2009
Nestlé Purina
Veterinary Symposium
on companion animal medicine

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WHAT'S INSIDE:

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Dietary treatment of diabetes mellitus
in dogs and cats

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Dietary therapy is the key to proper management of the diabetic cat and dog. Cats develop type II diabetes mellitus; therefore, feeding a low-carbohydrate, high-protein diet is essential for optimizing and utilizing the gluconeogenic capacity and obligate carnivore aspect of this species. In fact, cats fed low-carbohydrate, high-protein diets are more than twice as likely to go into remission and discontinue insulin injections compared with cats fed low-glycemic index, high-fiber diets. On the other hand, dogs develop type I diabetes mellitus which requires continuous insulin therapy. Dogs are more omnivorous than cats, and feeding a low-glycemic index, high-fiber diet is the key to good diabetic regulation. Dogs are susceptible to exocrine pancreatic disorders, such as pancreatitis and pancreatic insufficiency, therefore, fat content of the diet is important as well.

Feline diabetes mellitus

Diabetes mellitus is one of the most common feline endocrine diseases, affecting one in every 200 to 300 cats, or roughly 240,000 diagnosed cases per year.[1] Despite the increasing frequency of the disease in the cat population, treatment of diabetic cats is frustrating and often associated with serious complications.

While insulin therapy and high-fiber diets have been mainstays of diabetes treatment, many diabetic cats experience complications associated with this therapy, such as hypoglycemia and progressive neuropathy.[2-7] In a recent study, 10 percent of diabetic cats had documented hypoglycemia caused by an insulin overdose.[6] Obese cats (>6 kg) were more likely to become hypoglycemic and lack autonomic warning signs of hypoglycemia.[6] Because of the difficulty in achieving adequate glycemic control with insulin therapy in cats, diabetic neuropathy is a common finding in diabetic cats. In one study, all diabetic cats suffered from subclinical forms of diabetic neuropathy as evidenced by impaired motor and sensory peripheral nerve conduction.[7] In summary, current dietary and insulin therapy is associated with increased risk of severe hypoglycemia and often results in poorly-controlled diabetes and progressive neuropathy in cats with type II diabetes.

The latest clinical and histologic evidence suggests that type II diabetes is the most frequently occurring form of diabetes in cats and people.[2-4] Type II diabetes in cats is characterized by an impaired ability to secrete insulin following a glucose stimulus and is caused by both a defect in pancreatic beta cells and by peripheral insulin resistance.[2-4] The etiology of type II diabetes is undoubtedly multifactorial; obesity, genetics, diet, and islet amyloidosis are involved in the development of this form of diabetes in humans and cats.[2-4] It is now recognized that the classic metabolic abnormalities found in type II diabetes— decreased insulin secretion and peripheral insulin resistance—may be consequences of abnormal amyloid production by pancreatic cells.[2-4] Despite the prevalence of type II diabetes in cats, the advanced nature

of their disease (amyloid deposition, glucose toxicity) often requires that insulin therapy be instituted.[2]

Unique mammal

The cat is an obligate carnivore and, as such, is unique among mammals in its insulin response to dietary carbohydrates, protein, and fat. The feline liver exhibits normal hexokinase activity, but glucokinase activity is virtually absent.[8] Glucokinase converts glucose to glycogen for storage in the liver and is important in decreasing postprandial glucose. Normal cats are similar to humans in that glucokinase levels drop precipitously with persistent hyperglycemia in people with type II diabeties. Amino acids, rather than glucose, are the signal for insulin release in cats.[9] In fact, a recent publication demonstrated more effective assessment of insulin reserve in cats using the arginine response test rather than a glucose tolerance test.[10]

Another unusual aspect of feline metabolism is the increase in hepatic gluconeogenesis seen after a meal. Normal cats maintain essential glucose requirements from gluconeogenic precursors (e.g., amino acids) rather than from dietary carbohydrates. As a result, cats can maintain normal blood glucose concentrations even when deprived of food for more than 72 hours.[9] Furthermore, feeding has very little effect on blood glucose concentrations in normal cats.[2,11] In summary, the cat is uniquely adapted to a carnivorous diet and is not metabolically adapted to ingestion of excess carbohydrates.

Low protein and excess carbohydrates in the diet equal catabolism

When type II diabetes occurs in cats, the metabolic adaptations to a carnivorous diet become even more deleterious, leading to severe protein catabolism; feeding a diet rich in carbohydrates may exacerbate hyperglycemia and protein wasting in these diabetic cats. In fact, in people with type II diabetes, the first recommendation is to restrict excess dietary carbohydrates such as potatoes and bread and to control obesity by caloric restriction.[12] Furthermore, people with type II diabetes have improved glycemic control and nitrogen turnover during weight loss when a low-energy, high-protein diet is combined with oral hypoglycemic therapy.[13]

A low-carbohydrate, high-protein diet, which is similar to a cat's natural diet (mice), may ameliorate some of the abnormalities associated with feline diabetes. In initial studies using a canned high-protein, low-carbohydrate diet and the starch blocker acarbose, insulin injections were discontinued in 58 percent of cats, and those with continued insulin requirements were regulated on a much lower dosage (1 U twice daily).[14] Comparison of canned high-fiber vs. low-carbohydrate diets in 63 client-owned diabetic cats showed that those fed low-carbohydrate diets were three times more likely to discontinue insulin injections.[15]

The diet formulation is critical in that most dry cat food formulations contain excessive carbohydrates; therefore, canned cat foods and preferably high-protein formulations should be used for initial treatment of diabetic cats. Because weight reduction also decreases insulin resistance, cats should be fed no more than 30 kcal/lb of ideal body weight in two equal meals per day. Initially, caution should be used when changing from dry to canned foods, as insulin requirements may decrease dramatically, and a reduction in insulin dosage may be required. Feeding either dry or canned high-protein, low-carbohydrate diets can improve glycemic control; however, cats fed canned high-protein, low-carbohydrate (less than 10 percent on a dry matter basis) diets are two to three times more likely to no longer require exogenous insulin injections.[14-16]

Diabetic nephropathy

Diabetic nephropathy occurs in a large percentage of people with type II human diabetics.[17] Diabetic nephropathy, like other diabetic complications, is associated with poor glucose regulation. The earliest sign of diabetic nephropathy is microalbuminuria. Azotemia is a late consequence of diabetic nephropathy, but may be reversible with good diabetic regulation. Hyperglycemia increases the glomerular filtration rate and renal plasma flow, and may increase binding of plasma proteins to glomerular basement membranes.[17] Elevation of tissue polyol concentrations resulting from hyperglycemia contributes to renal dysfunction. Thickening of the glomerular basement membranes and glomerular

hypertension may also contribute to renal problems.[17] If diabetic nephropathy is identified early and glycemic control improves, glomerular damage may be reversed. The incidence of diabetic nephropathy in cats is unknown, but recent studies indicate that the incidence might approach 50 to 60 percent of diabetic cats.[18] Treatment using a low-carbohydrate diet, such as Purina Veterinary Diets DM Dietetic Management Feline Formula, combined with oral hypoglycemics or insulin may help prevent progression of nephropathy. Conversely, use of low-protein diets are contraindicated in diabetic cats with moderate or mild azotemia, as they may exacerbate the diabetes, leading to regulation problems. Unless the cat has advanced renal disease caused by infection, uroliths, or other disorders, dietary protein should not be restricted. Phosphorus binders may be indicated in severe cases of diabetic nephropathy.

Gastrointestinal complications

Concurrent gastrointestinal disease is very common in diabetics, particularly diabetic cats. In one study, fewer than one-third of the cats died of diabetes.[19] Thirty-nine of 42 cats presented with diabetic ketoacidosis had concurrent diseases, including hepatic lipidosis, cholangiohepatitis, pancreatitis, chronic renal failure, urinary tract infection, and neoplasia.[20] In a similar survey of 104 diabetic cats 22 percent had concurrent diseases, the most common being hyperthyroidism, inflammatory bowel disease, and eosinophilic granuloma complex.[21]

Dietary therapy for concurrent disorders such as pancreatitis or inflammatory bowel disease may benefit from a high-protein, low-carbohydrate diet (Purina Veterinary Diets DM Feline Formula or Purina Veterinary Diets EN Feline Formula) combined with probiotics (*e.g.*, Fortiflora—Nestlé Purina). Ancillary therapy should be directed at the specific underlying disorder.

Concurrent endocrinopathies

The most common endocrinopathies of diabetic cats are, in order of frequency, hyperthyroidism, acromegaly, and hyperadrenocorticism.[22-24] Diabetic cats suffering from hyperthyroidism will often have periods of hyperglycemia followed by periods of hypoglycemia as insulin dosages are adjusted upward. Serum fructosamine concentrations will be falsely lower in diabetic hyperthyroid cats due to increased protein turnover; therefore, poor regulation may be challenging to identify. Permanent therapy for hyperthyroidism, such as radioactive iodine or surgery, should be used to resolve the hyperthyroidism in cats with concurrent diabetes in order to improve diabetic regulation. In fact, many cats with concurrent hyperthyroidism and diabetes will go into remission and no longer require insulin following appropriate treatment of the hyperthyroidism.

Acromegaly in cats almost always presents first as insulin-resistant diabetes.[23] Acromegalic cats can be identified using a serum somatomedin (insulin-like growth factor) test and computed tomography or

magnetic resonance imaging of the pituitary gland.

Finally, hyperadrenocorticism is a rare cause of insulin resistance in the cat, but can be diagnosed using a low-dose dexamethasone suppression test (0.1mg/kg intravenously). Regardless of the concurrent endocrinopathy, cats with diabetes and concurrent disease should receive a high-protein, low-carbohydrate diet to ameliorate the consequences of high blood glucose.

