine. Carbohydrate supplements were administered at a rate of 1 g/kg BW/day. MOS supplementation decreased (P <0.05) fecal pH (6.6 versus 6.9), fecal ammonia excretion (78.4 versus 116 μmol/g feces), and apparent DM (81.9% versus 85.0%), CP (79.8% versus 82.5%), and nitrogen-free extract (83.1% versus 94.8%) digestibilities. By decreasing fecal pH and ammonia, MOS supplementation appeared to improve indices of colonic health. However, the decreases observed in apparent nutrient digestibilities resulting from MOS supplementation would increase fecal quantity and the cost of feeding the animal. The dose of carbohydrate supplements fed in this experiment (1 g/kg BW/day) was very high. Smaller doses of MOS may not have such negative effects on nutrient diges-tibility.

Two experiments were performed by Flickinger et al28 to evaluate the effects of scFOS and OF supplementation on nutrient digestibilities, fecal characteristics, fecal microbial populations, and fecal protein catabolite concentrations in adult dogs. In experiment 1, 16 dogs were randomly assigned to one of four treatments: 1) control (no OF); 2) 0.3% OF; 3) 0.6% OF; and 4) 0.9% OF. In this experiment, OF consumption decreased DM (P <0.05), OM (P <0.05), CP (P = 0.07), and fat (P <0.05) total tract digestibilities but did not influence fecal characteristics. Although OF supplementation did not alter concentrations of fecal branched-chain fatty acid (BCFA), biogenic amines, indole, or phenols, fecal ammonia was linearly decreased (P = 0.06). Fecal propionate (P <0.05) and total SCFA (P = 0.07) were increased with OF supplementation. In experiment 2, four ileal-cannulated adult dogs were used to test dietary inclusion of scFOS (0, 1, 2, and 3 g/day) in a 4 × 4 Latin square design. In this experiment, scFOS supplementation linearly increased ileal CP (P = 0.09) and fat (P = 0.07) digestibility. Supplementation of scFOS also linearly increased (P <0.05) total aerobes and decreased (P <0.05) C. perfringens populations. Fecal concentrations of fermentative end products were not influenced by scFOS in this experiment.

Grieshop et al29 tested the effects of chicory and/or MOS on nutritional and immunologic characteristics in geriatric dogs. After a 4-week baseline period, 34 senior dogs (beagles 9 to 11 years old; pointers 8 to 11 years old) were randomly allotted to one of four treatments: 1) control (no chicory or MOS); 2) 1% chicory; 3) 1% MOS; or 4) 1% chicory + 1% MOS. Dogs remained on treatment for 4 weeks. Increased (P = 0.07) food intake in dogs fed chicory + MOS and MOS alone resulted in increased (P <0.05) wet fecal output. Although DM, OM, and CP digestibilities were unchanged due to treatment, chicory supplementation resulted in increased (P = 0.07) total tract fat digestibility. While supplementation of chicory and MOS alone resulted in increased (P <0.05) bifidobacteria populations compared to control, the combination of these prebiotics failed to show this response. Supple-mentation of MOS alone also resulted in a decrease (P <0.05) in fecal E. coli populations. In agreement with previous experiments,9,12 prebiotic supplementation resulted in a shift in peripheral white blood cell populations. Supplementation of chicory and chicory + MOS tended to increase (P <0.10) neutrophil concentrations, while MOS (P = 0.06) and chicory + MOS (P <0.05) decreased lymphocyte concentra-tions. Finally, prebiotic supplementation altered proportions of lymphocytes expressing CD4 and CD8 cell surface markers. Chicory supplementation increased (P = 0.06) CD4-specific lymphocytes while chicory + MOS supplementation decreased CD8-specific lymphocytes. Results of this experiment support findings from previous experiments that in addition to altering gut microbial ecology, prebiotic supplementation may affect immune status.
Using a 7 × 7 Latin square design, Propst et al.\(^3^0\) tested the effects of three concentrations (0.3%, 0.6%, and 0.9%) of inulin and OF individually compared to a control diet (no OF or inulin) in adult ileal-cannulated dogs. Fecal characteristics, ileal and total tract nutrient digestibilities, and fecal fermentative end-product concentrations were the primary outcomes of this experiment. In this experiment, dogs fed OF tended to have an increased (\(P = 0.10\)) dry fecal output and decreased (\(P = 0.11\)) fecal DM%. Although OF and inulin supplementation did not affect food intake or ileal nutrient digestibilities, total tract DM, OM, and CP digestibilities were decreased (\(P < 0.05\)) with these prebiotics. Inulin and OF supplementation increased (\(P < 0.01\)) fecal acetate, propionate, butyrate, total SCFA, and ammonia concentrations compared to control. Similar to Swanson et al.,\(^9\) inulin decreased fecal phenol (\(P = 0.08\)) and total phenol (\(P = 0.04\)) concentrations, while OF decreased (\(P = 0.08\)) total phenol concentrations in a linear fashion. Finally, OF supplementation tended to increase putrescine (\(P = 0.11\)), cadaverine (\(P = 0.07\)), spermidine (\(P = 0.12\)), and total biogenic amine (\(P = 0.05\)) concentrations in a linear fashion.

### FELINE PREBIOTIC EXPERIMENTS

A literature search on prebiotic use in cats identified only four published papers where chicory, inulin, lactosucrose, OF, and FOS were tested. Again, the focus of these experiments has been very limited in scope, underscoring the need for more research in this area. The first experiment in felines was performed by Terada et al.,\(^3^1\) who fed 175 mg lactosucrose/day to cats for 14 days. As in their dog study, fecal and microbial characteristics were measured before lactosucrose administration, on day 7 and day 14 of lactosucrose administration, and 7 days after administration had ceased. Lactosucrose decreased fecal ammonia, indole, and ethylphenol concentrations after 7 and 14 days of supplementation. Urinary ammonia concentrations were decreased (\(P < 0.05\)) after 14 days, but not at 7 days, of lactosucrose supplementation. Similar to the experiment performed with dogs, all fecal and urinary compounds measured in this study returned to concentrations similar to that of baseline 7 days after supplementation ceased. Lactosucrose supplementation decreased (\(P < 0.05\)) fecal lecithinase-positive clostridia and Enterobacteriaceae concentrations and increased (\(P < 0.05\)) lactobacilli concentrations after 7 and 14 days of supplementation. Fecal fusobacteria, lecithinase-negative clostridia, and staphylococci concentrations were lower (\(P < 0.05\)) after 7 days, but not 14 days, of lactosucrose supplementation compared to baseline. Seven days after lactosucrose supplementation had ceased, all microbial populations were similar (\(P > 0.05\)) to baseline values.

After cats consumed a control diet for 8 weeks, a diet containing FOS (0.75% of diet) was fed for 12 weeks\(^3^2\) to measure changes in microbial populations. Fecal samples were collected after 8 weeks on control and 12 weeks on FOS diet. FOS supplementation resulted in greater (\(P < 0.05\)) lactobacilli (7.9 versus 5.7 \(\log_{10}\) CFU/g) and bacteroides (9.5 versus 8.0 \(\log_{10}\) CFU/g) concentrations and lower E. coli (6.3 versus 7.5 \(\log_{10}\) CFU/g) concentrations compared to control. Moreover, FOS also tended (\(P = 0.085\)) to decrease C. perfringens (4.9 versus 6.6 \(\log_{10}\) CFU/g) concentrations. The authors stated that they were unable to evaluate the effects of FOS on bifidobacteria populations because this microbe was only detectable in 1 of the 12 cats in the experiment, which may have been due to their housing and environmental conditions. Because bifidobacteria concentrations in the cats on this experiment were much lower than are commonly found in humans and dogs, it is unknown whether the same beneficial effects of FOS supplementation observed in humans and dogs also apply to cats.

Hesta et al.\(^3^3\) performed two experiments to evaluate the effects of various concentrations of inulin and OF on fecal characteristics, apparent nutrient digestibility, and fecal SCFA concentrations in cats. In the first experiment, a 4 × 4 Latin square design was used to test the effects of diets containing 0%, 3%, 6%, or 9% OF. In this experiment, a 7-day adaptation phase preceded a 5-day collection phase of all feces and urine. The greatest responses were observed in cats consuming diets containing 6% and 9% OF. In general, as percentage of OF in the diet increased, the number of defecations/day, amount of fresh feces excreted/day, and fecal moisture increased, while fecal score (1 = watery feces; 4 = constipation) and fecal pH decreased. In the second experiment, a 4 × 4 Latin square design was used to test the effects of diets containing 0%, 3%, 6% inulin and 3% OF. In this experiment, the 5-day collection phase followed a 12-day adaptation phase. Although water consumption was not different among groups, cats consuming inulin or OF had decreased (\(P = 0.01\)) urine production compared to those on the control diet. Increases in fecal moisture (\(P = 0.01\)) and fecal acetate (\(P = 0.004\)), valerate (\(P = 0.04\)), and total SCFA (\(P = 0.02\)) were observed in cats fed 6% inulin. Cats fed 6% inulin also had the lowest (\(P < 0.001\)) apparent CP and crude fat di-
gestibilities. In addition, cats fed 3% OF and 3% inulin had lower \(P < 0.001\) apparent CP and crude fat digestibilities compared to those on the control diet. CP digestibility was correlated with fecal bacterial nitrogen, as cats fed 6% inulin had the highest \(P < 0.001\) amount of bacterial nitrogen in feces. Cats fed 3% OF and 3% inulin had greater fecal bacterial nitrogen concentrations than those fed the control diet. When bacterial nitrogen was accounted for, apparent CP digestibility was not different among groups. Increased bacterial mass and consequent increased fecal nitrogen concentrations are commonly observed in animals consuming diets containing amounts of highly fermentable substrate\(^3\) resulting in decreased apparent crude protein digestibility.

Patil et al\(^3\) examined the effects of supplementing the diet with chicory, a natural source of fructans, on fecal microflora and odor components in cats. Following 15 days on a control diet, cats were assigned to one of four treatments for an additional 15 days: 1) control (no chicory); 2) 1% chicory; 3) 2% chicory; or 4) 3% chicory. Unlike data of Sparkes et al\(^3\) high concentrations of bifidobacteria were measured in this experiment. Although no differences \(P > 0.05\) were observed in fecal \textit{C. perfringens} concentrations, cats fed 2% chicory had greater \(P < 0.05\) fecal bifidobacteria concentrations compared to control. Cats fed 3% chicory had increased fecal lactobacilli compared to control. Fecal bifidobacteria and lactobacilli concentrations were unaffected by diets containing 1% chicory. When cats were fed 2% chicory, fecal benzothiazole, methyl sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide concentrations were lower \(P < 0.05\) than when fed the control diet. Inclusion of chicory to the diet did not affect food intake, apparent DM and protein digestibilities, or fecal characteristics.

### Prebiotic In Vitro Experiments Using Canine and Feline Fecal Inoculum

Hussein and Healy\(^3\) used canine and feline fecal inoculum to determine the fermentability of MOS. Substrates were incubated in 50-mL centrifuge tubes at 39°C for 6, 12, 18, and 24 hours. DM and OM disappearance, SCFA concentrations, and lactate concentrations were determined and used as indices of fermentability. No differences were observed in fermentability between dog and cat fecal inoculum. By examining DM and OM disappearance, it appeared that MOS was highly fermented. DM disappearance after 6, 12, 18, and 24 hours of in vitro fermentation was 54.3%, 57.9%, 60.7%, and 61.3%, respectively. OM disappearance was similar to that of DM (56.8%, 60.7%, 63.7%, and 64.1% after 6, 12, 18, and 24 hours of fermentation, respectively). However, DM and OM disappearance do not always reflect microbial fermentation due to the disappearance of soluble carbohydrates present in the substrates and not retained during filtering. Although soluble carbohydrates are available for fermentation, gravimetric methods cannot determine the proportion used by the microbes as an energy source. Therefore, the measurement of DM and OM disappearance is not as accurate as the measurement of the fermentation products (i.e., SCFA and gas), which is a direct measurement of fermentation. Concentrations of total SCFA, lactate, and propionate increased linearly over time. Moderate concentrations of total SCFA (10.1, 26.8, 36.7, and 49.7 mM) were produced after 6, 12, 18, and 24 hours. In comparison to total SCFA, lactate concentrations were fairly high (7.7, 8.7, 7.6, and 5.9 mM), suggesting fermentation by a lactate-producing species (e.g., lactobacilli, bifidobacteria). These data suggest that MOS is moderately fermentable by canine and feline microflora. The lactate produced during fermentation suggests that lactate-producing species are able to utilize MOS, possibly by acting as a prebiotic for these species.

