

Special topic: The association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation¹

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ABSTRACT: In July 2018, the Food and Drug Administration warned about a possible relationship between dilated cardiomyopathy (DCM) in dogs and the consumption of dog food formulated with potatoes and pulse ingredients. This issue may impede utilization of pulse ingredients in dog food or consideration of alternative proteins. Pulse ingredients have been used in the pet food industry for over 2 decades and represent a valuable source of protein to compliment animal-based ingredients. Moreover, individual ingredients used in commercial foods do not represent the final nutrient concentration of the complete diet. Thus, nutritionists formulating dog food must balance complementary ingredients to fulfill the animal's nutrient needs in the final diet. There are multiple factors that should be considered, including differences in nutrient digestibility and overall bioavailability, the fermentability and quantity of fiber, and interactions among food constituents that can increase the risk of DCM development.

Taurine is a dispensable amino acid that has been linked to DCM in dogs. As such, adequate supply of taurine and/or precursors for taurine synthesis plays an important role in preventing DCM. However, requirements of amino acids in dogs are not well investigated and are presented in total dietary content basis which does not account for bioavailability or digestibility. Similarly, any nutrient (e.g., soluble and fermentable fiber) or physiological condition (e.g., size of the dog, sex, and age) that increases the requirement for taurine will also augment the possibility for DCM development. Dog food formulators should have a deep knowledge of processing methodologies and nutrient interactions beyond meeting the Association of American Feed Control Officials nutrient profiles and should not carelessly follow unsubstantiated market trends. Vegetable ingredients, including pulses, are nutritious and can be used in combination with complementary ingredients to meet the nutritional needs of the dog.

Key words: dilated cardiomyopathy, dogs, feed formulation, grain-free, nutrition, pulse ingredients

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INTRODUCTION

In July 2018, the Food and Drug Administration (FDA) issued a statement relating dilated cardiomyopathy (DCM) in dogs to the consumption of foods that have potatoes and/or pulse ingredients, such as peas and lentils or their coproducts, as main ingredients (FDA, 2018). The FDA's statement, as well as media attention, has raised concern in some pet owners, veterinarians, nutritionists, and the pet food manufacturing and retail industry. The underlying cause for concern with pet food and DCM is that there is a link between nutrition that was previously tied to DCM and insufficient circulating taurine (Fascetti et al., 2003; Backus et al., 2006). The result was an increased need for dietary taurine or its precursor methionine due to higher fermentation of taurine and greater fecal excretion with dietary fermentable fiber (Kim et al., 1996a, 1996b). Whether this has any link to dietary pulses or the greater inclusion of pulses in grain-free dog food has yet to be directly demonstrated and mechanistic research is warranted.

Pulses are a subset of legumes, harvested as a dry crop, with low concentrations of lipid. They include peas, lentils, chickpeas, and dry beans (Marinangeli et al. 2017) which have been used as ingredients in dog food for their protein and fiber for more than 2 decades (Butterwick et al., 1994; Rice and Ihle, 1994). As a source of protein, the amino acid (AA) profile in peas, lentils, chickpeas, and beans is generally high in lysine and low in methionine (NRC, 2006) and serves as a complementary protein to both animal and plant-derived ingredients. As an example, soybean meal is derived from defatted soybeans and has an AA profile similar to pulses. In a 24-wk study that evaluated graded concentrations of soybean meal up to 17% (as-fed basis) in dog foods, soybean meal inclusion did not affect the nutrient status of dogs as indicated by serum biochemistry analysis (Menniti et al., 2014). However, Yamka et al. (2003) demonstrated that using soybean meal at more than 15% inclusion on a dry matter basis decreased crude protein digestibility. Based on the authors' assessment of current formulas in the market, there is a high likelihood that legume seed use in some foods may be greater

than 40%. This inclusion exceeds concentration of legumes previously investigated in dogs. When used to complement the nutritional profile of other ingredients, pulses can be used as nutrient-rich vehicles to meet the nutritional requirements of dogs and other companion animals. Given that companion animals most often consume static diets for long periods of time, overuse of any ingredient could facilitate higher risk of certain nutrient deficiencies if nutrient balance is not considered in the formulation. Thus, the formulation of static diets that use significant concentrations of a single ingredient, relative to other ingredients in the formulation, requires an in-depth knowledge of nutrient interactions, animal physiology, and effects of processing, beyond that of simply meeting minimum nutrient profiles stipulated in the Official Publication of The Association of American Feed Control Officials (AAFCO, 2018).