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Diabetes in dogs

Insulin-dependent diabetes mellitus (type I) is a diabetic state in which endogenous insulin secretion is insufficient to prevent ketone production. In type I diabetes, insulin secretion may be reduced or absent and can be readily corrected by exogenous insulin administration; in dogs, type I diabetes is caused by autoimmune destruction of the beta cells. Secondary diabetes in the dog may be due to pancreatitis or

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Table1. Macronutrient content of selected therapeutic dog foods suitable for treatment of diabetes mellitus								
		% nutrient dry matter				% nutrient Metabolizable energy		
Diet	Food form	Protein	Carbohydrate	Fat	Fiber	Protein	Carbohydrate	Fat
Purina Veterinary Diets								
DCO Canine Formula	Dry	25	48	12	8	24	46	29
OM Canine Formula	Canned	44	22	8	19	51	25	24
OM Canine Formula	Dry	31	44	7	10	34	48	19
Hill's Prescription Diet								
r/d Canine	Canned	25	39	9	21	30	46	24
r/d Canine	Dry	34	39	8	14	37	41	22
w/d Canine	Canned	18	53	13	12	18	52	30
w/d Canine	Dry	19	50	9	17	21	55	24
Royal Canin								
Diabetic HF 18	Dry	22	48	10	12	23	51	25
Digestive Low Fat LF 20	Canned	32	54	7	7	32	53	15
Digestive Low Fat LF 20	Dry	24	58	7	2	25	60	15
Eukanuba								
Eukanuba Weight Loss	Dry	25	58	9	2	Not listed		

concurrent endocrinopathies.

Dogs suffering from diabetes range in age from 4 to 14 years with a peak incidence at 7 to 9 years.[24] A genetic basis for diabetes is suspected in the keeshond and golden retriever. Other commonly affected breeds include miniature and toy poodles, dachshunds, miniature schnauzers, beagles, pulis, Cairn terriers and miniature pinschers. Most diabetic animals present with the classic clinical signs of polyuria and polydipsia. Weight loss is commonly observed in dogs. In some cases, polyphagia is also observed. In dogs, progressive polyuria, polydipsia and weight loss usually develop rapidly over a period of several weeks. Another common presenting complaint of diabetic dogs is acute onset of blindness caused by bilateral cataract formation. Physical examination findings of nonketotic diabetes in dogs are typically non-specific. In dogs, the most common physical examination findings are dehydration and muscle wasting or thin body condition. But about 25 to 30 percent of diabetic animals are obese upon initial examination. Hepatomegaly is usually observed in diabetic dogs. Cataracts are observed in approximately 40 percent of diabetic dogs. A diagnosis of diabetes should be based on the presence of clinical signs compatible with diabetes and evidence of fasting hyperglycemia and glycosuria. Common clinicopathologic features of diabetes in dogs include fasting hyperglycemia, hypercholesterolemia, increased liver enzyme activity (alkaline phosphatase, alanine aminotransferase),

neutrophilic leukocytosis, protein-uria, increased urine specific gravity, and glycosuria.[25]

Diet

The goals of dietary therapy in diabetes for both cats and dogs are to provide sufficient calories to maintain ideal body weight and correct obesity or emaciation, to minimize postprandial hyperglycemia, and to facilitate ideal glucose absorption by timing meals to coincide with insulin administration.[26] Caloric intake should be 60 to 70 kcal/kg per day for smaller dogs and 50 to 60 kcal/kg per day for larger dogs. Obese animals should have their body weight reduced gradually over a period of two to four months by feeding 60 to 70 percent of the calculated caloric requirements for ideal body weight. Underweight animals should be fed a high-caloric density food based on caloric intake for optimum body weight. *Table 1* lists the carbohydrate, fiber, and protein content of some commercially available dog foods.

The feeding schedule should be adjusted to the insulin therapy. Dogs should be fed within two hours of Lente (long-acting) insulin administration or six hours of protamine zinc insulin administration. Feeding usually elevates the plasma glucose for less than 90 minutes. Meals should be timed so that maximal exogenous insulin activity occurs during the postprandial period; therefore, when a twice-daily insulin dosing regimen is used, the dog should be fed immediately before or after the insulin injection. Diets formulated for canine adult maintenance with moderate dietary

fiber and carbohydrate content will be suitable for most diabetic dogs, for example, Purina Veterinary Diets DCO Dual Fiber Control canine formula. Most well-managed diabetic dogs require about the same amount of food per day as healthy nondiabetic dogs of similar age, gender, and lifestyle.

The protein content of the diet should be normal or increased in diabetic dogs, particularly in obese dogs. It has been shown that insulin resistance is reduced and lean body mass preserved in dogs fed higher-protein diets for weight loss.[27] Higher-protein formulations may also beneft underweight diabetic dogs with significant muscle wasting or those suffering from concurrent exocrine pancreatic insufficiency. Low-glycemic index carbohydrates such as sorghum and barley are recommended. Readily absorbed carbohydrates such as rice and corn syrup have high glycemic indices and should be avoided. Foods with high sugar content (semi-moist foods) should not be used to improve the palatability of food prescribed for diabetic dogs.

Dietary fat restriction (<12 percent on a dry matter basis or <30 percent ME) is recommended for diabetic dogs with concurrent chronic pancreatitis or persistent hypertriglyceridemia (miniature schnauzers). Foods with a high fat content should not be used to improve the palatability of food prescribed for diabetic dogs. Instead, the low-carbohydrate, low-fat food may be made more palatable with agents such as warm low-fat chicken or beef broth.

One of the approaches to manag-

ing diabetes in dogs combines the use of nutritional components such as starch blends, carboxymethyl-cellulose, and fermentable fiber blends. Barley and sorghum can be used to blunt the postprandial rise in blood glucose, adjust posprandial insulin to appropriate levels, and to help blunt glucose surge. Fermentable fibers, such as fructooligosccharides, beet pulp, and gum arabic, increase production of short-chain fatty acids from the large intestine, which in turn increases glucagon-like peptide-1 (GLP-1) secretion and activity. GLP-1 is necessary for normal insulin secretion and for normal timing of insulin secretion after eating. Carboxymethylcellulose delays gastric emptying, further blunting the glucose surge that occurs after feeding. Diets with moderate dietary fiber content are recommended for diabetic dogs especially those that are overweight (crude fiber maximum 10 to 15 percent dry matter). High-fiber, restricted-fat diets should not be routinely recommended for diabetic dogs with a body condition score of less than 4/9.

Hyperlipidemia

In a survey of concurrent disorders in 221 diabetic dogs, over 40 percent had lipemia.[28] Although hyperlipidemia can be caused by diabetes, a thorough evaluation of thyroid and adrenal status should be done to rule out common endocrinopathies as a cause of the hyperlipidemia, particularly if it is hypercholesterolemia. A fat-restricted diet should be considered for diabetic dogs with

Table 2. Causes of hyperglycemia and hypoglycemia in diabetic dogs

Causes of hyperglycemia in diabetic dogs
- Insufficient insulin dosage
- Insufficient duration of insulin action
- Outdated, inactive insulin
- Owner administration problems
- Overfeeding
- Stress
- Insulin resistance caused by: hyperadrenocorticism, infections, drugs, thyroid disease, pancreatitis, or acromegaly

Causes of hypoglycemia in diabetic dogs
- Insulin overdose
- Anorexia caused by (*e.g.*, oral disease, ketosis)
- Overlap of insulin action
- Maldigestion caused by exocrine pancreatic insufficiency or bacterial overgrowth
- Transient diabetes
- Malabsorption caused by inflammatory bowel disease, lymphangectasia, or lymphoma
- Somogyi effect
- Concentrated insulin (old)

persistent hypertriglyceridemia. This often involves breeds such as the miniature schnauzer that have a defect in lipoprotein lipase activity. Hyperlipidemia is a cause of insulin resistance; therefore, dietary restriction of fat is essential for good diabetic regulation in these patients.

Pancreatic disease

In the previously mentioned survey of concurrent disorders in 221 diabetic dogs, more than 70 percent had elevated liver enzyme activity, and 13 percent had acute pancreatitis.[28] Acute pancreatitis may be one of the most difficult diseases to manage nutritionally and is complicated further when the dog is also diabetic. Low-fat formulations (<12 percent on a dry matter basis or <30 percent ME) may be helpful to feed dogs after repeated bouts of pancreatitis.

A less commonly reported or identified pancreatic disorder in dogs is exocrine pancreatic insufficiency. These patients often present with repeated bouts of diarrhea, anorexia, and hypoglycemia. Unlike young German shepherds with exocrine pancreatic insufficiency, diabetic dogs may have borderline pancreatic enzyme activity resulting in carbohydrate and fat malassimilation and diarrhea, but not overt steatorrhea. The condition is diagnosed using the commercially available trypsin-like immunoreactivity test; I also measure cobalamin and folate concentrations to look for deficiency or excess of these vitamins. Adding pancreatic enzymes and probiotics to the diet may help in restoring normal digestion and microbial floral in these patients. Diabetic dogs with reduced exocrine pancreatic function have an increased caloric requirement compared with healthy dogs.

Concurrent endocrinopathies

In one study, 23 percent of the diabetic dogs had test results consistent with hyperadrenocorticism, and 9 percent were hypothyroid.[28] Hypothyroidism is the most common endocrinopathy accompanying diabetes in dogs and can result in insulin resistance.[29] Diabetic dogs should be evaluated for hypothyroidism at the time of diagnosis using thyrotropin (TT_4) and endogenous canine thyroid stimulating hormone (TSH) assays. Low TT_4 and high TSH results will confirm hypothyroidism, while low TT_4 and normal or low TSH results are indicative of euthyroid sick syndrome.