Similar to the experiment performed by Hussein and Healy,\(^3\) Vickers et al\(^3\) used canine fecal inoculum to determine the fermentability of several nondigestible oligosaccharides and fibers. Substrates were fermented at 39°C for 6, 12, and 24 hours. Lactate and SCFA (acetate, propionate, butyrate) concentrations were determined and used as a measure of fermentability. MOS fermentation produced moderate concentrations of total SCFA after 6 (0.49 mmol/g of OM), 12 (1.45 mmol/g), and 24 hours (2.40 mmol/g) of in vitro fermentation. In comparison, scFOS produced high concentrations of SCFA (0.97, 3.60, and 4.60 mmol/g). Beet pulp, a common fiber source in pet foods, produced similar SCFA concentrations (0.85, 0.92, and 2.60 mmol/g) to MOS after 6, 12, and 24 hours of fermentation. Very low concentrations of lactate were produced as a result of MOS fermentation. The microbial species responsible for MOS breakdown were not determined in this experiment.

### Conclusions

From the limited number of experiments published in this area, it appears that prebiotic supplementation has several beneficial effects in the GI tract of dogs and cats (e.g., positive shifts in microbial populations, decreases in fecal protein catabolites, changes in immune status). However,
more research is required to identify optimal doses, life stages most likely to benefit, and disease states likely to be avoided or treated with prebiotic supplementation. Most of the experiments performed to date have used healthy adult dogs and cats. In the future, experiments also must test prebiotic supplementation on animals of different life stages (e.g., weanlings, gestation/lactation, geriatric animals) and disease states. Although several hypotheses have been postulated, more research is needed to understand mechanisms by which prebiotics function. To completely understand these mechanisms, scientists must first accurately identify all microbial species inhabiting the gut and determine how they interact with one another and the epithelial cells of the gut. Although this task is far from complete, researchers have begun to make some progress with the use of molecular biologic techniques. In fact, several microbial genomes have already been sequenced. In addition to classical methodology, the use of molecular biologic techniques will accelerate the learning curve in several respects. First, scientists will be able to more accurately identify and quantify microbial species present in the gut, especially those that scientists are unable to grow in culture. Second, gene mapping will identify and locate genes of importance/interest. Third, by using microarray technology, which measures hundreds to thousands of genes simultaneously, gene expression profiles may be generated of microbial species or gut epithelium. This technology generates a global view of gene expression, enabling scientists to see the “big picture” as opposed to only a few genes of interest. The use of laser-capture microdissection and RNA amplification will allow researchers to generate gene expression profiles from minute quantities of gut tissue. This technology will enable researchers to measure responses of specific gut epithelial cells to gut microbes and metabolites. Finally, bioinformatic modeling and statistical analyses may be used to make sense of the enormous datasets generated by microarray technology. The completion of additional experiments using dogs and cats of different life stage and physiologic states, in combination with molecular biologic techniques, will greatly enhance our understanding of prebiotic function, perhaps enhancing the health and well-being of these important animal species.

REFERENCES


NUTRITIONAL ADVANCES IN GASTROINTESTINAL HEALTH AND DISEASE
AN ASSESSMENT OF PREBIOTIC USE IN COMPANION ANIMAL DIETS


Intestinal Immunity and Oral Tolerance

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The intestine is home to the largest collection of T-lymphocytes of any tissue in the body, and these and other leukocytes are separated from an enormous source of foreign dietary and microbial antigens by a single layer of epithelial cells. In addition, numerous lymphocytes reside within the epithelium itself where they are directly juxtaposed to the antigenic sea within the intestinal lumen. However, classical antigen-specific or innate immune responses to luminal antigens in the absence of pathogens is wasteful, pointless, and deleterious to the mucosa. For the most part in the normal intestine, such reactions do not occur, and this phenomenon is referred to as immunologic tolerance. The central hypothesis for the development and persistence of inflammatory bowel disease (IBD) is the loss of immunologic tolerance to the intestinal microflora and/or dietary antigens.

The concept of oral tolerance is not based on immunologic ignorance to antigens but on atypical antigen-specific responses within the organized and diffuse gastrointestinal-associated lymphoid tissues. At the same time, the intestine must preserve the ability to identify and respond to pathogenic, invasive, or adhesive organisms. Therefore, a careful balance between reactivity and tolerance must be established and maintained throughout the life of the animal for normal intestinal function and systemic health to be maintained.

PEYER’S PATCHES

The macroscopic lymphoid follicles within the submucosa of the small intestine are termed Peyer’s patches and are the primary inductive area of the intestinal immune system. Peyer’s patches are located most densely in the terminal ileum. The epithelium overlying the lymphoid follicles contains specialized epithelial cells that are devoid of the normal microvilli and are referred to as M-cells. M-cells sample unspecifically and by receptor-mediated uptake, particulate and insoluble antigens, and whole microorganisms from the lumens. Antigens and organisms are then transported to leukocytes that reside within basal membrane invaginations, namely B-cells, macrophages, and dendritic cells. In the normal intestine, these antigen-presenting cells (APCs) lack costimulatory molecules such as CD80 and CD86. Antigens processed by these “un-activated” APCs are then presented to naïve B and T cells either within the follicle or in the mesenteric lymph nodes following migration. This unique antigen presentation occurs within a local microenvironment that differs from other sites in the body and results in induction of hyporesponsive, Th3- or Th2-biased T cells that proliferate poorly.

LYMPHOCYTE MIGRATION

Activated cells leave the mucosa via lymphatics and pass via the mesenteric lymph nodes into the systemic circulation. A further unique feature of these cells is the expression of the integrin α4β7, which specifically engages with the cellular adhesion molecule Mad-CAM-1, specifically expressed by the high endothelial venules of mucosal tissues. Thus B and T lymphocytes activated within the mucosa reenter the lamina propria and other mucosal sites to await a secondary encounter with their specific antigen. Lymphocytes activated in peripheral sites are induced to express the integrin α4β1 and will therefore not enter mucosal sites producing an effective compartmentalization of the specific immune system. The CD4+ T cells are generally distributed throughout the lamina propria with a tendency to be more concentrated toward the distal villus; B lymphocytes tend to be preferentially located toward the crypt and villus base. The activated cells may secrete cytokines or antibodies immediately, and although this secretion will be more vigorous in response to secondary antigen exposure, further proliferation probably does not occur. Some activated cells will be nonresponsive to further antigenic stimulation and are termed anergic.
INTESTINAL IMMUNITY AND ORAL TOLERANCE

SOLUBLE VERSUS PARTICULATE ANTIGENS

A general tendency for discrimination between soluble and insoluble protein antigens exists within the intestine. In mammals, soluble antigens will be predominantly absorbed by intestinal epithelial cells and will induce tolerance, while insoluble, particulate antigens will be absorbed across M cells and tend to invoke more active responses.9,10 A study of Reovirus infection in mice supports this proposal.11 Reovirus type I infects IECs, whereas type III viruses gain entry via receptors expressed on M-cell membranes. Administration of Reovirus type I results in systemic tolerance, whereas type III virus administration results in an active IgA response. Thus a two-tiered response to innocuous antigens may be present. For soluble dietary antigens, immunologic tolerance may be characterized by the complete absence of an antigen-specific response, while insoluble, particulate, microbial antigens would elicit an IgA-dominated mucosal response.

RESPONSE TO PATHOGENS

It is important that the immune system reserves the ability to rapidly respond to pathogens. This ability to recognize pathogenicity is based on the engagement of evolutionarily conserved microbial molecular patterns with specific receptors. The best characterized receptors are the Toll-like receptors (TLRs), of which to date 10 variants have been identified.12 The membrane-associated TLR-4, along with other proteins (e.g., CD14), represents the lipopolysaccharide receptor. The TLR-2 homodimer binds to lipoteichoic acid and peptidoglycans derived from gram-positive bacteria.13 Engagement of TLRs activates an intracellular signaling cascade culminating in the release of the transcription factor NF-κB from inhibitory molecules (IkB), allowing translocation to the nucleus and inducing gene transcription by binding to specific DNA regulatory sites. Examples of TLR-induced genes are IL-1, IL-6, IL-8, IL-12, and CD80/CD86. Thus, TLRs represent the most important sensors for the presence of microorganisms and their by-products.

Predictably, expression of TLR-2 and TLR-4 is low to nonexistent in the mucosal cells of the normal human intestine, but they can be rapidly expressed in response to inflammatory cytokines.14 The absence of these “danger signals” results in relatively inefficient antigen processing by intestinal APCs, markedly reduced or absent TNF-α/IL-1/IL-12 production, and the absence of CD80/86 co-stimulatory molecule expression. T cells activated by such an APC will divide less, with most clones undergoing early deletion by apoptosis, while the surviving memory cells will tend to secrete IL-10, TGF-β, or no cytokines.15 This combination of apoptosis, functional defects in surviving clones, and T cells secreting the antiinflammatory and IgA-supporting cytokines is the general basis for immunologic tolerance to luminal antigens.

Thus oral tolerance is composed of a delicate balance between induction of IgA, T-cell deletion, anergy, and immunosuppression, and the retention of antigen-specific lymphocytes capable of responding to invasive pathogens through antibody isotype switching to IgM, IgE, or IgG and the production of inflammatory cytokines such as IFN-γ, IL-12, and IL-6. The exact nature of the response elicited to a given antigen is dependent upon many factors that include the solubility and dose of the antigen and the absence of co-stimulation by the particular antigen-presenting cell.

REFERENCES

5. Roccabianca P, Woo JC, Moore PF: Characterization of the diffuse mu-
NUTRITIONAL ADVANCES IN GASTROINTESTINAL HEALTH AND DISEASE

Feline Inflammatory Bowel Disease: Beyond Qualitative Histopathology

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Feline inflammatory bowel disease (IBD) is the term applied to a group of poorly understood enteropathies that are characterized by the infiltration of the gastrointestinal (GI) mucosa by inflammatory cells. The cellular infiltrate is composed of variable populations of lymphocytes, plasma cells, eosinophils, and neutrophils, and is often distributed throughout the GI tract. The infiltrate is variably accompanied by changes in the mucosal architecture such as villus atrophy, fusion, fibrosis, and lymphangiaectasia. IBD is likely the leading diagnosis in cats presented for the investigation of GI causes of vomiting, diarrhea, weight loss, and anorexia. Given its importance, there are surprisingly few original studies on IBD in cats, and much of what we know is descriptive.1–4 The median age for cats presenting with IBD is around 7 years. Purebred cats such as Siamese and Abyssinian cats may be over-represented. There is no reported predilection based on sex. The nature of the inflammation has not been defined beyond gross histopathology, and little is known about etiology and pathogenesis or the local and systemic consequences of IBD such as lymphoma or malnutrition.

Immune and Inflammatory Responses in IBD

Recent studies in experimental animals have shed light on the immunologic environment in the GI tract and reveal a complex interplay between the GI microflora, the epithelium, immune effector cells (e.g., lymphocytes and macrophages), and soluble mediators such as chemokines and cytokines.5–7 In health, this system functions to avoid active inflammation by antigen exclusion and the induction of immune tolerance. The development of mucosal inflammation in mice lacking the cytokines IL-10, TGFβ, or IL-2 indicates the central importance of cytokines in damping down mucosal inflammation. In many of these murine models, GI inflammation only develops in the presence of indigenous intestinal microflora, leading to the hypothesis that spontaneous IBD may be the result of a loss of tolerance to the indigenous GI microflora.