The present commentary discusses the following: 1) The limited data being used to support linkages between DCM and pulse ingredients; 2) The nutritional factors and physiological mechanisms that should be explored to establish causation between nutritional deficiencies and incidence of DCM; 3) The factors that nutritionists should consider when formulating complete diets destined for long-term consumption; and 4) The disadvantages of formulating protein and minimal AA recommendations rather than a balanced indispensable AA profile.

The Development of Canine DCM, Historical Linkages to Taurine Deficiency, and Pulses

Dilated cardiomyopathy is a disease of the myocardium that results in both mechanical dysfunction (enlarged heart cavities and congestion) and/or electrical dysfunction (arrhythmias and sudden death) (Sisson et al., 2000; Maron et al., 2006; Dutton and López-Alvarez, 2018). Development of DCM is slow and few clinical signs manifest over time. As DCM progresses, signs include lethargy, anorexia, shallow breathing, sudden fainting, and potential death. In some cases, animals may die from irregular heart rhythm without previous signs of the disease. In dogs, DCM can be

caused by various factors. Genetic predisposition is thought to play the most important role in the development of DCM in several dog breeds, mostly large and giant breeds. Genetic mutations associated with DCM have been discovered in American lines of Doberman and Boxer dogs (Meurs et al., 2012; Meurs et al., 2013). However, the Doberman variant's association was not upheld in a European population of Dobermans (Owczarek-Lipska et al., 2013). Similarly, a United Kingdom population of Boxers did not uphold their published DCM-associated variant (Cattanach et al., 2015). It is becoming increasingly clear that the genetic basis for DCM in dogs is not monogenic, but complex and polygenic. Breeds with the highest prevalence of DCM include Dobermans, Boxers, Great Danes, Newfoundlands, Irish Wolfhounds, English Cocker Spaniels, and Portuguese Water Dogs (Monnet et al., 1995; Borgarelli et al., 2006; Werner et al., 2008; Martin et al., 2009), and the genetic basis of DCM in each of these breeds has been investigated (Dutton and López-Alvarez, 2018). In addition, Golden Retrievers and American Cocker Spaniels appear to have breed predispositions to taurine deficiency (Kramer et al., 1995; Bélanger et al., 2005). When dogs are not genetically predisposed for developing DCM, diet and physiology are other factors that may be associated with the disease.

The first link between taurine deficiency and DCM was demonstrated in cats in 1987. Cats diagnosed with DCM recovered after taurine supplementation (Pion et al., 1987). Similarly, an inverse association between dietary taurine and the incidence of DCM in a population of foxes was documented by Moise et al. (1991) and

established the importance of taurine in the family Canidae. In dogs, DCM diagnoses related to low whole blood taurine concentrations have been reported in Cocker Spaniels, Dalmatians, Boxers, Newfoundlands, Portuguese Water Dogs, English Setters, Alaskan Malamutes, and Scottish Terriers (Freeman et al., 1996; Kittleson et al., 1997; Pion et al., 1998; Alroy et al., 2000; Fascetti et al., 2003; Backus et al., 2006). In all these cases, taurine supplementation improved cardiac function. However, dogs, in contrast to cats, can endogenously synthesize taurine from methionine and cysteine (Figure 1). Therefore, the above-mentioned data do not unequivocally establish taurine intake as the underlying mechanism for the development of DCM in dogs, whether they are genetically predisposed. Dietary supply of precursor AAs necessary for taurine synthesis (i.e., methionine and cysteine), metabolic intermediates, and cofactors (such as methyl donors) cannot be ruled out as factors that contribute to the susceptibility of dogs to developing genetic and diet-related DCM. When DCM is diet-related, the formulation and the provision of all nutrients, including indispensable AAs, to facilitate optimum health and wellbeing of dogs should be considered.

Recent reports, including the statement by the FDA (2018), have implicated that lentils, peas, and other legumes seeds could be responsible for the development of DCM in dogs not genetically predisposed to this disease. Such statements and associations between pulse ingredients and incidence of DCM are, at the present time, premature. Animals, including dogs, have no minimum or maximum requirements for ingredients. Ingredients serve