Hyperadrenocorticism is a less common endocrinopathy associated with diabetes, but often results in the development of ketosis. Most dogs with both diseases develop hyperadrenocorticism before the onset of diabetes. Testing for hyperadrenocorticism may be complicated in diabetic patients with a large number of false positives resulting from the low dose dexamethasone suppression test. I prefer to screen for hyperadrenocorticism using an ACTH response test in stable diabetic (*e.g.*, nonketotic) patients.

Hypoglycemia and hyperglycemia

The clinical signs of hypoglycemia in diabetics are initially consistent with epinephrine release to counter the hypoglycemia. Nervousness, anxiety, vocalization, muscle tremors, ataxia, and pupillary dilatation should alert the owner to the possibility of hypoglycemia. At this point, the animal should be offered food and the owner should seek veterinary advice. Late in the course of hypoglycemic shock, the animal may become recumbent, comatose, or experience seizure. If vascular access is not possible or if the owner is administering therapy, 50 percent dextrose (Karo syrup, pancake syrup) may be applied to the oral mucous membranes using a large syringe. When doing so, the owner should maintain a reasonable distance from the animal's teeth to prevent accidental injury from biting. The owner should then transport the animal to a veterinarian as soon as possible. At the veterinary office, the patient should receive a slow intravenous bolus of 50 percent dextrose (0.5 g/kg diluted 1:4).[30] Thereafter, a continuous infusion of 5 percent dextrose should be administered until the animal can be fed. Many animals that experience insulin overdose will suffer cerebral edema and temporary blindness or behavior changes; often these signs are temporary and resolve after several weeks or months. Endogenous glucose stores (hepatic glycogen) may have been depleted by the insulin overdose and it may take several days for hyperglycemia to recur. In these cases, insulin therapy should be discontinued until hyperglycemia recurs.

Generally, hyperglycemia problems can be differentiated by the characteristics of the blood glucose curve and the insulin dosage (per dosing interval). If the patient is receiving >2.2 U/kg of insulin per dose, insulin resistance should be investigated. Causes of insulin resistance in dogs include hypothyroidism, hyperadrenocorticism, acromegaly, estrus, drug therapy, and infections. If the animal is receiving <2.2 U/kg per dose, the blood glucose curve will usually indicate one of the following: insufficient dosage of insulin, short duration of action of insulin, insulin-induced hypoglycemic hyperglycemia (Somogyi effect) and, insulin overlap or prolonged insulin action. Corrective actions include, respectively, increasing the insulin dose, changing to a longer acting insulin or twice daily insulin regimen, reduction of the insulin dose by 25 percent, or changing to a shorter duration insulin or insulin mixture (30 percent regular, 70 percent NPH, respectively). Causes of hyperglycemia and hypoglycemia in diabetic dogs are listed in *Table 2*.

Exercise

Exercise should be kept constant in diabetic animals. The owners should be instructed to walk the animal daily and avoid intermittent or unplanned episodes of strenuous exercise. Increasing exercise in obese diabetic animals will reduce insulin resistance and improve glycemic control. In fact, the best way to reduce insulin requirements and maintain steady glucose concentrations throughout the day in dogs is to exercise the dog for 20 to 30 minutes before the evening meal

and insulin administration. I have been able to regulate very large and athletic breeds, such as racing greyhounds, by adjusting the insulin to the activity. For example, if a race or strenuous hike is planned, the morning insulin might not be given or only half the insulin is administered to avoid exercise-induced hypoglycemia.

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Managing chronic enteropathies
in dogs

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Dogs with intestinal disease typically present with clinical signs such as diarrhea, weight loss, or vomiting. Diarrhea that has lasted three weeks or more is usually considered chronic. The initial diagnostic approach to chronic diarrhea is based on determining the nature and severity of the diarrhea and the presence of specific or localizing clinical findings. The presence of additional clinical signs often points to the underlying cause (see *Table 1*). This information is integrated to determine whether diarrhea is most likely due to large bowel disease (dyschezia, tenesmus, increased frequency of defecation, small volume of feces with mucus and blood) or a consequence of small intestinal disease or exocrine pancreatic insufficiency (large volume of diarrhea, weight loss, possible vomiting). In patients with abdominal pain, dehydration, frequent vomiting, or localizing findings such as an abdominal mass, these problems are pursued ahead of an in-depth workup for chronic diarrhea. In patients with chronic diarrhea and no obvious cause, it is best to adopt a systematic approach, determined by the localization of diarrhea to the small or large bowel. Patients with signs of large or small bowel involvement are usually evaluated for diffuse GI disease. This presentation will review the diagnosis and management of dogs with chronic enteropathies that are predominantly associated with small bowel diarrhea.

Investigation of chronic small bowel diarrhea

The initial diagnostic approach to patients with chronic small bowel diarrhea is summarized in *Table 2*.

After the exclusion of infectious and parasitic agents, non-GI disorders, exocrine pancreatic insufficiency, and intestinal structural abnormalities requiring surgery, the most common group of intestinal conditions associated with chronic small bowel diarrhea are idiopathic inflammatory bowel disease (IBD), diet-responsive enteropathy, antibiotic-responsive enteropathy, and lymphangiectasia.

The diagnostic approach to this group of patients is usually determined by the severity of clinical signs and the presence or absence of hypoalbuminemia, intestinal thickening, or mesenteric lymphadenopathy. In patients with any of these abnormalities, intestinal biopsy is required to define the cause (*e.g.*, IBD, lymphangiectasia, lymphoma) and to optimize therapy. Controlled studies have shown that hypoalbuminemia is associated with a poor outcome in dogs with chronic enteropathy.[1, 2] Evaluation of hemostatic function is recommended to determine if hypo- or hypercoagulability have arisen as a consequence of enteric protein loss.

The clinical severity of intestinal disease can be quantified by determining the canine IBD activity index (CIBDAI) through evaluation of attitude, activity, appetite, vomiting, stool consistency, stool frequency, and weight loss.[3] Measurement of serum C-reactive protein (CRP) has been shown to correlate with CIBDAI and implies that severe clinical disease is accompanied by a systemic inflammatory response.[3] Measurement of CIBDAI or CRP can also serve as a baseline for determining the response to treatment.

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Table 1. Potential underlying causes of chronic diarrhea and commonly associated clinical signs

Underlying cause	Clinical Signs
large bowel disease	tenesmus and dyschezia
upper gastrointestinal (GI) bleeding	melena
structural disorders, perforation, thrombosis	abdominal pain
enteric protein loss	abdominal distension, difficulty breathing, and peripheral edema

In stable patients with chronic diarrhea (*i.e.*, good attitude, appetite, mild weight loss, normal serum proteins, and no intestinal thickening or lymphadenopathy), measurement of serum cobalamin and folate help evaluate disease severity, aid in localization of intestinal disease, and determine if supplementation is required. Low-serum cobalamin concentration (< 200ng/L) has also been associated with a negative prognosis.[2] Intestinal biopsy is indicated in dogs with low serum cobalamin concentrations to determine the nature of the intestinal disease. In stable patients with chronic diarrhea and normal serum cobalamin concentrations, the client can be given the option of empirical treatment (see below). Failure to respond to empirical therapy or worsening of disease is an indication for intestinal biopsy.

Intestinal biopsy

Intestinal biopsies can be acquired endoscopically or surgically. In patients without an indication for surgery (*e.g.*, intestinal masses, anatomic or structural disease, or perforation), the authors prefer to perform diagnostic endoscopy to visually inspect the esophageal, gastric, and intestinal mucosa, and to procure endoscopic biopsies. Guidelines for biopsy acquisition have recently been published.[4] Operator experience and biopsy quality and number are key in enabling effective histopathologic evaluation. Surgical biopsy is usually performed where intestinal disease is suspected to involve the submucosa or muscularis, and where the results of endoscopic biopsies do not adequately explain the clinical picture.

Unfortunately, the histopathologic interpretation of GI biopsies varies considerably among pathologists.[5] To try to correct this problem, a working group established by the World Small Animal Veterinary Association has created a series of images to standardize the evaluation of intestinal histopathology.[6] The ability of this scheme to increase agreement between pathologists, and the clinical relevance of the criteria it evaluates, remain to be determined, but it is a step in the right direction.

The most common histopathologic diagnoses in dogs with chronic diarrhea are IBD, lymphangiectasia, and lymphoma. The most commonly described histopathologic lesion in the intestines of dogs is increased cellularity of the lamina propria that is usually referred to as being consistent with IBD. The extent of inflammation is variable and ranges from focal to diffuse involvement of the small and large intestine. The degree of inflammatory cell accumulation is also variable and is subjectively categorized as normal, mild, moderate, or severe.

Increased numbers of lymphocytes and plasma cells, described as lymphoplasmacytic enteritis, is the most frequently reported form of IBD. Moderate to severe lymphoplasmacytic enteritis is often described in association with a protein-losing enteropathy. Predisposed breeds include basenji, Norwegian lundehund, and Chinese Shar-Pei. However, recent studies have called into question the appropriateness and clinical relevance of the term "lymphoplasmacytic" enteritis. Dogs have similar numbers of CD3+ T lymphocytes before and after clinical remission,[7] and cats with and without signs of intestinal disease have similar numbers of lymphocytes and plasma cells.[8]

The presence of moderate to large numbers of eosinophils in intestinal biopsies, which is often accompanied by circulating eosinophilia, suggests possible parasitic infestation or dietary intolerance. Moderate to high numbers of macrophages and neutrophils raise the possibility of an infectious process, and culture and special stains are indicated.