The basis of the immunologic response in feline IBD is unknown, and it remains to be determined if the inflammatory response is due to the presence of undefined pathogens or an inappropriate response to dietary antigens or intraluminal commensal bacteria. Determining the cytokine and immune-cell population in IBD is important from both a pathologic and therapeutic standpoint because treatment of IBD in cats is nonspecific and based on dietary modification, antibiotics, and suppression of the immune system. Recent studies in humans and experimental animals have resulted in the development of drugs and the identification of bacteria (e.g., Lactobacillus spp that modulate inflammation). Some of these are now in clinical trials (e.g., infliximab, etanercept, and probiotics for the treatment of human Crohn’s disease).7,8 A further benefit of characterizing the immune and inflammatory responses in cats with IBD would be to enable comparison with GI lymphoma. Distinguishing IBD from lymphoma by routine histology is difficult, and the potential transformation of IBD to lymphoma awaits critical evaluation.

One of the main factors limiting the study of the feline immune system to date has been the lack of reagents that react with feline immune cells and their products. It is only recently that the population of immune cells in the intestines and stomachs of healthy cats has been more clearly defined.9–11 The local immune and inflammatory responses in cats with gastritis and asthma have recently been studied using reagents that enable quantification of the inflammatory response by measuring the expression of cytokine mRNA, and the identification of immune cell subsets.11–13 Infection with Helicobacter pylori was associated with upregulation of mRNA for the proinflammatory cytokines IL-1, IL-8, and Interferon gamma, mucosal inflammation, lymphoid follicle hyperplasia, and the production of Helicobacter-specific IgG.11,14 Infection with
non-H. pylori gastric Helicobacter species is also associated with upregulation of mRNA of the pro-inflammatory cytokines IL-1 and IL-8, lymphoid follicular hyperplasia, and seroconversion, but mucosal inflammation is milder and upregulation of IFN-γ mRNA is absent (unpublished observations). These findings suggest a different immune response to a tightly adherent pathogenicity island containing H. pylori and non-adherent, commensal, large, gastric Helicobacter spp. By way of contrast to these proinflammatory Th-1-like responses, feline asthma is dominated by the up-regulation of IL-4, IL-6, 10, 12, and IFN-γ by a pathologist (mild, moderate, or severe) were evaluated. The mixed inflammatory pattern observed in cats with LPE is characterized by the upregulation correlated more strongly with villus atrophy and IL-1, -8, and -12; fusion and IL-1, -8, -10, -12, and IFN-γ. mRNA levels of IL-1 correlated with those of IL-8, 10, 12, and IFN-γ; IL-8 with IFN-γ, IL-10 with IFN-γ, mRNA by lamina propria cells and the intestinal epithelium was observed in the face of a luminal bacterial flora that was more numerous than that of control dogs. Levels of IL-10 were higher in cats with severe IBD. These results, summarized in Figure 1, indicate that lymphoplasmacytic enteritis (LPE) in cats is characterized by the activation of proinflammatory (IFN-γ, IL-1, -6, -8, and -12) and immunomodulatory cytokines (IL-10 but not IL-4). Cytokine upregulation correlated more strongly with villus atrophy and fusion, and epithelial changes, than the overall density of the cellular reaction. The mixed inflammatory pattern observed in cats with LPE is inconsistent with the classical descriptions of polarized Th1 or Th2 pathways in mice. The lack of IL-4 expression, eosinophils, and mast cells suggests that an immediate hypersensitivity or atopic environment is not present in cats with LPE. Our observations are perhaps more consistent with a model proposed for the mucosal response to gram-negative bacteria, whereby proinflammatory cytokines (e.g., IL-8, IL1-β), produced by epithelial cells in response to stimuli such as gram-negative bacteria, are modulated by the production of IL-10 by macrophages. Support for this concept in the canine GI tract is provided by studies in the small intestines of beagles, where expression of IL-10 and IFN-γ mRNA by lamina propria cells and the intestinal epithelium was observed in the face of a luminal bacterial flora that was more numerous than that of control dogs.

GI disease may decrease the availability of a number of micronutrients, such as vitamins and minerals, with important consequences for the pathogenesis, diagnosis, and treatment of GI disease. The diagnostic utility of measuring the serum concentrations of cobalamin (vitamin B12) and folate (vitamin B9) in cats with suspected intestinal disease has only recently been established, and the impact of deficiencies in cobalamin and folate is largely undetermined. The following summarizes recent research in cobalamin metabolism that impacts the diagnosis and treatment of IBD in cats. It has become clear that cats and dogs are very different from humans with respect to cobalamin metabolism. Cobalamin homeostasis is a complex, multi-step process that involves participation of the stomach, pancreas, intestines, and liver (Figure 2).

Following ingestion, cobalamin is released from food in the stomach. It is then bound to a nonspecific cobalamin-binding protein of salivary and gastric origin called haptocorrin. Intrinsic factor (IF), a cobalamin-binding protein that promotes cobalamin absorption in the ileum, is produced by the stomach and pancreas in dogs. In cats, it is produced in the pancreas but not the stomach. Humans produce only gastric intrinsic factor, and deficiency is usually associated with atrophic gastritis and the resultant lack of gastric IF production. The affinity of cobalamin for haptocorrin is higher at acid pH than for IF; so most is bound to haptocorrin in the stomach. Upon entering the duodenum, haptocorrin is degraded by pancreatic proteases, and cobalamin is transferred from haptocorrin to IF, a process facilitated by the high affinity of IF for cobalamin at neutral pH. Cobalamin-IF complexes traverse the intestine until they bind to specific receptors (previously called IFCR but recently dubbed cubilin)
located in the microvillus pits of the apical brush-border membrane of ileal enterocytes. Cobalamin is then transcytosed to the portal bloodstream and binds to a protein called transcobalamin 2 (TC II) that mediates cobalamin absorption by target cells. A portion of cobalamin taken up by hepatocytes is rapidly (within an hour in the dog) re-excreted in bile bound to haptocorrin.23 It is thought that cobalamin of hepatobiliary origin, in common with dietary-derived cobalamin, undergoes transfer to IF and receptor-mediated absorption, thus establishing enterohepatic recirculation of the vitamin. This situation of rapid turnover means that dogs and cats with cobalamin malabsorption can totally deplete their body cobalamin stores within 1 to 2 months.21,24 This is completely different from people in whom cobalamin depletion may take several years, possibly due to the presence of long-term storage enabled by the cobalamin-binding protein TC1, which is absent in dogs and cats.25

Selective cobalamin malabsorption and cobalamin deficiency was initially recognized in giant schnauzers with defective localization of the ileal cobalamin-intrinsic factor receptor.24 Cobalamin is an essential cofactor for the activity of methylmalonyl-CoA mutase and methionine synthase. Reduced activity of these two enzymes causes the biochemical signatures of cobalamin deficiency, methylmalonic acidemia/uria (MMA), and homocysteinemia/uria, respectively (Figure 3). Affected schnauzers have inappetance, failure to thrive, anemia, leukopenia, and methylmalonyl aciduria, which are completely reversed by the parenteral administration of cobalamin.

Recent investigation of the relationship of subnormal serum cobalamin concentrations to cobalamin deficiency and the effect of cobalamin deficiency on cats has revealed that cats with subnormal cobalamin concentrations are cobalamin deficient. In studies by Ruaux et al,22 cats with cobalamin deficiency (serum cobalamin was undetectable) had significant increases in mean serum concentrations of methylmalonic acid (9,607 nmol/L), compared with healthy cats (448 nmol/L). Affected cats also had substantial disturbances in amino acid metabolism compared with healthy cats, with significantly increased serum concentrations of methionine (133.8 versus 101.1 micromol/L) and significantly decreased serum concentrations of cystathionine (449.6 versus 573.2 nmol/L) and cysteine (142.3 versus 163.9 nmol/L). The circulating half-life of parenteral cyanocobalamin was shorter in two cats with IBD (5 days) than in four healthy cats (12.75 days). The rapid depletion of circulating cobalamin in cats suggests that cats may be highly susceptible to cobalamin deficiency.
micromol/L). There was no significant difference in serum concentrations of homocysteine between the two groups as indicated by the presence of elevated MMA. The metabolic consequences of cobalamin deficiency are reversed by parenteral cobalamin.

There is also emerging evidence that cobalamin supplementation may result in clinical improvement of cats with IBD, without recourse to immunosuppressive therapy. In this respect it is interesting to note that cobalamin deficiency is associated with altered immunoglobulin production and cytokine levels in mice. Serum C3, IgM, and IgG contents were lower in cobalamin-deficient mice than in the control mice. On the other hand, serum IgE content was significantly higher in cobalamin-deficient mice than in control mice, and CD4+IL-4+ cells were significantly lower in cobalamin-deficient mice. CD4+CD8- cells and CD4+IFN-gamma+ cells were significantly higher in cobalamin-deficient mice than in control mice. These results suggest that cobalamin-deficiency causes CD4+CD8-T cells to shift from the T helper type 1 to the T helper type 2, which participate in the IgE production and function.

In conclusion, much remains to be learned about the complex interplay between the GI microflora, dietary antigens, the epithelium, immune effector cells, and soluble mediators in the feline GI tract in health and disease. The development of feline-specific reagents and a growing realization of the nutritional consequences of IBD have precipitated a shift beyond reliance on qualitative histology and holds promise for improved understanding, therapy, and prevention in the future.

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REFERENCES


Inflammatory bowel disease (IBD) is the most common cause of chronic vomiting and diarrhea in dogs and refers to a group of idiopathic, chronic gastrointestinal (GI) tract disorders characterized by infiltration of the lamina propria by lymphocytes, plasma cells, eosinophils, macrophages, neutrophils, or combinations of these cells. The diagnosis of IBD requires the comprehensive exclusion of potential causes of GI inflammation, including intestinal parasites, small intestinal bacterial overgrowth, bacterial enterocolitis, dietary intolerances, and neoplasia. Failure to eliminate known causes of GI inflammation, which can mimic IBD, can result in frustration for the owner and clinician due to poor responsiveness of the animal to dietary or pharmacologic therapy.

Although the etiology of canine and feline IBD is poorly understood, most of the evidence for proposed causes in dogs has been extrapolated from humans with ulcerative colitis (UC) and Crohn’s disease (CD). Caution should be heeded in making extrapolations across species, because human and canine IBD are not synonymous. The main hypothesis for the etiopathogenesis of human IBD is that there is dysregulation of mucosal immune responses to intestinal microflora or potentially dietary antigens. In canine IBD, there is provocative evidence from clinical observations and animal models to incriminate normal luminal bacteria or bacterial products in the initiation and perpetuation of the disease. The clinical response to novel protein diets suggests that dietary antigens may influence the pathogenesis of canine IBD. Restriction or manipulation of individual dietary components is perhaps the single most important factor in the treatment of IBD. Despite this fact, there is a paucity of information pertaining to the nutritional requirements of dogs and cats with IBD.
diseases, including IBD. It is likely that mucosal repair is impeded in the initial management of IBD when B₁₂ is deficient and its absorption impaired; however, this has not been investigated. Consideration should be given to B₁₂ assays in the initial evaluation of dogs and cats with chronic intestinal disease, and parenteral administration during the initial management of IBD if low serum cobalamin is identified. Dogs and cats are typically supplemented with B₁₂ at a dose of 500 µg/dose SC for 4 to 5 weeks on a weekly basis.

**Vitamin K**

Vitamin K deficiency leading to coagulopathy has been reported to occur in cats in association with IBD and may also occur in dogs. In the cats reported, the coagulopathy responded to parenteral vitamin K₁ administration.

**Antioxidants**

In human patients with UC or CD, deficiencies in zinc and vitamins A, E, B₆, thiamine, and riboflavin have also been described and may contribute to mucosal oxidative damage, anemia, increased intestinal permeability, and persistent inflammation. A recent study assessed the plasma antioxidant status and proinflammatory cytokines of 26 CD patients. Decreased selenium concentrations and erythrocyte glutathione peroxidase activity was found in these patients. In addition, glutathione peroxidase activity was inversely correlated with plasma TNF-α concentrations, and serum selenium was inversely correlated with plasma levels of both TNF-α and the soluble receptor of IL-2. It is likely that similar deficiencies occur in severely affected feline and canine patients, and consideration of parenteral fat-soluble vitamin administration is warranted in severely malnourished cases.