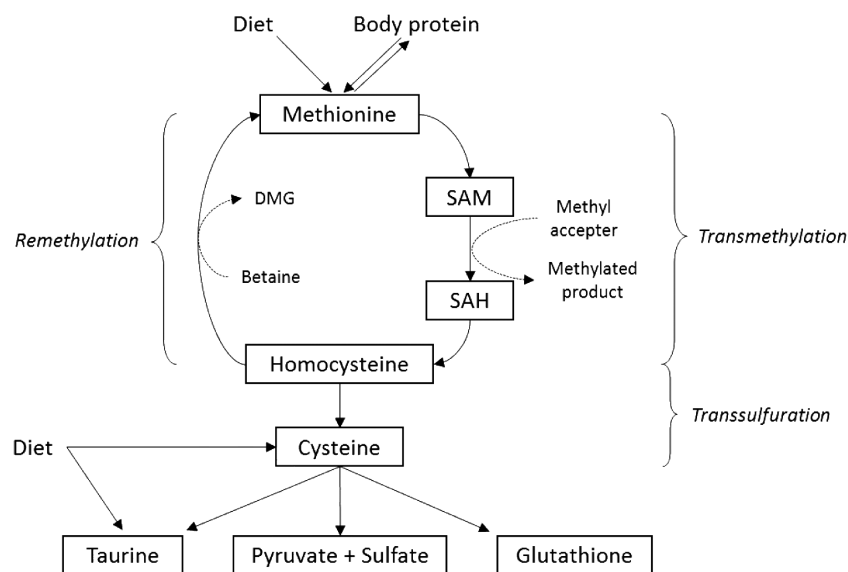


Figure 1. Metabolism of sulfur amino acids. DMG = dimethylglycine; SAH = S-adenosylhomocysteine; SAM = S-adenosylmethionine.

as the vehicle to providing nutrients to animals. As such, animals have nutrient requirements, not ingredient requirements. In diets that have nutrient deficits, imbalances, or exceed maximums, the final nutrient composition of the diet, not the ingredients, should be critiqued. In addition, animal nutritionists should consider that the nutrient concentration of ingredients can vary, nutrient availability is not 100%, and diets formulated to marginally meet requirements could actually be deficient. Overall, it is the responsibility of nutritionists to use different ingredients to formulate diets that can be produced and safely meet the nutritional needs of animals.

Taurine Deficiency and the Development of Canine DCM

For dogs, taurine is a dispensable AA synthesized from methionine and cysteine primarily in the liver (Figure 1). Taurine is not incorporated into proteins. Instead, it is used as a mediator for various biological processes and is the most abundant free AA intracellularly (Huxtable, 1992). In the heart, taurine represents ~60% of the total AA free pool (Huxtable, 1992). The high concentration of taurine in cardiac cells may explain the role of a taurine deficiency in the development of DCM. It has been speculated that taurine contributes to the reabsorption of calcium by the sarcoplasmic reticulum and increases the sensitivity of the myofilaments to calcium (Bakker and Berg, 2002). Thus, low dietary taurine intake and/or reduced synthesis of taurine from methionine and cysteine can deplete calcium pools in the cardiac cells and impede proper contraction of the cardiac muscle tissue, resulting in DCM in dogs.

For diagnosing DCM in dogs and cats, among other diagnostic methods including electrocardiograms and echocardiography, it is common to measure taurine concentration in whole blood. Whole blood samples, and not plasma samples, should be used to assess circulating taurine concentrations. In plasma, free taurine concentrations are much lower compared with intracellular taurine. This suggests that the plasma pool is not representative of taurine in other pools (Schaffer et al., 2010). In platelets, taurine concentration is high and is considered a marker of taurine status. Taurine concentration in platelets is captured when whole blood is analyzed (Huxtable, 1992). However, platelet count can vary depending on the immune status of the animal and whole blood taurine concentration can be affected. In this scenario, whole blood taurine may not represent concentrations of

taurine in muscle cells, including cardiac muscle. These additional variables related to the measurement of taurine status may explain why some dogs diagnosed with DCM have normal whole blood taurine concentrations.

As taurine can be synthesized endogenously in dogs, taurine is not considered an indispensable AA for the species Canidae. Thus, there are no recommendations on minimum dietary concentrations of taurine for dogs reported by the National Research Council (NRC, 2006) or AAFCO (2018). The lack of regulation on minimum taurine concentrations in commercial dog foods suggests that endogenous synthesis of taurine can meet the metabolic needs in all dogs and at all life stages. This assumption may not be accurate as studies have determined that synthesis of taurine is related to the size of dog (Ko et al., 2007), and some dietary factors can increase the physiological need for taurine (Story, 1978). Nutritional factors that increase the dietary requirement, reduce the supply, or increase the excretion of taurine in dogs are discussed in subsequent sections of this review and should be considered to avoid taurine deficiency in dogs and the risk of DCM.