Changes in mucosal architecture, such as villus atrophy and fusion, are less frequently commented on than cellularity, but appear to be

important indicators of disease severity. A recent study in cats with signs of GI disease measured mucosal cytokines levels to identify histologic correlates of mucosal inflammation. In this study, villus atrophy and fusion correlated with severity of clinical signs and degree of pro-inflammatory cytokine upregulation in the duodenal mucosa.[9]

Dilatation of lymphatics and the presence of crypt abscesses and crypts cysts are most commonly encountered in dogs with protein-losing enteropathies, and often are accompanied by lymphoplasmacytic inflammation of varying severity.[5,10]

Treatment

The clinical severity of disease, the nature and severity of histopathologic lesions, and the presence or absence of hypoalbuminemia guides treatment. For dogs with low clinical disease activity, normal intestinal histopathology, and serum albumin concentrations >2.0g/dl, the following empirical treatment protocol can be followed.

1. Empirical treatment for *Giardia* and endoparasites if not already performed (*e.g.*, fenbendazole 50mg/kg by mouth for five days)
2. Dietary trial (see *Table 3*). A positive response suggests diet-responsive enteropathy. If a good response, continue diet, consider re-challenge, and defining basis of dietary intolerance.
3. Antibiotic trial: tylosin (10 to 15mg/kg by mouth three times daily), oxytetracycline (20mg/kg by mouth three times daily), or metronidazole (10mg/kg by mouth twice daily). A positive response suggests antibiotic-responsive enteropathy, maintain on antibiotics for 28 days then discontinue. If a good response, consider transition to probiotics.
4. For poor response, reappraise, then consider other treatments.

Inflammatory bowel disease

Treatment of any disease is ideally directed at the underlying cause, which is problematic for IBD as the etiopathogenesis is unclear.

IBD in people and animals is increasingly considered a consequence of uncontrolled intestinal inflammation in response to a combination of elusive environmental, enteric luminal constituents (principally microbial and dietary), and immunoregulatory factors in genetically susceptible individuals.

In people, genetic susceptibility is linked increasingly to defects in innate immunity exemplified by mutations in the innate immune receptor NOD2/CARD15, which in the presence of the enteric microflora may lead to upregulated mucosal cytokine production, delayed bacterial clearance, and increased bacterial translocation, thereby promoting and perpetuating intestinal inflammation.[11] While the mucosa-associated flora is implicated frequently as a pivotal factor in the development of IBD in people and animals, the specific bacterial characteristics that drive the inflammatory response have remained elusive. The clinical responses of some dogs with idiopathic chronic diarrhea to antibiotics such as tylosin or oxytetracycline, and the predisposition of certain breeds, (*e.g.*, German shepherd), points to a similar interaction of host susceptibility and microflora in dogs.[12-14] As the numbers of cultivable aerobic and anaerobic bacteria in the duodenal fluid of dogs that respond to antibiotics is similar to dogs that respond to food or immunosuppression, it is plausible that dogs with antibiotic responsive enteropathy are more susceptible to their resident microflora, but this remains to be determined.[14,15]

Recent advances in molecular microbiology have enabled the analysis of complex bacterial communities without bacterial culture. Culture-

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The most common histopathologic diagnoses in dogs with chronic diarrhea are IBD, lymphangiectasia, and lymphoma.

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independent analyses of bacterial 16S rDNA libraries in people reveal that only 30 percent of the fecal flora appears cultivable, and there is significant variation in the flora in different gastrointestinal segments and luminal contents versus the mucosa of healthy individuals.[16] The application of these culture-independent techniques to people, dogs, and cats has revealed that intestinal inflammation is associated with a floral shift from gram-positive Firmicutes to gram-negative bacteria, predominantly Enterobacteriaceae.[9,17,18] It is noteworthy that increased numbers of Enteobacteriaceae have been

Table 2. Initial diagnostic approach to chronic small bowel diarrhea

Purpose of Test	Tests to perform
Integrate signalment, history and physical examination	Breed predisposition, environment, diet, other clinical signs, localizing findings
Detect endoparasites and enteric pathogens	Fecal analysis
Perform clinicopathologic testing to detect non-GI disease and to detect and characterize intestinal disease	Complete blood count, serum chemistry profile, urinalysis ACTH simulation test, bile acids assay, freeT4 concentration, thyroid-stimulating hormone assay, ± trypsin-like immunoreactivity
Perform diagnostic imaging to detect non-GI disease and to detect and characterize intestinal disease	Abdominal radiographs, ultrasound

found to correlate with mucosal inflammation and clinical signs in cats with signs of GI disease,[9] and a novel group of adherent and invasive *Esherichia coli* (AIEC) have been associated with intestinal inflammation in people and boxer dogs with granulomatous or histiocytic ulcerative colitis.[17,19] While it remains to be determined if these floral alterations are a cause or a consequence of the inflammation, their discovery has provided new opportunities for therapeutic intervention.

There is also growing evidence to support an important role diet plays in the development of canine IBD. In a controlled study of 65 dogs with diarrhea of at least six weeks duration, 39 of 65 dogs responded to dietary modification (10 days of Purina Veterinary Diets LA Limited Antigen [now known as DRM Dermatologic Management®]), and the remaining dogs were treated with corticosteroids (2mg/kg orally once daily for 10 days followed by a tapering dose over 10 weeks).[20] The CIBDAI and histopathologic scores

were similar (> 70 percent moderate to severe in each group) in dogs that did and did not respond to diet. Dogs that responded to diet tended to be younger and have higher serum albumin than dogs that did not respond to diet. Dogs that did not respond to diet were subsequently treated with steroids. Interestingly, intestinal histopathology results did not differ in either diet-responsive or steroid-responsive dogs before and after treatment.

Taken as a whole, the results of studies in dogs with chronic diarrhea have, to date, provided reasonable evidence that various subsets of dogs will respond to treatment with specific antibiotics, diet modification, or immunosuppressive therapy. At present there is no reliable means for predicting which dogs will respond to which treatment, and treatment consists of a series of therapeutic trials.

Standardized treatment protocol for canine IBD

The authors are prospectively evaluating a standardized treatment proto-

col for dogs with IBD:

• **Mild to moderate disease activity, mild to moderate histopathologic abnormalities (lymphocytes and plasma cells are the predominant cell type), serum albumin > 2.0g/dl:**

1. Empirical treatment for *Giardia* and helminths, if not already performed.
2. Dietary trial with a hydrolyzed soy diet for two weeks. If a good response then maintain on diet, consider re-challenge, and defining basis of dietary intolerance.
3. Antibiotic trial with tylosin for two weeks. If a good response, maintain on antibiotics for 28 total days then discontinue. Consider transition to probiotics.
4. Immunosuppression with glucocorticoids or azathioprine.
   • For dogs < 70lbs: 2mg/kg prednisolone orally once daily for 21 days, 1.5mg/kg orally once daily for 21 days, 1mg/kg orally once daily for 21 days.
   • For dogs > 70lbs: 2mg/kg azathioprine orally once daily for five days, then 2mg/kg orally

once daily every other day.

5. If poor response, reappraise before considering escalating immunosuppression (*e.g.*, adding azathioprine, or substituting with cyclosporine[21] if already on azathioprine).

6. If good response taper immunosuppression, then stop antibiotics.

• **Moderate disease activity, moderate to severe intestinal histopathologic abnormalities (villus atrophy and fusion, lymphocytes and plasma cells are the predominant cell type), serum albumin < 2.0g/dl:**

1. Empirical treatment for *Giardia* and helminths if not already performed.

2. Concurrent dietary modification to a hydrolyzed soy diet, antibiotics (tylosin), and immunosuppression (glucocorticoids or azathioprine).

   • The use of elemental (monomeric) diets and partial parenteral nutrition may be required in some dogs with severe protein-losing enteropathy.

   • Concurrent therapy with ultra low dose aspirin (0.5mg/kg orally once daily) may be indicated in patients considered at risk for thromboembolic disease.

   • Concurrent judicious use of diuretics (furosemide 0.5 mg/kg once or twice daily for 2 to 3 days, and spironolactone 0.5-1.0 mg/kg by mouth twice daily) is often recommended in patients with significant ascites. Monitor for hypokalemia and metabolic alkalosis.

3. Reappraise if poor response before considering escalating immunosuppression.

   • Consider failure to absorb

oral prednisolone and switch to injectable corticosteroids.

   • Dexamethasone may be preferable to prednisolone in patients with ascites to avoid increased fluid retention.

4. If good response taper immunosuppression, then stop antibiotics.

The authors are evaluating this approach in an ongoing prospective clinical trial: To date, in 16 dogs with a histopathologic diagnosis of IBD, 10 dogs were diet-responsive, three steroid-responsive, and three were partially responsive to a combination of food and antibiotics.

Lymphangiectasia

Intestinal lymphangiectasia is characterized by the abnormal distension of lymphatic vessels within the mucosa. Intestinal lymphangiectasia is a consequence of a localized or generalized lymphatic abnormality or increased portal pressure (*e.g.*, right-sided heart failure, caval obstruction, or hepatic disease). Lymphatic abnormalities are often associated with lipogranulomatous inflammation that is visible as small white granules on the intestinal mesentery. Tumor infiltration of lymphatics or lymph nodes can also cause lymphangiectasia. In some cases, lymphangiography reveals a generalized lymphatic abnormality. Dilatation of lymphatics is associated with the exudation of protein rich lymph into the intestine and severe malabsorption of long chain fats. Crypt cysts and crypt abscesses may also be observed in intestinal biopsies. Yorkshire terriers (10-fold relative risk), soft coated wheaten terriers (frequently with concurrent proteinuria), and Norwegian lundehund seem to be over represented, supporting a familial cause in some dogs.[5,10,22]

Clinical findings: The clinical findings are essentially a consequence of the intestinal loss of protein and can include weight loss, chronic diarrhea, ascites, edema, and chylothorax.