**Zinc**

The possibility that zinc deficiency might coexist in patients with IBD bears special consideration since zinc deficiency exacerbates diarrhea in humans and rodents. Oral supplementation improves histologic recovery, normalizes absorption, and decreases NF-κB nuclear binding in experimental models of diarrhea. In a study of CD patients with increased intestinal permeability, daily oral zinc supplementation improved symptoms and normalized the permeability in 80% of cases. Additional mechanisms for the effect of zinc treatment on the duration of diarrhea include improved absorption of water and electrolytes, increased levels of brush border enzymes, and faster regeneration of the intestinal epithelium.

**POTENTIAL ROLE OF DIETARY COMPONENTS IN THE PATHOGENESIS OF IBD**

**Abnormal Responses to Dietary Antigens**

Regardless of the underlying etiology for any given patient, exaggerated responses to dietary antigens are often suspected. This is assumed to be the result of the increased permeability and increased expression of co-stimulatory molecules on antigen-presenting cells (APCs) that commonly accompanies IBD. In human IBD patients, exaggerated humoral and cellular responses as well as clinical food intolerance have been recorded. However, the frequency with which this might occur in canine and feline IBD is unknown. Also unknown in any given patient is whether any abnormal immune response to the diet is the cause or the result of a mucosal infiltrate. If the cause, it is expected that removal of the inciting antigen would lead to improvement. If the effect, it still may be that removing the largest single source of antigen during an elimination-diet trial is sufficient to reduce the inflammatory stimulus allowing restoration of normal intestinal immunity.

Elimination diets have proven to be effective in dogs and cats with small and large intestinal lymphocytic-plasmacytic, eosinophilic, and mixed cellular infiltrates. In one study, Guilford et al found that in 16 feline cases of elimination-challenge proven dietary hypersensitivity with chronic GI signs, all 16 had mild to severe inflammatory infiltrates in at least one region of the bowel. The infiltrates were lymphocytic, lymphocytic-plasmacytic (most cases), or eosinophilic (two cases). All cases responded completely to the elimination diet alone and offending foods were identified in all cases. In a report of 13 dogs with lymphocytic-plasmacytic colitis, clinical signs resolved in all 13 with the introduction of an elimination diet, and of 11 dogs re-challenged with their original diet, 9 relapsed. In a further report of six cats with lymphocytic-plasmacytic colitis, all six responded completely to an elimination diet. A complete clinical response to an elimination diet has been reported in a cat with duodenal and ileal lymphocytic infiltrates so severe that a histologic diagnosis of intestinal lymphosarcoma was made.

The theoretic basis for the use of protein hydrolysate diets is that a reduction in immunogenic epitopes being presented to the mucosal immune system while dysregulation is present will increase the potential for resolution. Thus the argument for the use of a hydrolysate diet is independent of whether a dietary-specific immunologic response is suspected to be present or not. Experience with protein hydrolysate diets is increasing, and
anecdotaly they appear to be very effective adjuncts to pharmacologic therapy, even as sole therapy. Clinical resolution with histologic improvement has been reported in 4 of 6 dogs with refractory IBD when treated with a hydrolyzed soy protein diet alone.29 A similar study of dogs with IBD documented equally beneficial results utilizing a different hydrolyzed soy protein diet (Biouge V, personal communication).

Although small and uncontrolled, these results are encouraging, since five cases in the first study had previously failed elimination diet trials. However, it is possible that nutritional factors other than protein hydrolysis are responsible. These could include dietary digestibility, correction of vitamin or mineral deficiencies, a lowered n-6:n-3 fatty acid ratio, and the potential for an immunomodulatory effect of soy isoflavones within the diet.

One could argue that IBD should not be “diagnosed” if there is a complete response to dietary therapy alone, and a diagnosis of dietary intolerance should be made. However, this is probably more semantic than helpful, since it is equally possible that eliminating the quantitatively most significant antigen source is sufficient to eliminate clinical signs, reduce inflammation, and allow restoration of normal mucosal immunity, even if dietary hypersensitivity is not the primary pathogenic process.

NUTRITIONAL STRATEGIES FOR THERAPY OF IBD

Pre- and Probiotics

It is increasingly clear that dietary influences on the intestinal flora are involved in health and disease. On heating, the amino acid lysine reacts with reducing sugars to form Maillard compounds that cannot be digested or absorbed in a useable form.30 This serves as substrate for luminal bacteria in the small intestine, leading to quantitative and/or qualitative changes in the flora. This leads to increased bile acid deconjugation and loss of the bile acid conjugate taurine, thus increasing the dietary requirement for taurine in canned compared with dry diets. This effect is reversible with antibiotics.31 Additionally, fermentable fiber has been shown to profoundly affect intestinal flora, in addition to its effect on enterocytes, by promoting the development of beneficial species.32 This prebiotic effect reduces or prevents inflammation in experimental models of IBD.8,33 Therefore, a fermentable fiber source should probaly be included as part of dietary therapy, although information regarding which (e.g., resistant starch, fructooligosaccharides [FOS], inulin) and how much is lacking. FOS are carbohydrates that resist digestion by the enzymes in the GI tract and can be metabolized by the microbial species that colonize the distal small intestine and colon. The addition of FOS to feline diets at 0.75% (dry matter) did not affect duodenal flora, but it did increase the numbers of lactobacilli and reduce the numbers of Escherichia coli in the fecal flora of healthy cats.34,35 Healthy German shepherds believed to have bacterial overgrowth were supplemented with FOS at 1.0% (AF) of their diet.36 Changes were recognized in the duodenal bacterial flora, but these changes were of less magnitude than seen in normal dogs for these parameters. The clinical significance of these studies in cats and dogs with IBD is unknown.

A probiotic has been defined as “a preparation containing viable, defined microorganisms in sufficient numbers, which alter the established intestinal microflora by implantation or colonization in a compartment of the host, and by that exert beneficial health effects in the host.”37 Unfortunately, most commercial veterinary probiotic preparations are not accurately represented by label claims, reflecting the poor quality control for most commercial veterinary probiotics.38

Much work is required to define what constitutes optimal numbers and species of intestinal microorganisms. However, it is likely that through interaction with the gut flora, certain diets could protect against, while others actually predispose to, the development of IBD. Until further data is available, it is prudent to select diets with a high digestibility in the management of IBD with a source of fermentable fiber, and avoidance of canned diets in feline cases seems rational at present.

Glutamine

Glutamine is a conditionally essential amino acid that is utilized as a significant fuel source by mucosal leukocytes, in particular lymphocytes, and small intestinal epithelial cells. In addition, it serves as the dominant nitrogen source for purine synthesis, the requirement for which is relatively large given the mitotic rate within the normal mucosa and the greater rate during periods of mucosal repair. It has been proposed that gut mucosal turnover and barrier function is compromised during IBD due, in part, to a relative glutamine deficiency. This is supported by experimental studies that have demonstrated a reduction in mucosal inflammation and lipid peroxidation products following luminal glutamine supplementation in models of mucosal inflammation.39 Caution should be heeded in interpreting many of the experimental studies, as disparate dietary effects are often seen. It is clear that the availability of glutamine is probably benefi-
cial in all causes of acute and chronic enteritis. However, it is uncertain if any benefit will be provided by supplementation beyond that present in adequate amounts of intact protein. Studies of spontaneous IBD in human patients have yet to provide any evidence that “extra” glutamine provides any benefit over conventional levels.40

Arginine and Nitric Oxide

The main potential mechanism for the positive action of luminal arginine supplementation in IBD is via modulation of nitric oxide (NO) production within the mucosa. In the last 10 years, it has become clear that NO is an important molecule in normal intestinal homeostasis and the inflamed intestine. Numerous studies have attempted to elucidate whether NO production during intestinal inflammation is beneficial or deleterious, producing conclusions that range from bad, through indifferent, to essential.41 NO is produced in low amounts constitutively by endothelial and neuronal NO synthases (eNOS and nNOS). During inflammation, and under the transcriptional control of NF-κB, a third NO synthase enzyme is induced (iNOS) in most activated leukocytes and activated epithelial cells, which produce much greater amounts of NO than produced constitutively.42 It has recently been reported that iNOS is expressed in canine IBD, and NO-derived nitrite is increased in the colonic lumen of affected dogs.43

Constitutively produced NO serves to maintain intestinal perfusion, inhibit longitudinal smooth muscle contraction, inhibit the expression of broad-spectrum endothelial adhesion molecules, coordinate epithelial cell turnover, and promote barrier integrity.41 In large iNOS-dependent quantities, studies have shown that NO can scavenge free radicals; preserve epithelial integrity or promote epithelial apoptosis with loss of barrier integrity and increased bacterial translocation; induce or inhibit inflammatory cytokines; and lead to irreversible host-protein nitrosylation and dysfunction.45 Variables that affect the role of NO include the cellular source, timing of production in relation to the insult, chronicity of the disease, quantity produced, and the presence of superoxide leading to the formation of peroxynitrite. It is not surprising that such a heterogenous collection of responses under different experimental and clinical settings has lead to controversy about whether inhibition of iNOS in IBD might be beneficial or detrimental. Importantly, most experimental models of intestinal inflammation mimic human forms of IBD and probably do not reflect the same pathogenesis as that seen in feline and canine IBD. Further research, specific to feline and canine disease, needs to be performed before the use of iNOS inhibitors or even NO donors or precursors could be recommended therapeutically.

Antioxidants

Increased free radical production is a cardinal characteristic of almost any inflammatory disease and has been demonstrated convincingly in human IBD patients. In addition, as previously stated, deficiencies in vitamins and minerals associated with oxidant defense (vitamins A, E, C; Zn, Mn, Cu) are commonly associated with IBD, and their supplementation has been shown to be effective in reducing the effects of intestinal damage following experimental insults. Although it is expected that oxidative stress is a feature of canine and feline IBD, the absence of significant numbers of the major oxidant-producing species (neutrophils and macrophages) in the majority of intestinal infiltrates suggests it is less significant than in its human analogues. Nonetheless, supplementation of dietary antioxidants seems prudent until reasons are provided to suggest their lack of efficacy or detrimental effects. It is currently unknown what the optimal dose and combination of antioxidants is for patients with IBD.

Dietary Fat

A fat-restricted diet is important in the management of a variety of GI diseases in dogs, even though fat is a valuable caloric source and enhances the palatability of the diet. Fat delays gastric emptying,46,47 and fat-restricted diets appear to be better tolerated in a variety of GI diseases. The assimilation of dietary fat is a relatively complex process, and intestinal and colonic bacteria hydroxylate malabsorbed fatty acids. These hydroxy-fatty acids stimulate colonic water secretion and exacerbate diarrhea and fluid loss.48 Fat malabsorption can also be associated with malabsorption of bile acids, resulting in deconjugation of unabsorbed bile acids and increased mucosal permeability and secretion.49 Dietary fat restriction is particularly important in patients diagnosed with lymphangiectasia, with many patients needing restriction to less than 15% fat calories. Unfortunately, there are no commercial veterinary diets available that contain less than 15% fat calories. Commercial canine veterinary diets that are the most restricted in fat calories include Eukanuba Restricted-Calorie dry (15%), Nestlé Purina OM dry (16.3%), Waltham Low Fat canned (16.3%), and Waltham Low Fat dry (18.1%). The hydrolyzed protein diets contain between 24% and 30%
fat calories. It is the authors’ opinion that when severe lymphangiectasia accompanies IBD, priority should be given to the feeding of a restricted-fat diet over antigenic novelty. Further studies are warranted to document the touted benefits of medium-chain triglycerides (MCTs) because increasing evidence has highlighted their limitations based on high cost, low palatability, and evidence that at least in the dog, absorption still occurs via intestinal lymph.

Polyunsaturated n-3 Fatty Acids

The ability of dietary n-3 polyunsaturated fatty acids (PUFA) to compete with arachidonic acid for oxygenation by cyclooxygenase and lipoxygenase is well known. The resulting 3-series prostaglandins from n-3 PUFA-rich diets such as PGE₃ are less biologically active than the 2-series prostaglandins such as PGE₂ that are produced after oxygenation of arachidonic acid. However, the net effect of such manipulations is not always easy to predict, given that PGE₂ may exert distinct and at times opposing effects on leukocytes depending on their activation phenotype. However, it is not too simplistic to expect low n-6:n-3 diets would have a beneficially immunosuppressive effect in established IBD. The prominent role of NF-κB in the inflammatory process in IBD is emphasized by consideration of specific genes responsive to or dependent on its nuclear binding. These include the inflammatory cytokines IL-1, IL-6, IL-8, IL-12, and CD80/CD86, the inflammatory adhesion molecules VCAM-1, E-selectin, and ICAM-1, iNOS, and cyclooxygenase-2. Induction of these products leads to loss of T-cell tolerance, increased leukocyte trafficking, epithelial apoptosis, and increased permeability. Thus the search for and evaluation of specific inhibitors of NF-κB is intense, and preliminary trials in human and experimental IBD are promising.