Physiological factors can increase taurine utilization in dogs, and endogenous synthesis of taurine could be insufficient for meeting taurine requirements. For example, compared with smaller size dogs, synthesis of taurine in large dog breeds is up to 50% lower per unit of metabolic body weight (Ko et al., 2007). These results demonstrate that larger dogs are at higher risk for insufficient endogenous taurine synthesis, and dietary supplementation or fortification may be required, even when there is no minimum dietary taurine concentration according to current recommendations (AAFCO, 2018). Obesity and diabetes have also been related to lower concentrations of taurine in blood in humans and rats, respectively (Merheb et al., 2007; Nardelli et al., 2011; Ito et al., 2012), and may increase the requirement for sulfur AAs necessary for endogenous taurine synthesis. This is of importance given that approximately half of dogs in North America are obese (Linder and Mueller, 2014). Data from rats and cats suggest that age and sex could also affect whole body taurine status. Hepatic activity of cysteine sulfonate decarboxylase, the enzyme responsible for taurine synthesis, was shown to be 16 times higher in adult male rats vs. female rats. In the same study, the activity of cysteine sulfonate decarboxylase was higher in 5- to 6-wk-old kittens compared with 15-mo-old cats and in 8-wk-old mice compared with 16-wk-old mice; changes of

the enzyme activity in dogs have not been tested (Worden and Stipanuk, 1985). Overall, these studies suggest that, despite some capacity for endogenous synthesis, physiological need of taurine can be heavily dependent on breed, age, sex, and physiological status. These physiological factors could help us to predict the risk for developing DCM when genotypic and environmental factors, such as diet, are simultaneously considered to ensure that dogs maintain adequate concentrations of taurine and other sulfur AAs.

Given that there are no recommendations for the minimum concentration of taurine in dog food, the concentration of taurine in dog foods can vary substantially depending on the ingredients used. Taurine is very low in plant-based ingredients (Table 1) but is higher in some algae and fungi species and is ubiquitously found in animal tissues, especially in the heart, brain, and white blood cells (Huxtable, 1992). This is relevant, as many grain-free and/or high legume dog foods attempt to limit the use of animal byproducts, which can substantially decrease the levels of dietary taurine. In the context of providing adequate and preventive nutrition, dog foods should include organ meat

or animal byproducts or be fortified with taurine and/or its precursors (methionine and/or cysteine) to ensure the delivery of sufficient levels of taurine.

Effect of Dietary Fiber on Taurine Status and Risk of Canine DCM

Dietary fiber has been shown to affect the taurine status in dogs. For example, commercial diets formulated with lamb meal and rice bran were shown to cause taurine deficiency in part because of low bioavailable cysteine from lamb meal and possibly more importantly due to the effects of rice bran fiber on gastrointestinal metabolism of taurine (Johnson et al., 1998; Tôrres et al., 2003). It has been hypothesized that high-fiber diets can increase susceptibility to taurine deficiency by 2 mechanisms of action linked to obligatory bile acid conjugation with taurine in dogs (O'Máidille et al., 1965) and reliance on enterohepatic circulation for the reabsorption of bile acids and taurine. First, high-fiber diets may increase fecal output and losses of taurine-conjugated bile. This would require higher synthesis rates of bile in the liver, and consequently, higher utilization of taurine

Table 1. Crude protein (CP), fiber, selected amino acids, and carnitine contents in the principal legumes, cereals, and animal-derived ingredients used in dog food formulation

Ingredients	CP, %	Crude fiber, ¹ %	α -amino acids, mg/g protein ¹			Tau, mg/kg ²	Carnitine, mg/kg ³
			Lys	Met	Cys		
Legumes	Fava beans	27.2	8.55	23.9	7.0	12.5	–
	Phaseolus beans	22.9	NR	72.9	12.7	12.7	–
	Kidney beans	20.0	6.40	26.5	14.0	12.0	–
	Lentils	26.0	NR	65.8	6.9	10.4	–
	Lupins	32.4	14.25	48.7	6.5	14.2	–
	Chick peas	20.3	6.16	69.4	14.8	21.6	–
	Soybean meal	47.7	3.89	62.0	13.8	14.7	–
Grains	Barley	11.3	3.90	35.3	17.7	22.9	–
	Corn, yellow dent	8.2	1.98	30.3	21.8	23.1	–
	Oats	11.2	2.20	43.9	60.9	32.3	–
	Rice	7.9	0.52	44.5	31.8	22.9	–
	Rye	11.7	2.71	36.9	13.7	16.3	–
	Sorghum	9.4	2.14	21.4	17.1	19.2	–
	Wheat hard, red	14.5	2.57	27.0	15.2	22.8	–
Animal-derived ingredients	Beef, meat	15.0	–	77.3	28.7	15.3	296
	Chicken, meat and skin	17.6	–	81.3	26.7	13.1	159
	Chicken, by product	59.0	–	48.1	17.3	16.8	3049
	Lamb, ground	16.6	–	88.0	25.9	12.0	473
	Rendered meat	54.1	2.50	53.8	14.2	11.3	NR

Cys = cysteine; Lys = lysine; Met = methionine; NR = not reported; Tau = taurine.