Diagnosis: Lymphangiectasia usually presents with clinical signs of a protein-losing enteropathy. It appears endoscopically as white blebs on the mucosa (dilated lymphatics). Endoscopic biopsies are often adequate. Surgical biopsy should be undertaken carefully with appropriate precautionary measures to avoid dehiscence.

Treatment: The underlying cause of lymphangiectasia is not usually determined. Treatment is supportive and symptomatic.

1. Dietary recommendations are similar to those for the other causes of chronic small bowel diarrhea listed, but fat restriction may have to be more severe.

   • Medium chain triglyceride (*e.g.*, coconut oil 0.5 to 2ml/kg body weight per day added to food) can be added to the diet, or a diet already containing them (*e.g.*, Purina Veterinary Diets EN Gastroenteric® canine formula) can be fed to provide an easily assimilable source of calories.

2. Treatment with prednisolone is often necessary (1 to 2mg/kg by mouth twice daily) and may work by decreasing lipogranulomatous inflammation or concurrent mucosal inflammation. The authors try to avoid high-dose, long-term immunosuppression with corticosteriods due to risk of sepsis in these patients. Prednisolone is tapered to the

Table 3: Options for dietary trials

Global modification of diet
• Switch to a different diet or different manufacturer

Optimize assimilation of diet
• Highly digestible (usually rice-based)
• Fat restricted (<15% DM)
• Easily digested fats (*e.g.*, medium chain triglyceridies)
• Restrict fiber

Antigenic modification of diet
• Novel protein source
• Protein hydrolysate

Immunomodulation of diet
• Altered fat composition (*e.g.*, Omega 3:6, Fish oil)
• Prebiotics [*e.g.*, inulin (fructans), fructooligosaccharides]

lowest effective dose once remission is achieved.
  • In patients with severe malabsorption, parenteral glucocorticoids may be required, and a switch to dexamethasone may be made in patients with ascites or edema.
3. Escalation of immunosuppression may be tried if unresponsive.
4. Adjunctive therapy with metronidazole or tylosin is often employed to decrease the risk of bacterial translocation through the markedly impaired gut.
5. Ultra low-dose aspirin and diuretic therapies are employed as in treatment of IBD with albumin < 2.0g/dl).

  Prognosis: The response to therapy is variable, with some dogs staying in remission for several years while others pursue a path towards fulminant hypoproteinemia or thromboembolic disease. The prognosis is always guarded.

Disclosure:
K. Simpson is a member of the Nestle Purina Advisory Council.

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GI flora: Understanding the role of probiotics **in veterinary medicine**

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The mammalian intestinal tract contains a complex, dynamic, and diverse society of pathogenic and nonpathogenic bacteria. Researchers have estimated that the human body contains 10^{14} cells—only 10 percent of which are not bacteria and belong to the human body proper.[1] A large body of research has focused on the mechanisms by which pathogenic bacteria influence intestinal function and induce disease; however, recent attention has been focused on the indigenous nonpathogenic microorganisms and how they may benefit the host. Initial colonization of the sterile newborn intestine occurs with maternal vaginal and fecal bacterial flora. The first colonizers can include species of enterobacteria, streptococci, and staphylococci. These bacteria metabolize oxygen and favor the growth of anaerobic bacteria, including lactobacilli and bifidobacteria. This overview will introduce the history of probiotics, their safety and regulatory background, provide information on their mechanisms of action, and introduce possible ways probiotics may be used to treat clinical diseases.

History of probiotic health claims

Documentation of the healthy consumption of bacteria in food dates back to the Old Testament (Gen. 18:8). Plinius, a Roman historian in 76 B.C., recommended the use of fermented milk products for the treatment of gastroenteritis.[2] In the early 20th century, Elie Metchnikoff, a Nobel Prize-winning Russian scientist, suggested that the ingestion of *Lactobacillus*-containing yogurt contributed to the longevity of Bulgarian peasants by decreasing the pathogenic bacteria in the intestine.[3] These observations led to the concept of a "probiotic," derived from the Greek, meaning "for life." The term probiotic was first used in 1965 to define substances secreted by one microorganism that stimulates the growth of another. The meaning of the word probiotics subsequently evolved to apply to those bacteria that contribute to intestinal balance. The World Health Organization defines probiotics as "live microorganisms, which when administered in adequate amounts, confer a heath benefit on the host.".[4]

Different strains of probiotic bacteria may exert different effects based on specific capabilities and enzymatic activities—even within one species.[5,6] Different microorganisms express habitat preferences that may differ in various host species. The four habitats in the gastrointestinal tract are: the surface of epithelial cells; the crypts of the ileum, cecum, and colon; the mucus gel that overlays the epithelium; and the lumen of the intestine.[7] The luminal content of bacteria depends greatly on bowel transit, resulting in a relatively low microbial density in the small bowel.

Because probiotics have multiple mechanisms of action, many have potential applications for managing various diseases. Those probiotics that have undergone the most clinical testing in people and livestock and are used widely include *Lactobacillus* species (such as *L. acidophilus*, *L. rhamnosus*, *L. delbrueckii* ssp. *bulgaricus*, *L. reuteri*,

Table 1. Mechanisms of probiotic action

A) Probiotics block intestinal bacterial effects

Probiotics have been identified to mediate maintenance of the gastrointestinal microbial balance via two mechanisms: production of antibacterial substances, such as bacteriocins (*e.g.*, lantibiotics) and acids (*e.g.*, acetic, lactic, and proprionic) and competitive inhibition of pathogen and toxin adherence to the intestinal epithelium.

B) Probiotics regulate mucosal immune responses

Both in vitro and in vivo studies show the effects of probiotics on host immune functions, including upregulation of immune function that may improve the ability to fight infections or inhibit tumor formation and downregulation of immune function that may prevent the onset of allergy or intestinal inflammation. One mechanism of probiotics regulating immunomodulatory functions is through activation of toll-like receptors (TLRs).

C) Enhancing host innate immunity

Probiotics have the potential to stimulate innate immune responses against microorganisms and dietary antigens that are newly encountered by the host through several mechanisms. Intestinal dendritic cells can retain commensal bacteria by selectively activating B lymphocytes to produce IgA (to reduce mucosal penetration by bacteria). The dendritic cells carrying commensals are restricted to the intestinal mucosal lymphoid tissues and thus avoid potential systemic immune responses.

D) Modulation of pathogen-induced inflammatory responses

The host's innate defenses must modulate responses appropriate to the level of threat provided by a given pathogen. If the response is too weak, the infection may not be cleared, leaving the host susceptible to systemic infection. However, if it is too strong the result may be excess tissue damage. A mechanism of probiotic protection from pathogen-induced injury and inflammation is modulating the balance of pro- and anti-inflammatory cytokine production.
• Increasing anti-inflammatory cytokine production
Probiotics can induce dendritic cells to produce anti-inflammatory cytokines.
• Suppressing proinflammatory cytokine production
Probiotics have been shown to inhibit lipopolysaccharide and *Helicobacter pylori*-stimulated TNF production by murine macrophages.

E) Upregulation of host-immune responses to defend against infection

Probiotics and commensal microflora may regulate a balance between pro- and anti-inflammatory mucosal responses leading to intestinal homeostasis. Probiotics facilitate this important function by stimulation of host immunological functions.

F) Regulation of immune responses by probiotic DNA

There have been a number of intriguing studies documenting the beneficial immunomodulatory properties of probiotic DNA in people and murine models.

G) Differential activation of TLRs by probiotics in immune cells

Probiotic bacteria possess molecular recognition patterns similar to pathogenic bacteria; however, the probiotic organisms do not normally initiate pathogenic inflammatory responses. It appears that probiotics exert both up- and downregulatory effects on immune responses, and TLR-regulated signaling pathways appear to be one of the mechanisms for these immunoregulatory actions.

H) Probiotics regulate intestinal epithelial cell functions

Substantial evidence indicates that probiotic bacteria stimulate intestinal epithelial cell responses, including restitution of damaged epithelial barrier, production of antibacterial substances and cell-protective proteins, and prevention of cytokine-induced intestinal epithelial cell apoptosis. Many of these responses result from probiotic stimulation of specific intracellular signaling pathways in the intestinal epithelial cells.

and *L. casei*); *Bifidobacterium* species; *Enterococcus faecium* and *Saccharomyces boulardii*, which is a nonpathogenic yeast. In dogs and cats, lactobacilli and bifidobacteria have also been used clinically. However, *Enterococcus faecium* has also garnered attention in clinical use in Europe and in the United States (see *Figure 1*). Despite the interest in probiotics for clinical use, understanding their clinical application in veterinary medicine has been limited by the paucity of well-

> Although… nonviable bacteria may mediate a physiologic benefit, they are not considered to be probiotics under the present definition.

designed laboratory, translational, and clinical studies.

Safety and regulation of probiotics

The Food and Agriculture Organization of the United Nations and the World Health Organization have published recommended guidelines for what constitutes a probiotics product intended for use in humans. Requirements allow that the included strains be designated individually and speciated appropriately. The formulations must retain viable strain counts at the end of their shelf lives and must confer a proven clinical end-point.[8] Some human products continue to be of dubious quality and carry unsupported health claims, which suggests that some manufacturers are not enforcing the recommended guidelines. This problem is compounded by the diverse categories that encompass probiotic products for humans, including food, functional food, novel food, natural remedy (Denmark, Sweden, and Finland), natural health product (Canada), dietetic food (Italy), direct fed microbial (animal feed), dietary supplement (human food) (USA), and biotherapeutic and pharmaceuticals (probiotic pharmaceuticals are available in Canada, China, and a variety of European countries).