Another class of transcription factors that may have an important role in IBD is the peroxisome proliferator-activated receptors (PPARs). Upon binding with their ligands, PPARs translocate to the nucleus and bind to PPAR-response elements. Although the understanding of the range of action of PPARs and their ligands in cats and dogs is rudimentary, it is interesting to note that NF-κB-dependent gene transcription is decreased by PPAR-γ ligands. It has been shown that PPAR-γ ligands can potently inhibit NF-κB-dependent cytokine production by the murine colonic epithelium and significantly decrease intestinal inflammation in an experimental model of IBD. This is especially interesting given that certain n-3 fatty acids are known PPAR-γ ligands.

Lastly, although as yet unproven, aberrant immunologic responses to enteric flora are considered a key component to the dysregulation of immunity in feline and canine IBD. If this is the case, the recent finding that n-3 PUFA are capable of acting as competitive agonists of the bacterial lipopolysaccharide receptor complex (Toll-like receptor 4) is another potential mechanism by which these PUFA could be beneficial in IBD.

Fish oil supplementation has been reported to be beneficial in ulcerative colitis and Crohn’s disease patients, but the results are controversial. One study of 18 patients with ulcerative colitis demonstrated a reduction in the number of CD3-positive cells within the intestinal mucosa, reduced expression of MHC II antigens, and reduced plasma cell numbers following treatment with fish oil extract compared with placebo. However, a larger, randomized, double-blind trial comprising 96 patients with ulcerative colitis failed to reveal any benefit in remission maintenance or treatment of relapse on 4.5 g of eicosapentaenoic acid daily, despite a significant reduction in LTB₄ synthesis by blood peripheral polymorphonuclear cells. The differences between the reports regarding study design, supplement composition, dose, whole diet n-6:n-3 ratios, and assessment of clinical improvement may in part explain the conflicting results. A recent study compared the efficacy of fish oil to sulfasalazine in the treatment of mild to moderate active ulcerative colitis in humans. Treatment with fish oil resulted in greater disease activity as detected by a significant increase in platelet count, erythrocyte sedimentation rate, C-reactive protein, and total fecal nitrogen excretion. Often overlooked is the increase in lipid peroxidation after fish oil supplementation is instituted. Antioxidant supplementation may be able to counteract the potentially adverse effects of n-3 fatty acids. Most of the literature regarding n-3 fatty acid administration fails to address the amount of attendant antioxidant supplementation. There are no reports in the veterinary literature demonstrating the efficacy of n-3 fatty acid supplementation in managing canine or feline IBD. Studies in healthy dogs fed diets with n-6 to n-3 ratios of 5:1 and 10:1 demonstrated a decreased production of LTB₄ in plasma, neutrophils, and skin. Increases in certain long-chain n-3 fatty acids and decreases in arachidonic acid were identified in the small intestine and colonic mucosa of healthy beagles fed the same ratios. Further research is necessary to determine the clinical benefits in dogs and cats with IBD, and currently no effective, established dosages exist.
CONCLUSION

The optimal nutritional approach for dogs and cats with IBD remains to be determined and probably varies from animal to animal. Our approach to dietary therapy will be impeded until the etiopathogenesis of canine and feline IBD is better elucidated. Future trials must compare formulas in which a single modification has been introduced, with control diets identical in other respects, including caloric density and protein content. There is little doubt that proper dietary management is underutilized in small animals with IBD, and that implementation of sound nutrition could result in decreased utilization or dosage of pharmacologic therapy.

REFERENCES


Long-term food consumption and body weight changes in a controlled population of geriatric cats

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The objective of this study was to evaluate food consumption and body weight (BW) in a population of 85 senior cats on a long-term feeding study. Cats between the ages of 10 and 15 years were selected. Cats were fed a standard fish-based American Association of Feed Control Officials (AAFCO) maintenance canned diet for up to 3.3 years. Individual weekly BW and daily food consumption were monitored and average monthly values analyzed for overall trends. There was a significant increase in total kcal/kg BW (TKC/BW) from 10 to 15 years of age (p <0.0001). There was an indication (p <0.10) that the increase after 13 years (0.63 increase in TKC/BW per month of age) was larger than the increase between 10 and 13 years (0.13 increase in TKC/BW per month of age). Total daily food consumption in kcal also increased significantly with age (p <0.0001). BW from these same cats showed a significant decrease with age (p <0.0001), with the greatest decrease after 13 years of age, demonstrating that food consumption increased with age independent of BW. A potential decline in nutrient digestibility in older cats, particularly cats over the age of 12, may explain this increase in caloric intake despite progressive weight loss. Cats over the age of 12 could benefit from energy-dense, highly digestible diets to help maintain BW, lean tissue, and a good quality of life.

Effect of age on fecal microflora of cats

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The objective of this study was to evaluate the effect of age on fecal microflora (bifidobacteria, *Clostridium perfringens*, and lactobacilli) of cats. Data from 115 cats ranging in age from 1 to 16 years were examined. Cats were fed a wheat, corn, and poultry meal-based dry diet or a fish-based canned diet for a period of 14 days. On day 15, fresh fecal samples were collected from individual cats. Fecal microflora was quantified by a series of dilutions and plating on selective and differential media. The fecal microflora of cats in six different age groups was compared: 1 to 3 (n = 16); 3 to 5 (n = 27); 5 to 7 (n = 23); 7 to 9 (n = 26); 9 to 11 (n = 12); and >11 years (n = 9). Also, fecal microflora of male (n = 61) and female (n = 54) cats were compared. Fecal bifidobacteria and lactobacilli concentrations for cats >9 years of age were approximately 1 log unit lower than younger cats. Conversely, fecal *C. perfringens* were approximately 0.9 log units higher for cats >9 years of age than cats in other age groups. No significant effect (P >0.05) of gender was observed on fecal bifidobacteria and lactobacilli levels. Fecal *C. perfringens* concentrations were numerically higher for male cats than for female cats. The results of this study indicate that aging has an impact on fecal microflora of cats. The wet and dry cat foods fed differ considerably in their nutrient profile, which may have a significant impact on fecal microflora. Thus, the interaction between age and diet type on fecal microflora of cats deserves further study.
**INTRODUCTION:** Midazolam is a benzodiazepine. If administered at subtherapeutic doses, it acts directly on the neurotransmitter gamma-aminobutyric acid (GABA), which stimulates the appetite center. **OBJECTIVES:** Evaluate the efficacy of midazolam for stimulating food intake in anorexic cats while avoiding adverse side effects. **MATERIAL AND METHODS:** The study included 50 hospitalized cats of different breeds, 30 males and 20 females, with more than 2 days of anorexia. Anamnesis, physical examination, laboratory tests, and necessary diagnostic or complementary tests were completed. Fluid, electrolyte, and acid–base imbalances were corrected. The energetic and metabolic requirements were calculated depending on the disease. Midazolam was administered at 2 to 5 µg/kg, through IV doses, and cats were offered a Purina Veterinary Diet appropriate to their condition. Cardiac and respiratory frequencies, pulse oximetry, and a visual analogue scale (VAS) were evaluated during the orexigenic stimulation on the cats. For cats stimulated on multiple occasions (up to 10), hepatic and renal functions were monitored by laboratory tests. **RESULTS:** After 2 minutes, a strong appetite was observed. No alterations in cardiac and respiratory frequencies, aggressiveness, incoordination, or ataxia were observed. Patients that were stimulated on different occasions with midazolam also did not develop aggression or other adverse effects with this drug administration. **CONCLUSION:** Midazolam is a hydrosoluble benzodiazepine used in veterinary medicine worldwide. Benzodiazepines such as diazepam have previously been used as an appetite stimulant on cats. However, diazepam cannot be used during prolonged periods of time because it produces hepatic damage, collateral effects (aggression), its vehicle is cardiotoxic and irritating, and its orexigenic time is minor compared with midazolam. As authors of the present research, we recommend midazolam administration at the previously described doses for anorexic cats because it is a simple technique, of easy application, without collateral effects, and is inexpensive.
Older cats with gastrointestinal disease are more likely to be cobalamin deficient