Values are presented on as-fed basis.

¹NRC, 2006; NRC, 2012.

²Spitze et al. 2003.

³Arslan, 2006.

(Story, 1978). Second, high consumption of fermentable fibers may increase the abundance of microbial populations that degrade taurine in the intestinal lumen (Kim et al., 1996a, 1996b). Either alone or together, increased excretion or degradation of taurine from high-fiber diets may decrease enterohepatic circulation and recycling of taurine. Given that taurine is the only AA used for bile acid conjugation in dogs, over time, high-fiber diets could increase the risk of taurine insufficiency in dogs and lead to DCM.

This should not be interpreted as dietary fiber being deleterious to the health of dogs. However, there may be a limit to the benefit for soluble fibers. Legume seeds contain an appreciable quantity of oligosaccharides which are known to be fermentable (Tosh and Yada, 2010). Thus, by a similar mechanism as described above, high levels of legume seed oligosaccharides could ostensibly contribute to taurine depletion via excretion in the feces as bile conjugation and degradation by colonic bacteria. In addition to the physiological benefits of high-fiber diets in certain dogs, formulators should also be cognizant of possible nutritional risks associated with high concentrations of fiber in dog foods. Consequently, dog foods with high concentrations of dietary fiber should be accompanied by higher supplies of taurine or sulfur AAs for endogenous taurine synthesis. Overall, the digestibility and bioavailability of taurine in ingredients used and the effect of other nutrients in taurine metabolism should be considered to avoid taurine deficiency and the development of DCM.

Carnitine Deficiency and Risk of Canine DCM

Carnitine is not nutritionally indispensable since it is endogenously produced in the liver and kidneys from lysine and methionine; it can also be attained exogenously from animal-based products. Carnitine is highly abundant in skeletal and cardiac muscles. Together, these represent >95% of the total carnitine in the body. Carnitine is essential for metabolism of fatty acids used for energy production (Hoppel, 2003). In the heart, where 60% of the energy is derived from fatty acid oxidation, carnitine facilitates the uptake of free fatty acids into the mitochondria to produce ATP (Hoppel, 2003). Plant-based ingredients do not contain carnitine (Table 1). Therefore, in commercial dog foods with reduced inclusion of animal-based ingredients, intakes of carnitine could be decreased if diets are not fortified. Reduced dietary carnitine intake

translates into increased reliance on endogenous synthesis to meet physiological requirements.

Given that carnitine is required for sufficient energy production in cardiac muscle, it is not surprising that carnitine deficiency is associated with DCM. In 1991, a family of Boxers diagnosed with DCM were also diagnosed with carnitine deficiency (Keene et al., 1991). In dogs, carnitine deficiency can occur with aberrations of carnitine regulation in disorders such as cardiomyopathy (including DCM), diabetes, sepsis, and malnutrition (Flanagan et al., 2010). However, carnitine deficiency as a causative factor in the development of DCM or a consequence of cardiac malfunction remains as a subject of debate (Freeman and Rush, 2006). Despite the interest in this metabolite, little progress has been made on determining the effect of carnitine supplementation on alleviating risk of DCM. However, both taurine and carnitine are often supplemented in suprphysiological concentrations once DCM is diagnosed. This practice is supported by positive clinical outcomes, albeit without comparison groups (Kittleson et al., 1997; Sanderson et al., 2001). Concentrations of carnitine in the plasma are relatively insensitive to dietary carnitine, and more invasive techniques (biopsies) are required to determine the concentration of carnitine in muscle tissue (Flanagan et al., 2010; Rășanu et al., 2012). The invasive nature of testing for carnitine status is likely the reason why carnitine is rarely explored when investigating possible causes of canine DCM.

Preventing Diet-Mediated DCM in Dogs by Providing Adequate Sulfur AAs and Maximizing Endogenous Taurine Synthesis