A true probiotic contains live microbes having a substantiated beneficial effect. Although a preparation of nonviable bacteria may mediate a physiologic benefit, they are not considered to be probiotics under the present definition. Furthermore, any strains that do not confer benefits should not be referred to as probiotics. In vitro testing to establish mechanisms of action are insufficient substantiation for classifying a microbial strain as a probiotic. The basis for a microbe being termed a probiotic should be proven efficacy and safety under the recommended conditions of use, with considerations given to target population, route of administration, and dose applied.[8]

Despite prolonged marketing of probiotic products, a relatively large number of human products are mislabeled with inaccurate use of nomenclature for genus and species, inaccurate cell count, or unsubstantiated structure and function statements.[9] From a scientific perspective, the suitable description of a probiotic reflected on the label should include the following information:

• genus and species identification, with nomenclature consistent with current scientifically recognized names
• strain designation
• viable count of each strain at the end of shelf life
• recommended storage conditions
• recommended dose
• an accurate description of the physiological effect (as far as is allowable by law)
• contact information for post-market surveillance.

In the U.S., probiotic preparations labeled for use in dogs or cats are classified by AAFCO (Association of American Feed Control Officials) and FDA's Center of Veterinary Medicine (CVM) as direct fed microorganisms or microbials, not pharmaceutical products. CVM has listed bacterial species that are considered safe when used in direct fed microbial (DFM) products. These include multiple species of *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. In addition to using approved species and defined fermentation product ingredients, AAFCO has established labeling requirements for DFM products such as probiotics.

All products that are sources of DFM are required to provide potency guarantees for each microorganism in colony forming units per gram (CFU/g), along with a distinct

listing of the microorganism species. Additionally, AAFCO requires the statement "contains a source of live (viable) naturally occurring microorganisms" as an indication of safety and efficacy.

Although these requirements apply to all such products, there is not universal compliance in the industry. This important point is best illustrated by a recent study in which nineteen commercially available canine and feline diets purporting to contain probiotics were evaluated bacteriologically.[9] Quantitative bacterial cultures were performed on all products and the labeling claim of each product was compared to the qualitative and quantitative culture results. None of the products were found to contain all of the claimed organisms, while one or more of the listed contents were isolated from 10 of 19 (53 percent) products. Eleven (58 percent) diets contained additional, related products, and five (26 percent) diets did not contain any relevant growth. The diets tested contained between 0 and 1.8×105 CFU/g , with the exception of *Enterococcus faecium* in dogs and cats. No dose-response trials appear to have been carried out in people or animals, and the question of what constitutes an effective dose of a probiotic has yet to be defined for most probiotic strains.

Antibiotic resistance

Antibiotic resistance screening has shown that the spontaneous mutation rate to antibiotic resistance among lactobacilli can be as high as 2×10^5, depending on the strain.[10] Several animal isolates of *L. acidophilus* and *L. reuteri* were tested for antibiotic resistance and all 16 *L. reuteri* strains

were resistant to vancomycin and polymyxin B irrespective of their source. Only four of 30 *L. acidophilus* strains were vancomycin-resistant and seven were chloramphenicol-resistant.[11] Antibiotic resistance plasmids from lactobacilli have been detected in a number of studies. Although enterococci are normal inhabitants of the gastrointestinal tract and are widely used as both human and animal probiotics, in vivo conjugative transfer of antibiotic resistance plasmids from *L. reuteri* to *Enterococcus faecalis* has been demonstrated in germ-free mice.[12] In most cases, antibiotic resistance in lactic acid bacteria is not transmissible, but represents an intrinsic species or genus-specific characteristic of the organism. Knowledge of the ability of a proposed probiotic strain to act as a donor of conjugative antibiotic resistance genes is a prudent precaution. Safety of some probiotic strains has been extensively studied. For example, it has been proven that *Enterococcus faecium* SF68 (NCIMB 10415) does not acquire or transmit antibiotic resistance.

Probiotics therapy

One important characteristic of probiotics is their ability to suppress the proliferation and virulence of pathogenic organisms. This characteristic is increasingly well documented in probiotic bacteria in the gastrointestinal tract and genitourinary tract. However, probiotics are also thought to have direct effects on human physiology and immunity, including allergic disease (*e.g.*, asthma, hay fever), autoimmune diseases (*e.g.*, multiple sclerosis and type I diabetes), diseases of the

Criteria for probiotics

A probiotic should:
- be nonpathogenic in nature
- be resistant to destruction by technical processing
- be resistant to destruction by gastric acid and bile
- adhere to intestinal epithelial tissue
- colonize the gastrointestinal tract, if even for a short time
- have a benefit to the host. For example:
  - produce antimicrobial substances
  - modulate immune responses
  - influence gut metabolic activities (*e.g.*, cholesterol assimilation).

oral cavity (*e.g.*, periodontal disease and caries), and the nervous system (*e.g.*, autism and depression).[13] *Table 1* gives an overview of the mechanisms of action of probiotics.

Probiotic therapy in dogs

To date, the study of probiotic efficacy in dogs is still in its infancy, and the small number of studies that have evaluated the effects of probiotics in dogs were designed to screen for potential new activity of new probiotics. In these early studies, most have been focused on the intestinal microflora in apparently healthy dogs. Specifically, probiotic strains of human or canine origin (lactobacilli, bifidobacteria, and enterococci) were used in adult dogs to assess effects on intestinal microbial populations, reduction of specific pathogens in feces, and immunomodulation.[14-20] In many of these studies, the effect of probiotics added to the food in healthy dogs had an equivocal effect

in veterinary medicine

on fecal microflora and pathogens.[18,21] Further, it is important to note that studies designed to determine the potential of a new probiotic were not randomized, controlled trials, and the strains of probiotic varied from study to study, therefore making generalization more difficult to make. In addition, many studies focused on fecal isolation and quantitative cultures of putative pathogenic bacteria such as *Clostridium perfringens*, rather than evaluating more meaningful end points such as shifts in the microbial flora, mucosal immunopathology, and alterations in intestinal integrity. Only two studies addressing the role of probiotics in management of dietary sensitivity and food-responsive diarrhea have been published to date, both with positive results.[15,17] Only one of these studies was a randomized, placebo-controlled clinical trial.[15] The results of that study were clinically positive because all dogs in the study improved after being placed on the elimination diet. However, the results showed no specific changes in the inflammatory cytokine patterns or a specific benefit of the probiotic.[15] The immunomodulatory effects of *E. faecium* SF68 have been studied in dogs. The results of one study showed that the probiotic was associated with increased fecal IgA concentrations and increased vaccine-specific circulating IgG and IgA concentrations.[22] These puppies also had improved fecal quality and decreased variability in fecal quality when compared to the control puppies. An additional study with elderly dogs showed that elderly dogs fed *E. faecium* SF68 maintained higher IgA levels than control dogs.[23] While increased immunoglobulins may sug-

gest enhanced immune response and should signal an enhanced ability to handle new immunological challenges, proving the clinical relevance of increased IgA is difficult to prove without purposely exposing dogs to infectious diseases. Additional studies are warranted in dogs to further assess the immunomodulatory effects of probiotics. Because of the numerous published studies proving beneficial effects of probiotics in people and livestock, tremendous interest has been shown among commercial pet food companies that market probiotics for use in dogs or cats. However, most of the evidence surrounding the use of probiotics in puppies or adult dogs with stress colitis or antibiotic-responsive diarrhea is anecdotal, with no prospective, randomized, placebo-controlled studies in these disorders published to date.

Probiotic therapy in cats

There is also a paucity of published information pertaining to probiotic use in cats, and there are no clinical studies reporting a beneficial effect of probiotic therapy for any feline disease.[24] One study evaluating the effect of dietary supplementation with the probiotic strain of *L. acidophilus* (DSM 13241) administered in 15 healthy adult cats demonstrated the recovery of the probiotic from the feces of the cats in association with a significant reduction in *Clostridium* spp. and *E. faecalis*.[25] However, immunomodulatory effects were reported based on decreased lymphocyte and increased eosinophil populations, and increased activities of phagocytes in the peripheral blood. This study was not a randomized trial and the changes reported in the pop-

ulations of peripheral blood cells cannot be extrapolated into evidence of systemic health benefits. Evaluation of the effect of supplementation with *E. faecium* SF68 on immune function responses following administration of a multivalent vaccine was evaluated in specific pathogen-free kittens.[26] This prospective, randomized, placebo-controlled study resulted in the recovery of *E. faecium* SF68 from the feces of seven of nine cats treated with the probiotic, and a nonsignificant increase in feline her-

> The study of probiotic efficacy in dogs is still in its infancy, and the small number of studies… were designed to screen for potential new activity….

pesvirus-1-specific serum IgG levels. Concentrations of total IgG and IgA in serum were similar between the probiotic and placebo groups and the percentage of CD4+ T lymphocytes was only significantly increased in kittens at 27 weeks (and no other time). Probiotics have also been evaluated in juvenile captive cheetahs, a population with a relatively high incidence of bacterial-associated enteritis. Administration of a species-specific probiotic containing

Lactobacillus Group 2 and *E. faecium* to 27 juvenile cheetahs was associated with a significantly increased body weight in the treatment group, while there was no increase in the control group.[27] In addition, administration of the probiotic was associated with improved fecal quality in the probiotic group. All studies were performed in healthy kittens or cats, and there are no published studies to date evaluating the use of probiotics in cats with gastrointestinal disorders such as bacterial or parasitic-associated diarrhea, food allergy, antibiotic-associated diarrhea, or inflammatory bowel disease. However, despite the paucity of studies of probiotics in cats, clinical use of probiotics in practice for prevention of diarrhea in kittens or cats receiving antibiotics, for kittens or cats undergoing diet changes, and for kittens with parasitic diarrheas is likely to be safe, and in many cases, may be effective for management of diarrhea.