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Elderly humans are at higher risk for cobalamin deficiency. The purpose of this study was to investigate the relationship between age and cobalamin status in cats with serum samples submitted to the Gastrointestinal Laboratory at Texas A&M University. Serum cobalamin concentration and age data were available for 2,067 feline samples submitted in a 4-month period. Ages were normally distributed with a mean age of 9.8 years and a median of 10.5 years. The sample population was divided at the median value, and the frequency of occurrence of serum cobalamin concentrations lower than 290 and 100 ng/L (representing the lower limit of the normal range and a state of extreme cobalamin deficiency, respectively) were calculated for each group. Differences in the probability of a cat presenting with serum cobalamin concentration = the cut-off values were assessed by chi-squared analysis, with values of p <0.05 considered significant. The percentage of cats presenting with serum cobalamin <160 ng/L (the concentration at which most cats show biochemical abnormalities due to cobalamin deficiency) and with serum cobalamin <100 ng/L was calculated for each year of age, and the relationship between age and the proportion of cats presenting with cobalamin deficiency assessed using Pearson’s correlation analysis. Cats aged greater than median were significantly more likely to present with serum cobalamin concentrations <290 and 100 ng/L (p <0.0001 and p <0.05, respectively). A strong, significant linear correlation was found between age and the proportion of cats presenting with serum cobalamin concentrations <160 ng/L (p <0.0001, Pearson r² = 0.6697) and between age and proportion of cats presenting with serum cobalamin concentrations <100 ng/L (p = 0.0065, Pearson r² = 0.3613). These data indicate that older cats with gastrointestinal disease are at significantly greater risk of developing cobalamin deficiency.
The incidence of obesity in cats is over 25%. Furthermore, a number of studies have suggested that obesity is linked to impaired immunity. Although the hormonal and lipid changes in obese cats have been rigorously studied, little is known about the effects of obesity on the immune system. In spite of the suggestive evidence reporting a link between impaired immunity and obesity, direct comparisons of specific immune responses have not been published. To address this question, obese and lean cats were used to conduct a study of the adaptive and innate branches of their immune system. Obese and lean animals were divided into four groups: two obese groups of 14 cats each and two lean groups of 6 cats each. The lean cats weighed 3.3 ± 0.5 kg and had a body mass index (BMI) of 34.5 ± 2.75, whereas the obese cats weighed 5.5 ± 1.0 kg and had a BMI of 55.7 ± 7.1. One group each of obese and lean cats were fed diets containing polyunsaturated (A) or saturated fatty acids (B), respectively, for a period of 2 months prior to testing. A total of 10 randomly selected cats (which included animals from each of the groups) were bled each day for a double-blind analysis of immune parameters. Blood samples were subjected to a complete (differential) cell count and lymphocyte distribution was determined by flow cytometric analysis. The proliferative activity of different cellular fractions was tested with polyclonal mitogens (lipopolysaccharide, phytohemagglutinin, phorbol myristate acetate, Ca ionophore, and concanavalin A). Neutrophil phagocytosis and natural killer cell (NK) cytotoxicity were assessed as measures of innate immune function. There were no significant differences between groups (lean or obese) or diets with respect to cell counts, cellular subsets, phagocytosis, NK cell cytotoxicity, or lymphocyte proliferation. The results from this research indicate that obesity to the degree studied has no effect on feline immune function as assessed in this study.
Propofol is a short-acting anesthetic agent which, at subtherapeutic doses, stimulates neurotransmitter gamma-aminobutyric Acid A (GABA-A) and neuropeptide Y and inhibits the serotonin receptors to stimulate the appetite center and food intake. The study objective was to evaluate the efficacy of propofol for stimulating food intake in anorexic dogs while avoiding adverse side effects. The treatment and control group included 70 and 35 hospitalized dogs, respectively, of different breeds with more than 2 days of anorexia. Anamnesis, physical examination, laboratory tests, and necessary diagnostic or complementary tests were completed. Fluid, electrolyte, and acid–base imbalances were corrected. Patients were treated on the basis of laboratory and complementary tests. The energetic and metabolic requirements and specific diet (Purina Veterinary Diets) were determined depending on the disease. Propofol was administered at 1 to 2 mg/kg IV. Saline was administered to the control dogs. Cardiac and respiratory frequencies, pulse oximetry, and visual analogue scale (VAS) were observed during all the orexigenic stimulation on the dogs. For dogs stimulated on multiple occasions (up to 9), hepatic and renal functions were monitored by laboratory tests. Following a brief, mild sedation, a strong appetite was observed in all stimulated dogs. No alterations were observed on cardiac and respiratory frequencies or pulse oximetry parameters. No appetite stimulation was observed in control dogs. No secondary adverse effects were observed in dogs stimulated multiple times with this drug. Propofol provides effective stimulation of appetite to prevent malnutrition and stimulate the fastest recovery of the disease. Propofol administration is recommended at the previously described doses for anorexic dogs, because it is a simple technique, of easy application, without collateral effects, and it is inexpensive.
Labrador retrievers (48) were used to assess effects of lifetime diet restriction on metabolizable energy (ME) requirement for maintenance (MER). The diet contained 14.8 MJ ME/kg, and the control-fed (CF) dogs’ dietary intake was fixed at 0.26 MJ ME/kg of estimated ideal body weight (BW). Diet-restricted (DR) dogs were fed 25% less of the same diet consumed by CF pair-mates. From age 6 to 12 years, lean body mass (LBM) was estimated annually using dual-energy x-ray absorptiometry. Average ME intake/dog was calculated (Kienzle et al, 1998) using in vivo energy digestibility estimates. ME intake was 21% greater (p <0.05) for CF than DR dogs (6.527 versus 5.151 MJ/d, respectively). ME intake decreased with age for CF dogs but increased for DR dogs (p <0.05). MER, expressed on a BW basis, was 8% lower (p <0.05) for CF versus DR dogs (0.200 versus 0.216 MJ/kg BW, respectively) and decreased (p <0.05) with age in a cubic (R² = 0.81), rather than linear, manner. There was no difference (p >0.05) between CF and DR dogs when MER was expressed on a metabolic BW basis (0.477 versus 0.476 MJ/kg BW⁻⁰·⁷⁵, respectively), but the cubic (R² = 0.84) age-associated decline (p <0.05) remained. When expressed on a LBM basis, CF dogs had 17% higher (p <0.05) MER compared to DR dogs (0.307 versus 0.255 MJ/kg LBM, respectively) and increased (p <0.05) with age. The response was quadratic (R² = 0.85) with diet restriction affecting MER response (p <0.05). Expression of MER per these body mass components affects interpretation of results. Use of highly metabolic tissue (LBM) as basis of MER comparison reveals the effects of body condition (fat versus lean) differences and may help explain the health and survival benefits of feeding dogs for leaner body condition.
During lactation, milk FA originate from the adipose tissue, de novo synthesis in the mammary gland, and/or the diet. Using dietary long-chain FA as markers, the origin of milk FA in cats during early and late lactation and the importance of LPL for milk fat synthesis was determined. Eight normal (3 to 5 years of age, 3.9 ± 0.1 kg) and 14 LPL-deficient (2 to 6 years of age, 3.0 ± 0.1 kg) intact queens were continuously fed for ≥4 months an “all-stages” commercial dry-expanded diet supplemented with 30 g of linseed oil (56% C18:3 n-3)/kg of diet. For lactating queens (five normal, five LPL-deficient), linseed oil was substituted with an equivalent weight of DHA oil (33% C22:6 n-3) after milking on day 4 of lactation. Milk samples were collected on days 4, 9, and 42 of lactation. FA profiles in non-esterified FA, triacylglycerol (TG), and phospholipids of diet, milk, and plasma were determined by gas chromatography. With progression of lactation (day 4, 9, 42), the fractional percent of C18:3 n-3 in milk TG decreased (P <0.0001) for normal (10.3%, 5.1%, 2.3%) and LPL-deficient queens (5.3%, 0.6%, 0.4%), while that of C22:6 n-3 increased (P <0.0001) for normal (0.24%, 3.5%, 5.8%) and LPL-deficient queens (0.27%, 4.4%, 4.87%). Milk saturated to polyunsaturated FA ratio (SFA:PUFA) increased with stage of lactation in all cats. Milk of LPL-deficient queens was lower in total fat (3.0% to 4.3% versus 8.5% to 11.4%), higher in SFA (35% to 41% versus 25% to 35%), and lower in PUFA (23% to 25% versus 29% to 31%) than that of normal queens at all sample times (P <0.0001). The increase in C22:6 n-3 in milk TG indicates a high dietary FA contribution to the milk of queens. FA contribution to milk de novo synthesis appeared to increase as lactation progressed, especially in LPL-deficient cats. LPL appears important for provision of FA for milk fat synthesis.
Maternal dietary alpha-linolenic acid during gestation and lactation does not increase canine milk docosahexaenoic acid content

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The effect of feeding alpha-linolenic acid (ALA)-enriched diets on docosahexaenoic acid (DHA) enrichment of canine milk was investigated. Three bitches per group (n = 12) were fed one of four experimental diets from breeding through gestation, parturition, and lactation. Diets containing sufficient linoleic acid and 14% total fat were formulated with different fatty acid (FA) profiles by using beef tallow (TAL), linseed oil (LIN), and high (HMH) or low (LMH) levels of menhaden fish oil as the primary fat source but otherwise identical. After parturition, milk samples were collected by manual expression on day 4, 10, 16, and 28 of lactation. Milk total lipids were extracted, FA methyl esters prepared, and FA profiles determined. Total phospholipid FA profiles were determined on plasma samples at these same times. Canine milk averaged 9% to 10% total fat (as-is), and no diet differences were observed. However, differences in individual FAs were seen. At all time points, milk ALA was highest in dogs fed LIN. Arachidonic acid was moderately decreased in LMH and HMH, especially during later lactation, compared with TAL or LIN. Eicosapentaenoic acid content of milk from dogs fed LMH or HMH was increased compared with TAL or LIN. Milk from dogs fed the menhaden oil diets was enriched in DHA in a dose-dependent fashion. Changes in plasma phospholipid FA profiles mimicked those seen in the milk. Most striking was that no enrichment in milk DHA was observed in animals fed the LIN diet. These findings, following long-term supplementation, are similar to those recently reported in women supplemented with flaxseed oil on a short-term basis during their lactation period. It appears that dietary ALA is an ineffective means of increasing milk DHA content for neonatal nutritional modification.
The use of endogenous strains as probiotics is a most promising concept. Re-introducing native strains is a natural and safe way to influence the gut environment. Furthermore, the strains are well adapted and therefore likely to persist and show in vivo activity. The challenge of this approach is to develop an effective and meaningful system for the selection of strains with probiotic properties. About 75 lactobacilli strains from dog feces were isolated and typed by biochemical methods. All strains were screened in both gastric and small intestinal models mimicking canine in vivo conditions. The models allowed simple and efficient testing for (1) resistance to canine gastric conditions, (2) resistance to canine small intestinal conditions, (3) adaptation to dog food (utilization of carbohydrates), and (4) bactericidal activity against enterotoxic *Escherichia coli*. Sixteen strains exhibited bactericidal activity against the pathogen in addition to resistance to canine gastrointestinal conditions and physiologic activity. Strains typed *Lactobacillus acidophilus* or *Lactobacillus johnsonii* were best adapted to pet food because they were able to ferment starch, the main carbohydrate of the diet. These strains also had the highest antimicrobial activities. The main active agents were identified as lactic acid and hydrogen peroxide. In order to further characterize the strains and design probiotic cocktails, additional in vitro studies were performed: Molecular typing allowed strain-specific identification and development of analytical methods; activity against other enteric pathogens was tested; cytokine expression profiles of stimulated canine peripheral blood mononuclear cells gave first insights in possible effects on the immune system; resistance against frequently used antibiotics was measured as a safety aspect; and storage tests were performed to test for stability during in vivo trials. The results enabled us to select promising pet strains for evaluation of probiotic efficacy in clinical studies.
Use of herbal antidiarrheal compounds in canines—A novel approach

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There are many traditional Indian herbal antidiarrheal compounds useful in gastrointestinal (GI) infections of dogs such as Holarrhena antidysenterica, Acacia catechu, Aegle marmelos, and Punica granatum. Preparations made up of extracts of various parts of these herbs in an aqueous solution are used in the treatment of GI disorders. Lack of scientific approach and absence of an effective medicinal value delivery system have limited their use in modern medicine. In vivo and in vitro effects of herbal preparation were studied in dogs by using a novel approach in drug delivery systems. Dog biscuits containing these compounds were prepared and used in the study. Blind trials were conducted to study palatability and in vivo effects of these compounds in clinical cases of GI disorders in dogs. A dose of two biscuits containing 10 mL of aqueous extracts of herbs twice a day for 3 successive days was used. Results were recorded by recording consistency of the feces. The sensitivity pattern of the herbal preparation against pathogenic Escherichia coli organisms was also studied using standard methods. The herbal preparation was serially diluted and placed in wells in an agar plate containing pathogenic E. coli culture. The plate was incubated at 37°C overnight, and a zone of inhibition was measured around wells containing the herbal preparation. The herbal preparation was found to be highly effective against E. coli in in vitro sensitivity studies. Dog biscuits containing herbal antidiarrheal compounds were found to be palatable and effective in control of clinical cases of diarrhea in dogs. The above stated herbal preparations can be added to dog food to treat certain types of diarrhea.
Evaluation of two diets in the management of cats with naturally occurring chronic diarrhea

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The objective of this study was to evaluate the clinical responses of cats with chronic diarrhea fed a highly digestible, moderate-carbohydrate diet (Diet A: Prescription Diet i/d, Hill's Pet Nutrition) or a high-protein, low-carbohydrate diet (Diet B: Purina Veterinary Diet DM, Nestlé Purina PetCare).

Pet cats with chronic (>1 month) diarrhea, recruited through primary care veterinary practices, were randomized to receive either Diet A or Diet B as the sole diet. After 1 month, cats that had not improved adequately, using pre-defined criteria, were switched to the alternate diet for another month. Primary outcome measures included frequency of defecation and fecal score (Purina scale 1 to 100), based on daily entry in client diaries.

Twenty-nine cats completed both test periods and two additional cats completed only period 1. Both diets were well accepted by the cats. During period 1, 19 cats were fed Diet A. Of these, four (21.1%) remained on Diet A. Twelve cats were fed Diet B in period 1. Of these, six (50.0%) remained on Diet B (p = 0.199 for diet effect). Fecal frequency was not affected by diet. Fecal scores for all cats averaged 33.6 ± 20.6 at entry, increasing to 53.2 ± 23.0 after Diet A and 54.5 ± 25.3 after Diet B. Fourteen of 24 (58.3%) cats on Diet A and 15 of 26 (57.7%) cats on diet B showed a significant improvement in fecal score. Overall, 22 (71.0%) cats showed a positive response to at least one of the diets as the sole therapy for diarrhea. No patterns in trypsin-like immunoreactivity, serum cobalamin or folate were observed between responders and non-responders. However, initial fecal score was lower for responders than for non-responders.