Although taurine is considered a dispensable AA in dogs, endogenous taurine synthesis requires an adequate supply of bioavailable sulfur AA precursors cysteine or methionine (Figure 1). Thus, providing marginal concentrations of these 2 sulfur AAs, or providing sources with lower bioavailability, could increase the risk of taurine deficiency and facilitate the development of DCM. Contrary to taurine, methionine cannot be synthesized endogenously in dogs (NRC, 2006). Therefore, dogs depend on the provision of dietary methionine to meet daily sulfur AA requirements, which includes production of taurine. From an ingredient perspective, methionine and lysine are usually the first or second limiting AAs in dog diets formulated with soybean meal and rendered meats (NRC, 2006). In addition, methionine is particularly susceptible to damage, and subsequent reduction in bioavailability,

secondary to heat processing (Marshall et al. 1982; Hurrell et al., 1983). This suggests that the risk of methionine deficiency is more likely than any other indispensable AA in commercial dog diets. Although the primary role for methionine is protein synthesis, in pigs at least 50% of absorbed methionine acts as a methyl donor and a precursor in the production of cysteine, taurine, sulfate, and pyruvate (Robinson et al., 2016a; Figure 1). These functions of methionine become more crucial when dietary intake of cysteine, taurine, and/or dietary methyl donors (e.g., folate, betaine, and their precursors) is limited (Robinson et al., 2016b), and they need to be considered when nutritionists set criteria for delivery of sulfur AAs in pet foods.

Methionine and cysteine both contribute to the total sulfur AA requirements for humans and animals. For adult dogs at maintenance, the latest guidelines from the NRC (2006) recommend that adult dog foods contain 0.33% (on dry matter basis) methionine when cysteine is provided in excess, and 0.65% for methionine + cysteine. These NRC (2006) recommendations are not based on dose–response studies, but on a 4-yr study where adult dogs were fed low-crude protein diets (Sanderson et al., 2001). In that study, the lowest concentration of methionine in the diet that reported no observable deficiencies was used as the recommended requirement. As companion animals are typically fed a single static diet during adulthood, and for most of their lifespan, it is necessary that AA requirements of dogs should be measured empirically (Baker, 1986). In addition to the lack of empirical data corresponding to the AA requirements of dogs, it is equally important to understand how other dietary (e.g., dietary fiber), environmental, other physiological variables, and breed/genotype may alter AA requirements. The lack of recommendations for taurine in commercial dog food puts a higher stress on accurately meeting requirements for sulfur AAs, not only for protein synthesis, but also for the endogenous synthesis of taurine, for support of optimal methyl status, and for the synthesis of secondary metabolites.

Rethinking Indispensable AA Targets in Commercial Dog Foods

Currently, the ingredients permitted in pet foods and the corresponding nutrient targets are guided by recommendations made by AAFCO (2018). These recommendations are based on the peer-reviewed scientific literature and represented in the Nutrient Requirement of Dogs and Cats

(NRC, 2006). However, AA recommendations made by AAFCO correspond to total AA content within the formulation and do not consider the true ileal digestibility of ingredients. True ileal digestibility of AAs is more representative of nutrient absorption capacity and bioavailability compared with fecal digestibility or total AA content in the diet (Columbus and de Lange, 2012). To account for the reduced digestibility and bioavailability of protein-bound AAs in food ingredients, AAFCO arbitrarily increases AA recommendations relative to those from the NRC to ensure that an adequate supply of AAs is provided, regardless of the ingredients and effects of processing (Table 2). However, this increment is only applied to lysine, threonine, and tryptophan and not applied to other indispensable AAs, including methionine (AAFCO, 2018). For example, the recommended allowance for lysine reported in NRC (2006) is 0.35% for adult dogs at maintenance, whereas the minimum content of lysine to meet AAFCO (2018) recommendations is 0.63%. Nonruminant animals, including dogs, absorb AAs from the duodenum to the terminal ileum (Columbus and de Lange, 2012). Hence, feeding diets with lower ileal digestibility coefficients could decrease actual concentrations of available indispensable AAs, even when meeting AAFCO recommendations. This is of special concern for dietary taurine and other sulfur AAs, considering that there is no regulated minimum threshold for taurine in dog foods and that AAFCO (2018) recommendations for sulfur AAs are not increased compared with NRC (2006) recommendations to account for potential ileal digestibility coefficients. There is a dearth of data in this area to justify empirical adjustments based on different dietary variables. As such, future research should pursue how AA requirements change under different dietary variables that can affect small intestinal digestibility and whole body availability.

It is worthwhile to note that minimum dietary nutrient contents for dog foods, as reported in AAFCO (2018), only consider differences between growth/reproduction and adult life stages. This lack of data places the pregnant bitch in the same group as growing animals. Moreover, most studies on nutrient requirements in dogs have been established using Beagles as a proxy for all dogs. Using a single breed creates a homogenous sample and likely does not account for nutritional variability across pure and mixed breeds, or those of different sizes. Unpublished data from Shoveller et al. investigated the minimum methionine (with excess

Table 2. Recommended allowance (RA) and minimum dietary content suggested by AAFCO for crude protein and essential amino acids in dog food, and their physiological roles and potential interactions