Future considerations

The potential benefits and specific indications for probiotics in dogs and cats have yet to be clearly defined, and our understanding of the nature and diversity of the canine and feline intestinal microflora during health and disease is slowly expanding. The diverse microbial content of the intestinal tract is not adequately reflected by fecal analysis, which has been the predominant sample analyzed to date. The application of genome analysis to the study of the microbial ecology of the gastrointestinal tract should facilitate the identification of major culturable and nonculturable populations, and provide a tool for studying shifts in these populations over time and under different conditions. The completion of prospective, randomized, placebo-controlled studies in dogs and cats that rely on clinically relevant end points that relate to particular physiologic or pathologic conditions is needed to define what role probiotics will have. Probiotics do appear to have a potential role in the prevention and treatment of various gastrointestinal illnesses, but it is likely that benefits achieved are specific to the bacterial species used and to the underlying disease context. Further work will help us better define the appropriate probiotic species and the specific indications for their use.

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Figure 1. Fortiflora for dogs and cats

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A mulitmodal approach to treating canine osteoarthritis
beyond NSAIDs

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Osteoarthritis, the most common orthopedic disease we see in dogs today, is an insidiously progressive disease of diarthrodial joints that can profoundly impact a dog's quality of life. It has been reported that one in five adult dogs experience osteoarthritis.[1]

The disease is characterized clinically by pain, limitation of movement, effusion, and variable degrees of inflammation of the affected joints. Clinical signs may initially be limited to occasional stiffness, difficulty rising, or reluctance to exercise, but as osteoarthritis progresses, the clinical signs of stiffness, lameness, loss of range of motion, and muscle atrophy in the region of the affected joint(s) become easily identifiable. Osteoarthritis causes pain and discomfort to the dog and can be an emotional and financial burden to the owner.

Because there is no cure for osteoarthritis, managing it is crucial and should involve a multimodal process in which practitioners seek to manage pain; maintain or improve range of motion of affected joints; maintain or improve muscle mass; return working and performance dogs to their previous levels; and control the progression of the disease process.

Several factors can complicate the successful management of osteoarthritis and assessment of treatment, including:
- owner compliance
- affordability of treatment plans
- recognition of the location of the affected joints and cause of lameness
- method of assessing success (subjective vs. objective measurements)
- owner perceptions and expectations for the pet.

The initial treatment plan needs to be safe, noninvasive, easy for the owner to manage, and produce visible results. Fortunately, most osteoarthritis patients can be managed with one or more of the following: weight management, physical activity, disease-modifying osteoarthritis drugs (DMOADs), and nonsteroidal anti-inflammatory drugs (NSAIDs). Physical therapy, a developing modality in veterinary medicine, can also be beneficial. This multimodal approach to the osteoarthritis patient will ideally increase pain-free movement, decrease inflammation, decrease stress on joints, and have some chondroprotective attributes.

The normal joint

To understand the effects of osteoarthritis on a pet's mobility and well-being, it is helpful to review the anatomy of a normal joint. Diarthrodial joints are composed of a joint capsule, synovial fluid, articular cartilage, and subchondral bone. The synovial joint has two functions—to facilitate predictable, energy efficient, and pain-free movement, and to support the musculoskeletal system and transmit load. Synovial fluid is an ultrafiltrate of plasma containing the glycosaminoglycan (GAG) hyaluronic acid. The fluid serves as lubrication, has viscoelastic properties, and provides nutrients to the cartilage, as well as removing metabolic waste products from cartilage.

Chondrocytes are the cellular component of articular cartilage and

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Figure 1

Fatty acid	Description	Role in inflammation
Eicosapentae-noic Acid (EPA)	Omega-3 fatty acid; long chain	Anti-inflammatory Converted to PGE_3 which is weak inflammatory (PGE_2 is thought to be 100x more inflammatory than PGE_3)
Docosahexae-noic Acid (DHA)	Omega-3 fatty acid; long chain	Helps reduce inflammation Inhibits production of COX-2 enzymes

are responsible for the synthesis and maintenance of the extracellular matrix (ECM). Chondrocytes are imbedded in the ECM, which is a hydrated proteoglycan gel and fibrous collagen framework. The ECM is composed of primarily type II collagen and proteoglycans with a small percentage of other glycoproteins.[2,3]

Proteoglycans consist of a central protein core to which one or more GAG side chains are attached. Proteoglycans provide the articular cartilage with selective permeability and compressive stiffness, while collagen fibers provide tensile strength. Destruction of proteoglycans or collagen framework inhibits the stress-absorbing capacity of the articular cartilage and contributes to inflammation and further damage of the cartilage.[2]

Osteoarthritis

Understanding the pathophysiology of osteoarthritis is important for selecting appropriate treatment regimes. Osteoarthritis affects not only the cartilage, but also the entire joint structure, including the synovial membrane, subchondral bone, ligaments, and periarticular muscles. In osteoarthritis, the synovium

undergoes inflammatory changes that include synovial hypertrophy and hyperplasia with an increased number of lining cells, and also an infiltration of the sublining tissue with a mixed population of inflammatory cells.[4] Most specialists agree that the synovial inflammation frequently associated with osteoarthritis is secondary to the destruction of cartilage and the release of cartilage breakdown products into the synovial fluid.[4]

Osteoarthritis is biochemically characterized by a reduction of proteoglycan concentration in cartilage, alterations in the size and aggregation of proteoglycans, increased water content of the cartilage, collagen fibril disruption, and an imbalance in synthesis and degradation of matrix macromolecules. The affected cartilage has increased production and concentrations of inflammatory mediators such as: interleukins (IL-1, IL-6 and IL-8), prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α), nitric oxide (NO), and matrix metalloproteinases (MMP-2, MMP-3, MMP-9 and MMP-13). The major catabolic cytokines involved in the destruction of articular cartilage are IL-1 and TNF-α. Both are produced

by synovial cells and chondrocytes. These inflammatory mediators stimulate an increase in MMPs, which degrade GAGs and collagen, thus damaging articular cartilage. They also reduce hyaluronic acid concentrations in synovial fluid leading to less viscous synovial fluid.[2,3]

Cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) enzymes produce eicosanoids, which include: PGE_2, thromboxane A_2 (TXA_2), and leukotriene B_4 (LTB_4). The activity of these enzymes, and resulting eicosanoids, are increased in osteoarthritis and osteoarthritis cartilage spontaneously releases 50 times more PGE_2 compared with normal cartilage.[4,5]

Multimodal treatment

Multimodal treatment objectives are designed to affect the multiple pathways that cause an osteoarthritic dog to have pain and limited function.

Weight management: Obesity is the most common form of nutritional disorder in dogs with an estimated prevalence of 28 percent.[6] International studies have estimated the incidence of obesity in dogs to be 21 to 40 percent.[7,8]

Excess body weight increases stress on weight-bearing joints in which excessive cyclic stress contributes to degradation of articular cartilage and remodeling of subchondral bone. Studies have noted excessive body weight to be a risk factor for osteoarthritis development in people, guinea pigs, mice, and dogs.[9-15]

In one study of dogs with hip osteoarthritis, a weight reduction of 11 percent showed significant improvement in severity of hind limb lameness.[11] Another study reported body weight to be a predisposing

factor in humeral condylar fractures, cranial cruciate ligament rupture, and intervertebral disc disease in cocker spaniels.[16]

A study looking at 48 Labrador retrievers over their lifetime showed that restricted feeding had a profound effect on radiographic signs of hip osteoarthritis.[17] This study showed that clinical signs of osteoarthritis were seen in dogs that did not have their diet restricted, on average, almost two years earlier than in their diet-restricted counterparts.[10]

Being overweight increases the load placed on joints, which increases stress and could possibly hasten the breakdown of cartilage. For example, it is estimated that a force of nearly three to six times one's body weight is exerted across the human knee while walking; therefore, an increase in body weight increases the force placed on the joint by three to six times the amount of weight gained.[18]

Of the nonpharmaceutical, non-nutritional therapies for osteoarthritis, weight reduction is probably the single most efficacious. The weight reduction protocol needs to be tailored toward the individual patient. Total calorie restriction can successfully lead to weight loss, but has the potential, depending on the diet, to cause excessive protein (and thus lean body mass) loss.[12] One study compared high-protein and low-fiber diet to a commercial weight reduction diet that was lower in protein and higher in fiber.[19] Both diets worked in reducing the overall weight of the dog but the diet with a higher protein minimized the amount of lean tissue lost.[19] Therefore, I recommend using purpose-formulated diets or weight-reduction diets that,

when overall calories are decreased, still provide appropriate protein levels and micronutrients.

Weight-loss programs that encourage the owner and the dog to visit the clinic need to be established. Veterinarians need to scrutinize the dog's weight frequently and follow through with the owner to help ensure that the pet reaches its ideal weight.

Physical activity and physical therapy: Exercise is an effective intervention in osteoarthritis and important in its prevention. Dogs need muscular balance and well-conditioned muscles to attenuate impact loads, provide joint stability, and to support overall function of the body. Several human studies have shown the benefits of exercise for people with osteoarthritis.[20] Regular activity replenishes lubrication to the cartilage of the joint and reduces stiffness and pain. The goals of an exercise program for the canine patient with arthritis are to:

• preserve or restore range of motion and flexibility of the muscles around affected joints

•increase muscle strength and endurance; and

•increase overall conditioning to decrease health risks associated with a sedentary lifestyle.