Based on this study, a dietary change would be an appropriate therapy for cats with chronic, nonspecific diarrhea.
Effect of age and sex on feline skin

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**OBJECTIVE:** To determine the effect of age, sex, ambient temperature, and humidity on skin parameters in cats. **METHODS:** Sixty-six cats ranging in age from 1 to 15 years were fed canned cat food for 15 weeks. Of these cats, 24 were between the ages of 6 and 11; 31 were between the ages of 2 and 5; 10 were 1 year of age; and 1 was 15 years of age. Forty-four of the cats were male (38 neutered, 4 intact, 2 vasectomized) and 22 were female (9 spayed, 13 intact). Skin pH, elasticity, hydration, transepidermal water loss, and skin-fold thickness were measured pretrial and monthly during the trial. Skin-fold thickness was measured using calipers. Skin pH and hydration were measured using Courage-Khazaka Skin pH meter pH900 and Corneometer CM825, respectively. Courage-Khazaka Cutometer SEM575 measured skin elasticity. Analysis of covariance was used to examine effects of age, sex, ambient temperature, and humidity. Correlations between age, temperature, humidity, and the skin parameters were also calculated. **RESULTS:** There were no significant correlations ($P<0.05$) between age of cat and skin elasticity, skin hydration, transepidermal water loss, or skin thickness in this study. There was a significant positive correlation ($R=0.283$, $P=0.0053$) between skin pH and age. Male cats had significantly higher skin hydration and skin thickness but lower skin pH than females in this study. There was a positive correlation between ambient humidity and skin hydration ($R=0.293$, $P=0.0012$). **CONCLUSIONS:** In this study, it was observed that skin pH was correlated with age. Males had higher skin hydration and skin thickness than females, but females had higher skin pH. Skin hydration measurements are influenced by ambient humidity.

Body composition changes in aging cats

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The objective of this study was to evaluate the effect of age on body weight (BW) and body composition (body fat, lean and bone mineral density) of cats. Data from 256 cats ranging in age from 0.5 to 20 years were examined. In some cases, body composition was measured for the same cat on more than one occasion during the study. Cats were fed either extruded dry or retorted canned cat food. Data were catalogued into different age groups including: 1 to 4 years ($n=263$, $BW=3384±750$ g); 4 to 8 years ($n=62$, $BW=3946±1116$ g); 8 to 12 years ($n=61$, $BW=4311±1587$ g); 12 to 16 years ($n=172$, $BW=3055±1011$ g); and >16 years ($n=25$, $BW=2710±669$ g). Body composition was measured using dual energy x-ray absorptiometry. Bone mineral density ranged from 0.56 to 0.6 g/cm$^2$ and was not statistically affected by age ($P>0.05$). Percent body fat was highest and percent body lean was lowest for cats belonging to the 8- to 12-year age group. Average BW, grams of body lean, and grams of body fat declined sharply in cats >12 years of age. The reason for this sharp decline is unclear and may be unique to cats among domestic pets. This may be related to some compromised ability of cats >12 years of age to absorb nutrients and needs to be studied further.
The objective of this study was to evaluate the incidence of impaired nutrient digestibility associated with aging cats. Nutrient digestibility data from 188 cats housed in four different colonies were examined. Cats were fed either a wheat, corn, and poultry meal-based dry diet or a fish-based canned diet for a period of 10 days. Fecal samples were collected from day 6 to 10 and composited. Apparent nutrient digestibilities were determined. Cats were subdivided into different age groups: 1 to 7 years (n = 7), 8 to 10 years (n = 18), 10 to 12 years (n = 32), 12 to 14 years (n = 37), and >14 years (n = 53) for cats consuming wet diets; and 1 to 7 years (n = 8), 12 to 14 years (n = 29), and >14 years (n = 19) for cats fed dry diets. Approximately 33% of cats 12 years and older had apparent fat digestibility below 80%. Similarly, 22% of cats >14 years showed apparent protein digestibility below 77%. The cats in the 1 to 7 years of age group showed fat digestibility >90% for the wet diet and >85% for the dry diet. A large population of cats exhibits declining nutrient digestibility after 12 years of age. This reduction in nutrient digestibility in the older population of cats can affect their nutritional status and predispose to significant health problems in these cats. Although reduction in digestibility of senior cats has been reported previously, this work provides novel information on the incidence, extent, and consistency of these phenomena as well as the specific age ranges affected.

Diets rich in animal protein, such as commercial canned foods, have previously been associated with increased fecal clostridia counts in dogs. However, similar studies have not been reported with cats. Therefore, the objective of this study was to evaluate the effect of diet type on fecal microflora (bifidobacteria, lactobacilli, and Clostridium perfringens) in cats. A total of 115 cats were fed either an extruded dry cat food (approximately 30% to 33% protein on dry matter basis; n = 71) or fish-based canned cat food (approximately 50% to 52% protein on dry matter basis; n = 44) in a series of trials. Using a switchback design, cats were fed each diet for 14 days before switching to the alternative product form. Fecal microflora were quantified by sequential dilutions on selective media. Cats had significantly (P <0.0001) higher fecal C. perfringens when fed the high-protein canned food. Fecal bifidobacteria and lactobacilli were significantly (P <0.0001) higher in cats fed dry cat food. Although high fecal C. perfringens levels have not been linked to any adverse health conditions in cats, these data suggest that the use of nutritional interventions to modulate gut microflora may have a beneficial effect in cats fed diets rich in animal protein.
Extrusion effects on in vitro fermentation profiles and viscosity measures of selected fibrous substrates

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An in vitro experiment was conducted to establish the effects of extrusion conditions on the fermentation profile and viscosity measures of selected fibrous substrates. Six fibrous substrates (barley grits, corn meal, oat bran, soy flour, soy hulls, and wheat bran) were extruded under mild, moderate, and extreme conditions. Extrusion conditions were defined by a screw profile containing 1, 3, or 5 reverse diameter lobes, respectively. Viscosity of the unextruded and extruded fiber sources in a 2% solution of hydrolytic digestion fluids was measured. In vitro fermentation was conducted using porcine fecal inoculum to determine organic matter disappearance (OMD) and short-chain fatty acid (SCFA) production on residues recovered following hydrolytic digestion. Wheat bran, barley grits, corn meal, and oat bran became more viscous as severity of extrusion conditions increased. Oat bran tended to have increased OMD and SCFA production with more severe extrusion conditions, indicating greater fiber fermentation. In contrast, barley grits had lower OMD and SCFA production with increasing severity of extrusion conditions, indicating a capacity for greater hydrolytic digestion with less substrate available for fermentation. Extrusion conditions had little effect on OMD and SCFA production for corn meal, soy flour, soy hulls, and wheat bran. These data suggest that the effects of extrusion conditions on substrate viscosity and fermentability are influenced by the unique characteristics of individual fibrous substrates.

Effect of chicory on fecal quality in dogs and cats

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Fiber can help stabilize the digestive tract, promote regularity, and improve fecal quality during bouts of diarrhea. Fecal quality varies between animals on the same diet and can vary in healthy animals in relation to the diet they are fed. The purpose of this study was to evaluate the effect of the ingredient chicory, a natural source of the soluble fiber inulin, on fecal quality in healthy adult dogs and cats fed a variety of diets. Eighty healthy adult cats and 344 healthy adult dogs from the Nestlé Purina PetCare Research colony were fed a variety of dry or canned pet foods with or without added chicory. Each animal served as its own control, being fed both the chicory-free and the chicory-containing diet. Chicory inclusion level was 1% to 2% in dry dog and cat foods and 0.5% to 1% in canned dog and cat foods. Diets were fed at least two weeks before fecal quality was scored. A four-point fecal scoring scale was used with a score of one (1) representing firm, well-formed feces and a score of four (4) representing liquid feces. Feces were scored daily for 5 consecutive days. Chicory had no effect on fecal quality in dogs producing close to 100% firm feces when fed the control diets. However, when feces were less firm, chicory ingestion improved fecal quality (P <0.05). Likewise, chicory ingestion tended to improve fecal quality in cats with soft feces. These results indicate that addition of the soluble fiber chicory to pet foods can help improve fecal quality, a benefit important to pet owners.
Effect of aging on blood metabolites in the cat

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To improve quality of life and longevity for elderly cats we must first understand their metabolism. The purpose of this study was to evaluate the effect of aging and body condition on blood metabolites in cats. Normal blood values for frail, elderly cats have never been published. Blood samples were obtained from 10 young adult cats (average age 2.75 years) and 20 elderly cats (average age 15 years) from the Nestlé Purina colony. Two different groups of elderly cats were examined: 1) healthy elderly cats with good body condition (body condition score 5 to 6) and 2) frail looking, but otherwise healthy, elderly cats (body condition score 2 to 3). Classification as frail was based on general body and hair coat condition; frail cats were thin and had poor coat condition but had no clinically obvious disease condition. Only two cats had elevated T4 levels, and only one of these cats was frail. Therefore, hyperthyroidism did not seem to be the cause of the poor body condition observed in many of the elderly cats in this study. Serum calcium was significantly (P<0.05) lower in both frail and healthy elderly cats than in young adult cats. There was a trend for magnesium to decrease in elderly cats (P<0.06). Taurine status was not affected by age or body condition. Hemoglobin, hematocrit, and red blood cells were significantly (P<0.05) lower in elderly cats than in young adults. Total serum protein, hematocrit, and albumin were significantly (P<0.05) lower in frail elderly cats than in healthy elderly cats. In addition, albumin levels and the albumin/globulin ratio were significantly (P<0.05) lower in both frail and healthy elderly cats than in young adults. This may indicate increased protein needs or impairment in protein absorption or utilization in these animals. The reduced muscle mass of frail, elderly cats provides further evidence for this hypothesis.
Eighteen cats were used to compare the urine acidifying properties of sodium bisulfate to phosphoric acid when included in a diet at 0.4%, 0.6%, or 0.8% (as-is basis). Cats were acclimated to diets for 6 days, and urine samples were collected on day 7 at 0, 4, and 8 hours post-feeding to obtain pre- and postprandial urinary pH data. Intakes of diets containing sodium bisulfate tended ($P < 0.07$) to be lower than those containing phosphoric acid. Cats consuming the 0.8% phosphoric acid diet had higher ($P < 0.05$) food intakes than cats consuming either the 0.4% or 0.6% phosphoric acid-containing diets. There was a significant ($P = 0.01$) linear and quadratic response in food intake for cats consuming sodium bisulfate-containing diets. Cats consuming the 0.4% and 0.8% phosphoric acid-containing diets tended ($P = 0.07$) to have higher water intakes than cats consuming the 0.6% phosphoric acid-containing diet. There were no differences ($P > 0.05$) in urine pH and specific gravity between cats fed the different acidifiers. Cats consuming the 0.6% phosphoric acid-containing diet tended ($P = 0.07$) to have a higher urine pH 8 hours post-feeding than cats consuming the 0.4% and 0.8% phosphoric acid-containing diets. Urine pH was highest at 4 hours post-feeding except in cats fed the 0.4% sodium bisulfate- and the 0.6% phosphoric acid-containing diets. Cats consuming the 0.6% phosphoric acid diet tended ($P = 0.06$) to have a lower fecal score than cats consuming the 0.4% and 0.8% phosphoric acid diets. A linear increase was detected in fecal dry matter content in cats consuming the sodium bisulfate ($P = 0.08$) and phosphoric acid-containing ($P = 0.04$) diets. Sodium bisulfate and phosphoric acid behaved in a similar fashion when incorporated in dry cat diets.
Influence of fatty acids on glucose clearance, insulin secretion, and lipid metabolism in cats

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Research has shown that the type of fatty acid within the diet contributes to insulin sensitivity. The effect of polyunsaturated fatty acids (PUFA) on glucose tolerance, insulin secretion, and lipid metabolism in cats was investigated. Twenty-eight neutered lean adult cats (14 male, 14 female) were used for these studies and were equally and randomly assigned to one of two diet groups: saturated fatty acids (SFA) group or a PUFA group. The cats were maintained in the lean state for 10 weeks (L) and then fed the same diets ad libitum for 21 weeks (OB). Intravenous glucose tolerance tests were performed at the conclusion of the feeding period. Glucose, insulin, glucagon, and nonesterified fatty acids (NEFA) were measured before and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after glucose injection. Glucose concentrations versus time data from each individual intravenous glucose tolerance test (IVGTT) were fit to a monoexponential equation by nonlinear regression. The percent glucose disappearance/minute and percent NEFA suppression were also calculated. The data were evaluated by ANOVA and differences within a group were evaluated by student’s T-test for paired analysis. Weight, body mass index (BMI), girth, and percent fat were significantly higher in OB than in L (p <0.0001) in both diet groups. There was no difference between diet groups in any of the parameters that were evaluated. The obese cats in this study showed a greater glucose area under the curve, k-value, and glucose concentration at 120 minutes of the IVGTT compared to lean cats regardless of diet. Lean and obese cats of the SFA group had higher glycosylated hemoglobin concentrations than the cats of the PUFA group. This was only significant in the obese state. The cats fed the SFA diet showed a significantly higher insulin area under the curve (AUC) in the obese state than in the lean state. Additionally, the obese cats fed the PUFA diet had a lower insulin AUC than obese cats fed the SFA diet. A diet high in PUFA is beneficial for obese cats because it prevents hyperinsulinemia and improves long-term glucose control.
Intra- and extra-myocellular lipid content increased with weight in cats