Nutrient	NRC RA ¹ , % DM	AAFCO ² , % DM	Important physiological roles and potential interactions
Crude protein	10	18	Necessary for synthesis of nonessential amino acids
Arginine	0.35	–	Competes with lysine absorption, arginine should be increased when high lysine concentrations in the diet
Histidine	0.19	–	
Lysine	0.35	0.63	Highly reactive to reducing sugars during heating (Maillard reaction), reducing bioavailability
Methionine	0.33	0.33	Requirement increases when methyl donors/acceptors and cysteine are reduced in the diet
Methionine + cystine	0.65	0.65	Requirement is increased with low supply of taurine and during immune challenge
Phenylalanine	0.45	0.45	
Phenylalanine + tyrosine	0.74	0.74	
Threonine	0.43	0.48	Abundant in mucosal proteins (mucin), requirement increases when feeding high fermentable fibers
Tryptophan	0.14	0.16	Precursor for serotonin synthesis. Ratio of Trp: LNAA should be considered; lower ratios may deprive appetite
Valine	0.49	0.49	Abnormal Increment of valine, leucine, or isoleucine (BCAA) will cause catabolism of the other BCAA in the muscle
Isoleucine	0.38	–	
Leucine	0.68	0.68	

AAFCO = The Association of American Feed Control Officials; BCAA = branched chain amino acids; DM = dry matter; NRC = National Research Council; RA = recommended allowance; Trp:LNAA = tryptophan to large neutral amino acid ratio.

¹Recommended Allowance requirements for adult dogs at maintenance, Nutrient Requirements of Dogs and Cats (NRC, 2006).

²Minimum dietary content, AAFCO (2018).

cysteine) requirements of Miniature Dachshunds, Beagles, and Labrador Retrievers as proxies for small, medium, and large dog breeds and found that methionine requirements may differ across breeds or size of dogs and be greater than previously estimated. Thus, given the methods of derivation, single indispensable AA requirements for all dog populations, as presented in AAFCO (2018), may not consider variable AA requirements across dog phenotypes. Moreover, it is widely assumed that endogenous synthesis of dispensable AAs, such as taurine in the dog, is sufficient for meeting metabolic demands. However, recent studies suggest that under some metabolic conditions, dispensable AAs may also be required in diets (Hou et al., 2015). Taurine, as described in this commentary, is a clear example of this paradigm shift. Dietary taurine or the capacity for its adequate endogenous synthesis, especially in circumstances where excessive losses might occur, should be considered in the final formulation of dog foods to decrease the risk of canine DCM.

Nutritionists and regulatory agencies should be aware that, in the spectrum of nutrient requirements, dog populations with higher AA requirements relative to energy intake and other factors could be at a higher risk for a taurine deficiency. More precise categorization of requirements among different canine

populations would help us to optimize nutritional adequacy and decrease risk of diseases, such as DCM, that are possibly linked to nutrient deficiencies.

Effect of Processing on Antinutritional Factors in Plant-Based Ingredients

Just as understanding the inherent nutritional characteristics and the interaction between ingredients is important for preventing nutritional imbalances in pet foods, the effects of processing on these factors are equally important. Raw cereals and legumes contain antinutritional factors such as trypsin inhibitors, phytates, hematoglutinins, and polyphenols that can decrease protein digestion, nutrient absorption, and/or cause illness. Some of these antinutritional factors are thermolabile and, under the right conditions, can be effectively destroyed during the extrusion process improving the overall quality of plant-based ingredients and the final diet (Patterson et al., 2017). Recent reviews across a variety of legumes and legume-derived ingredients show that the activities of trypsin inhibitor, chymotrypsin inhibitor, and hemagglutinating activity were decreased by up to 95% across a variety of thermal treatment conditions, including extrusion (Patterson et al., 2017; Avilés-Gaxiola et al., 2018). Extrusion had modest effects on levels of phytate with reductions ranging from 7% to 26% and varied