In veterinary medicine today we have Certified Canine Rehabilitation Therapists who design and perform exercises that are specifically designed for the particular dog and orthopedic problem(s). Modalities used such as underwater treadmills, hydrotherapy, and electrical stimulation are just a few modalities that will improve muscle range of motion and stability.

Disease-modifying osteoarthritis drugs: DMOADs have become an appealing primary or adjunct treatment for osteoarthritis in both people and dogs. These substances modify the course of osteoarthritis by improving the health of articular cartilage or synovial fluid.

Many studies have explored the effects of DMOADs in people.[22,23] In these types of studies, a significant difference compared to placebo needs to be demonstrated and benefits are measured by three co-primary end-points—joint space narrowing (JSN), pain, and function.

> The weight reduction protocol needs to be tailored toward the individual patient.

In canine patients, these parameters are difficult to ascertain. We can't evaluate joint space accurately in dogs because our patients are not weight-bearing during radiographs. Owners usually provide us with our patients' relative pain and function scores. Objective measurements such as force plate analysis and kinematics would be ideal; however, subjective assessments by owners and veterinarians are the most common method for evaluating treatment modalities for osteoarthritis. Therefore, when choosing

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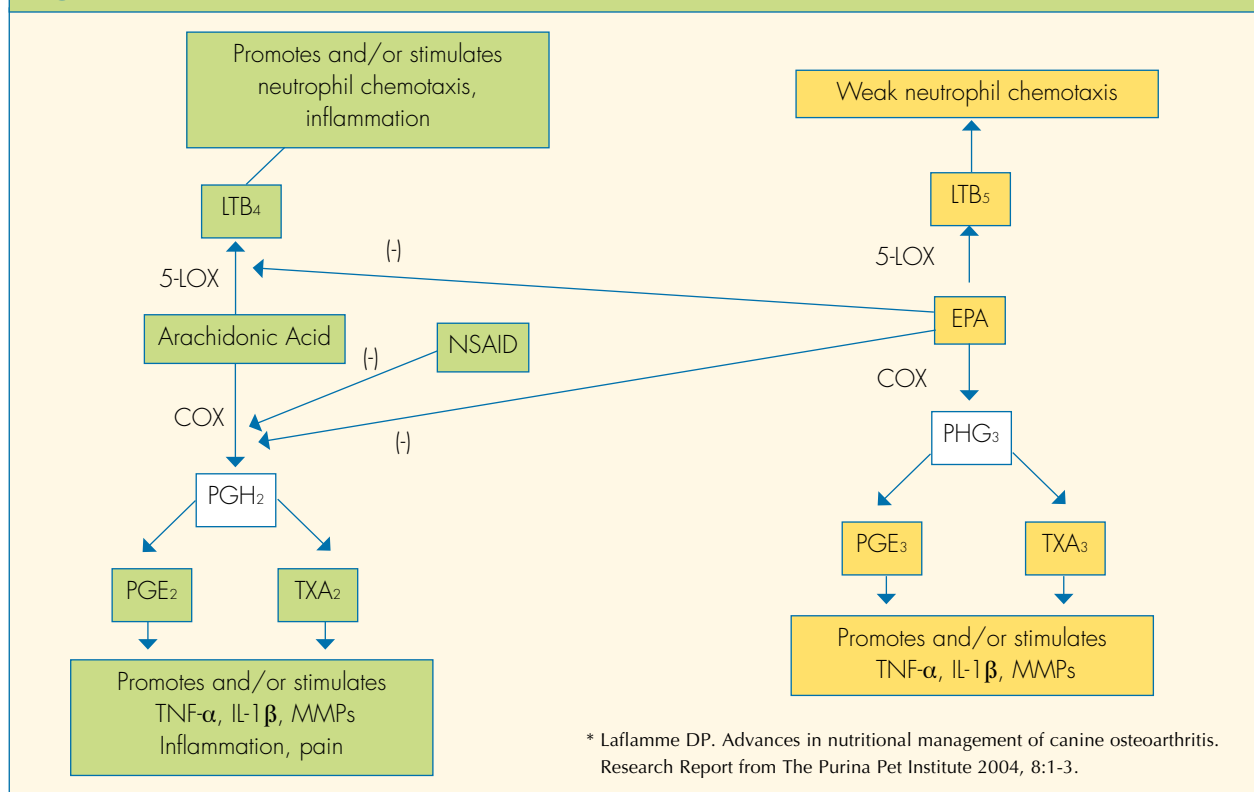
a DMOAD, it is important to rely on evidence-based medicine as a guide to efficacy.

Some common DMOADs used in veterinary medicine include polysulfated GAGs (glucosamine, chondroitin sulfate, or a combination of the two) omega-3 fatty acids, and methyl-sulfonyl-methane (MSM). For this article we will discuss glucosamine, chondroitin sulfate and fatty acid DMOADs.

Glucosamine:
Glucosamine is an amino sugar that is a precursor to GAGs that are present in the ECM of articular cartilage. Osteoarthritic cartilage appears to have a decreased ability to synthesize glucosamine compared to healthy cartilage. Glucosamine salt supplements are readily available

and are most commonly found as glucosamine hydrochloride or glucosamine sulfate. The hydrochloride form is most commonly used in published studies and was used in one of the largest studies in people.[21]

Chondroitin sulfate (CS):
CS is the predominant GAG found within the ECM. CS has been found to decrease IL-1 production, inhibit MMPs, and stimulate GAG and collagen synthesis.[24] However, the form and source of chondroitin sulfate influences its pharmacokinetic profile.[3] Chondroitin-4-sulfate, which is sulfated on the fourth carbon of the N-acetylgalactosamine residue, is derived primarily from mammalian tissue. Chondroitin-6-sulfate, which is sulfated on the sixth carbon, is derived primarily from shark carti-

lage. These two types of chondroitin sulfate may vary greatly in molecular weight, which can potentially influence their bioavailability and purity.[25] Pure low-molecular weight chondroitin sulfate displays significant accumulation upon multiple dosing in beagle dogs.[26]

When used together, the effects of glucosamine and chondroitin sulfate combine to:
- stimulate the metabolism of chondrocytes and synoviocytes
- inhibit degradative enzymes
- reduce fibrin thrombi in periarticular microvasculature.

Extrapolation from some animal and human trials suggests that there can be some benefit from these compounds.[3,25,27] Because they are relatively harmless and potentially hold a

promise of protecting articular cartilage and relieving clinical signs, they can be a valuable additional treatment modality for dogs with osteoarthritis.

Omega fatty acids: Polyunsaturated fatty acids (PUFAs) are classified as omega-3, omega-6, or omega-9, depending on the position of the last double bond along the fatty acid chain. The main dietary PUFAs are omega-3s [*e.g.,* linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)] (*Figure 1*) and omega-6s (*e.g.,* linoleic acid and arachidonic acid)]. Omega-3 and omega-6 fatty acids are metabolized by the COX and 5-LOX pathways into distinct eicosanoids (*Figure 2*). The omega-6-derived eicosanoids—PGE_2, TXA_2, and LTB_4—tend to be proinflammatory; whereas the omega-3-derived eicosanoids tend to be anti-inflammatory. A study using long-chain omega-3 PUFAs showed improved biochemical parameters in associated with canine osteoarthritis.[28]

Since the omega-3 PUFAs EPA and DHA act as competitive inhibitors of the conversion of arachidonic acid to proinflammatory eicosanoids, dietary habits may have a considerable influence on an individual's propensity to become and remain inflamed. Long-chain omega-3 fatty acids are available in fish and plant sources and commercially available as nutraceutical supplements and veterinary diets, such as Purina Veterinary Diets JM Joint Mobility Canine Formula.

NSAIDs: NSAIDs are designed to reduce proinflammatory mediators, such as prostaglandins, by inhibiting cyclooxygenase 1 and 2 (COX-1 and COX-2). The six registered NSAIDs for the treatment of canine osteoarthritis commonly used in veterinary medicine were developed to preferentially inhibit COX-2.[23] NSAIDs are the most widely used analgesics in veterinary medicine, and all have some toxic potential. The most common adverse effects of NSAIDs are gastrointestinal, renal, hepatic, and coagulation disorders. The effectiveness of using NSAIDs in managing osteoarthritis should be balanced with their potential to cause side effects, and they should be titrated to their lowest effective dosage. The effectiveness of NSAIDs can be enhanced by physical therapy, use of DMOADs, and diet and exercise to control weight. With a multimodal treatment regime, one may even be able to exclude the use of NSAIDs.

Since osteoarthritis is a chronic disease, it may be the perfect paradigm of a pathology where multimodal management is better positioned to provide long-term benefits. For example, a nutritional compound may have significant benefits that are reached gradually; however, many nutritional compounds have the benefit of containing active compounds that target multiple pathways. Pharmacologic interventions often have monomodal modes of action, which may explain why they can fail to completely control the clinical signs of osteoarthritis.[29] Therefore, multimodal management could provide a welcome alternative to pharmacologic therapy for osteoarthritis.

Implementing multimodal management

Canine osteoarthritis is an enormous subject to discuss, and many points regarding its pathophysiology and treatment are still being debated. Multiple pathways contribute to the clinical signs of canine osteoarthritis, and it is important to understand that there is no cure or silver bullet for treatment. In order to be most effective, after identifying and localizing osteoarthritis in our canine patients, we must address it on many fronts, including:

- weight management (by using targeted diet modification and moderate low-impact activities)
- use of DMOADs
- use of physical therapy to aid in increasing range of motion and muscle mass
- limitation of NSAID usage to treating acute flare ups (*e.g.,* weekend-warrior syndrome).

The plan must be customized for each patient affected by osteoarthritis, depending on the joints affected and the severity of symptoms.

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