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With obesity, insulin is not as efficient in its activation of the uptake of glucose in liver, skeletal muscle, and adipose tissue. This diminished effect of insulin is referred to as insulin resistance. Insulin resistance influences not only glucose but also lipid metabolism. The effects of saturated and polyunsaturated fatty acids on intra-myocellular (IMCL) and extra-myocellular (EMCL) lipid accumulation were investigated. Twenty-eight neutered lean adult domestic shorthair cats (14 male, 14 female) were used for this study and were equally and randomly assigned to one of two diet groups: a saturated fatty acid (SFA) group or a polyunsaturated fatty acid (PUFA) group. Cats were maintained in the lean state for 10 weeks (L) and fed either SFA or PUFA. They were then fed ad libitum for 21 weeks (OB). The procedures used to test glucose tolerance and insulin secretion are described elsewhere, and lipid accumulation was determined using magnetic resonance. There was no significant difference in the integrated intensity of the peak of the total IMCL and EMCL concentrations between PUFA and SFA in the lean (L) and obese (OB) state. Thus, the data for both diets were combined. Comparing the integrated intensity of the peaks of IMCL and EMCL of all cats resulted in lower values in L than in OB in both IMCL and EMCL; however, only significant for EMCL (p <0.0018). The EMCL/IMCL ratio did not change significantly between L and OB and the total increase in lipid in muscle regardless of location was significantly different in L versus OB (p = 0.0179). EMCL correlated significantly and positively with IMCL (p = 0.002), with the fasting insulin/glucose ratio ($r^2 = 0.271, p = 0.011$), and insulin 120-minute concentration and insulin AUC ($r^2 = 0.237, p = 0.016; r^2 = 0.339, p = 0.003$, respectively). IMCL correlated significantly with insulin baseline concentrations ($r^2 = 0.302, p = 0.008$) and the fasting insulin/glucose ratio ($r^2 = 0.261, p = 0.013$). In conclusion, obese cats showed an increase in both IMCL and EMCL with no effect of diet and no change in the ratio of EMCL/IMCL due to diet. The increase in both IMCL and EMCL suggests that lipids are not preferentially partitioned into the intramyocellular space in obese cats.
Pyruvate is a natural metabolite involved in the citric acid cycle. Clinical studies in humans have shown that dietary supplementation with pyruvate-enhanced weight loss and resulted in a greater reduction of body fat in overweight adults consuming a low-calorie diet. The objective of this study was to evaluate the effect of pyruvate on weight and fat loss in dogs. Thirty-three overweight adult dogs with body condition scores (BCS) of 6 or greater were randomized into two groups based on breed, baseline maintenance energy requirement (MER), and percent body fat. MER was determined in a 6-week pre-test and body composition was determined by dual energy X-ray absorptiometry. Sixteen dogs received a diet containing 21% protein and 8% fat (control) and 17 dogs received an identical diet except it contained 0.6% pyruvate. The supplementation level of pyruvate was determined based on the recommended level of 2 g/day for human consumption. The metabolizable energy of both diets was 3.6 kcal/g. Each dog was fed 70% of its individual MER for the first 5 weeks, then reduced to 60% MER for another 11 weeks. At the end of the study, there were no significant differences between the two groups in food intake, body weight loss, or changes in body composition. The average body weight lost was 16.9% for the control and 15.9% for the pyruvate group. The percentage of body fat changed from 32.2% to 22.8% in the control group and from 32.3% to 23.8% in the pyruvate group. Percent lean body mass increased in both groups, from 64.9% to 73.9% in the control group and from 64.6% to 72.8% in the pyruvate group. We conclude that pyruvate supplementation to dog food at 0.6% inclusion does not enhance body weight loss or fat loss in overweight dogs during calorie restriction.
Influence of lifetime diet restriction on lean and fat body composition of Labrador retriever dogs: Predictive aspects

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A lifetime study of 25% restriction of food intake was conducted with 48 Labrador retriever dogs from 7 litters, in a paired feeding design.1 Longitudinal assessments that were made during the study included body composition by dual energy x-ray absorptiometry done at the birth anniversary from age 6 to mortality.2 Response variables were examined with a mixed-effects ANOVA model for repeated measures. The relationship of body lean and fat mass to survival was evaluated using Cox proportional hazards regression models.

Longitudinal evaluation of body composition from age 6 to mortality revealed 26% lower (p <0.01) body weight among 25% food-restricted dogs.2 Restricted dogs had lower (p <0.01) lean mass, but lean mass percentage was higher (p <0.01). Lean mass declined in each group as mortality approached, but the decline was nearly 2 years later among the restricted group, which lived a median of 1.8 years longer.2 Fat mass and percentage of fat mass were consistently higher (p <0.01) in control-fed dogs, with gradually increasing fat mass (p <0.05) in both groups.2

Cox proportional hazards models revealed that high fat mass (p <0.001) and declining lean mass (p <0.002) were hazard-predictive of mortality, most strongly at one year prior to death.

REFERENCES
A lifetime study of 25% restriction of food intake was conducted with 48 Labrador retriever dogs from 7 litters, in a paired feeding design. During this study, longitudinal skeletal assessments included dual-energy x-ray absorptiometry, radiography, and serum levels of alkaline phosphatase, total and ionized calcium, phosphorus, and parathormone. Response variables were examined with a mixed-effects ANOVA model for repeated measures. The relationship of bone mass to survival was evaluated using Cox proportional hazards regression models. Bone mineral density (BMD) and bone mineral content (BMC) decreased (p < 0.01) during ages 6 to 12 years in control-fed dogs but not (p > 0.05) in restricted dogs. BMD was greater (p < 0.01) in restricted dogs during ages 6 to 12 years. BMC was greater (p < 0.01) in control-fed dogs during ages 6 to 10 years. Bone percentage of total body composition was lower (p < 0.05) just before mortality but was not hazard-predictive of death at 1 or 2 years prior. Serum ionized calcium increased (p < 0.01) with time due to lower values at age 5, but without difference by feeding group. No linear trend or difference (p > 0.05) by feeding group was found for serum parathormone. Linear trends (p < 0.05) were found for serum alkaline phosphatase, total calcium, and phosphorus, primarily because of high serum levels during growth. Feeding group effects were found for serum total calcium (p < 0.05), but the numerical difference between means was small. Clinical evaluation, plain film radiography, metabolic testing, and postmortem evaluation revealed no evidence for bone loss disease. **CONCLUSIONS:** (1) Control-fed dogs had greater bone mineral content (ages 6 to 10 years) and lower bone-mineral density (ages 6 to 12 years). No anatomic or biochemical evidence was found for bone loss disease in either group of dogs. (2) Restriction of food intake by 25% for lifetime did not adversely affect skeletal growth, skeletal structure, or bone metabolism.
Many dewormers can cause stress on the host and may lead to clinical complications. Anthelmintic medicinal plants can be the solution. Many medicinal plants are referred to as anthelmintic in Ayurveda. The present investigation was planned to assess the anthelmintic efficacy of a newly formulated herbal formulation.

Each 100 mL of the formulation contained aqueous extract of 4 g *Butea frondosa*, 4 g *Embellia ribes*, 1 g *Ferula foetida*, and 2 gm *Artemisia maritime*. Sixty naturally worm-infested mongrel dogs were selected for the study and divided into groups of 20 dogs:

- **Group I**: Untreated control
- **Group II**: Treated with herbal formulation (1 mL/10 kg body weight [BW] twice a day for 2 days)
- **Group III**: Treated with mebendazole (25 mg/kg BW twice a day for 2 days)

Skin coat shine, BW changes, and growth rate were observed weekly throughout the experimental period.

The hematologic parameters, namely hemoglobin and differential leukocyte count (DLC), and blood biochemical observation such as total proteins, were examined 7 days prior to treatment and on days 0, 3, 7, 15, and 30 post-treatment. Fecal samples were examined on the same days for egg per gram (EPG) count.

No significant change was observed in BW and growth rate of all the animals. However, improvement in skin coat shine of both the treatment groups was observed from the seventh day of the post-treatment period. No significant change in DLC of all the animals was observed. However, hemoglobin count was elevated in both the treatment groups on day 15 and 30 post-treatment. The EPG count was observed to be increasing in Group I animals. However, significant reductions in EPG of Group III animals from day 3, and from day 7 in Group II animals, were observed. The tested herbal combination can be added to the pet food to control intestinal parasites.
Ayurveda, the ancient Indian system of medicine, advocates Rasayana therapy, which is recognized as adaptogenic compounds in modern medicine and characterized by improvement of endurance against stress, stimulation of metabolism and catabolism, and promotion of vitality and immune status, thereby stimulating general resistance, activation of nervous system, and retardation of the aging process. The present investigations were carried out using the herb ashwagandha, known for the immunostimulating properties recommended in Ayurveda. Thirty-six pups 1 month of age were selected for the study. Animals were screened for infectious diseases and only healthy pups were used. Pups were housed in a hygienic environment with free access to food and water up to the age of 3 months. All the pups were dewormed, vaccinated against rabies, and divided in two groups, each containing 18 pups. Group I was the control and Group II was the treatment group. Complete and balanced diets were fed to the pups in both groups. However, the treatment group pups were fed with the herbal formulation at the dose rate of 1 g/kg body weight (BW) in regular food. Weekly BW changes and growth rate were observed. The blood biochemical parameters, including serum total protein (TP), total immunoglobulins, serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) were studied on days 0, 15, 30, 45, and 60 of the post-treatment period. Significant increase in the BW and growth rate of the treatment group animals with no significant alterations in SGOT and SGPT was observed. The treatment group animals showed marked elevation in TP and total immunoglobulins from day 30 of the post-treatment period. However, a significant change was observed on days 45 and 60 of the post-treatment period, indicating immunostimulant properties of the herbal formulation. The addition of this herb in a normal diet of the growing pups may stimulate immune response.
Plasma total cholesterol concentrations (TC) and lipoprotein (LP) distributions of neonatal mixed-breed dogs were investigated during suckling. One of four diets varying in fatty acid composition was fed to 12 bitches (3 per group) from breeding through gestation, parturition, and lactation. Diets containing sufficient linoleic acid and 14% total fat were formulated with different fatty acid profiles using beef tallow (TAL), linseed oil (LIN), and high (HMH) or low (LMH) amounts of menhaden fish oil as the primary fat source. All puppies used were healthy and ingested colostrum. Puppies were weaned to their respective mothers’ diet on day 29 postpartum and plasma lipoprotein electrophoresis was performed on days 4, 10, 28, and 70 postpartum. Prior to blood sampling, puppies were separated from their mothers for 2 to 3 hours (days 4, 10, 28) or fasted overnight (day 70). Relative LP distributions were quantified by scanning densitometry, TC were determined, and concentrations for chylomicrons, beta (LDL), pre-beta (VLDL), alpha1, and alpha2 fractions (HDLs) were calculated. Repeated measures ANOVA indicated significant diet and time main effects (p <0.05). TC and beta LP were elevated on days 4 and 10. Chylomicra and pre-beta fractions were elevated on day 4. Alpha fractions were elevated during suckling: alpha2 decreased at weaning while alpha-1 remained essentially unchanged. Decreased TC and LP fractions were observed in puppies whose mothers were fed LMH or HMH compared to TAL or LIN groups. LP distributions of puppies during suckling in both menhaden oil groups were similar to normal adult dogs, and a menhaden oil dose response was noted. This work is the first report of increased low-density LP elevations in canine puppies during the early suckling period and their modification by maternal dietary fatty acids. The findings are consistent with cholesterol lowering with dietary marine oils and reduction of post-prandial LP fractions reported in adult humans and other species.