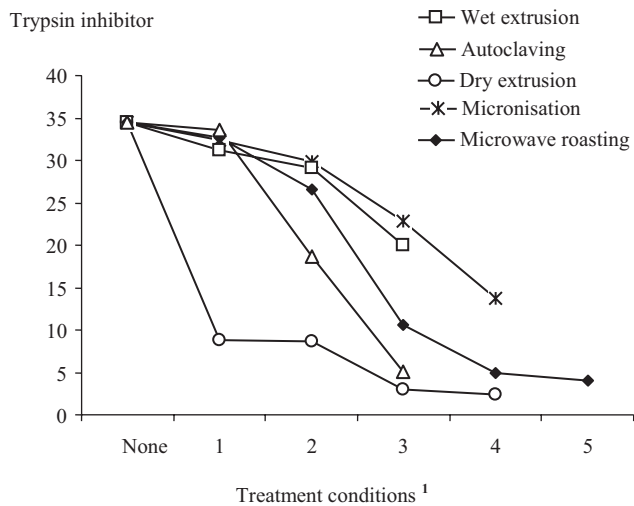


Figure 2. Effect of thermal processing methods on trypsin inhibitor levels (mg/g) soybean kernel. ¹Treatment conditions: None = no treatment; dry extrusion for 25 to 30 sec (1 = 100 °C; 2 = 125 °C; 3 = 140 °C; 4 = 150 °C); wet extrusion for 25 to 30 s with 6% to 8% added moisture (1 = 100 °C; 2 = 125 °C; 3 = 140 °C); micronization with near-infrared rays wavelength of 1.8 to 3.4 μ m for 90 s (1 = 100 °C; 2 = 125 °C; 3 = 140 °C; 4 = 150 °C); microwave roasting at 800 W and 2450 MHz (1 = 1 min [kernel temp = 57 °C], 2 = 2 min [kernel temp = 88 °C], 3 = 3 min [kernel temp = 108 °C], 4 = 4 min [kernel temp = 121 °C], 5 = 5 min [kernel temp = 132 °C]); Autoclaving at 120 °C and 1.2 bars (1 = 10 min, 2 = 20 min, 3 = 30 min). Reprinted with permission from Žilić et al. (2012).

by legume and extrusion conditions (Patterson et al., 2017). Figure 2 highlights the variability between processing methods and thermic conditions for decreasing antinutritional factors. For example, when soybeans were subjected to extrusion at increasing temperatures that ranged from 100 to 150 °C, trypsin inhibitor levels were incrementally decreased. At 140 °C, dry extrusion was considerably more effective at decreasing trypsin inhibitors (−91%) compared with wet extrusion (−44%). When the dry extrusion temperature was increased to 150 °C, reductions in trypsin inhibitors were further decreased by 94% (Žilić et al., 2012). Other thermal treatments, such as micronisation, microwave roasting, and autoclaving, also facilitated incremental reductions in trypsin inhibitors with increasing temperatures (Žilić et al., 2012). When formulating foods with higher concentrations of plant-based ingredients, consideration should also be given to the processing methods and the parameters used to effectively optimize the nutritional density and decrease antinutritional factors.

It is important to mention that, while temperature and pressure processing can greatly decrease antinutritional factors, they can also negatively affect bioavailability of AAs. The Maillard reaction is a well-known example of heat-damaged protein (Teodorowicz et al., 2017). In this reaction, lysine interacts with reducing sugars present in the

diets forming the Maillard product. The complex formed can be digested and absorbed by the animal but cannot be utilized for metabolic processes (e.g., protein synthesis). Thus, in heat-damaged proteins, digestibility of AAs can greatly overestimate bioavailability (Moehn et al., 2005). Other products of heat damage on proteins include racemization of AAs (alteration from L to D form) and the formation of cross-linked AAs. Such components can decrease bioavailability of AAs and digestibility of proteins, and their effects on protein quality cannot usually be determined using conventional methods of AA analysis. Pet foods with higher levels of plant-based ingredients may also require optimization of processing methods to maximize their nutritional density and nutrient bioavailability.

Recommendations for Formulating Dog Food With Novel Ingredients

Considering the AA profile of dog foods. Feed formulation for agricultural and companion animals should be based on the ideal protein concept (Baker, 1991; Swanson et al., 2013). The ideal protein is defined as that in which all AAs are in perfect balance compared with the animal's AA requirements (mg/g protein). Hence, all indispensable AAs are equally limiting. However, this is impossible to achieve in practical animal feed formulation, and diets should be formulated considering the first limiting indispensable AA. The first limiting indispensable AA refers to the indispensable AA that is present in the lowest proportion compared with the animal's requirement. By meeting the first indispensable limiting AA requirement, requirements for all other indispensable AAs are also inherently satisfied. Moreover, to avoid the formulation of diets with excessive protein concentration or an excess of indispensable AAs relative to the requirements of dogs, animal nutritionists combine multiple ingredients that are complementary in their AA profiles. Commonly, dog foods are formulated with a higher proportion of animal-derived ingredients, and a lower proportion of plant-based ingredients to meet nutrient recommendations. More recently, however, cereal grains have been removed in some diet formulations or the proportion of animal-based ingredients has been reduced. The production of these types of formulations is often driven by consumer perception, rather than scientific evidence. Allowing consumers to direct the ingredient composition of dog foods, or other pet foods, could perpetuate nutrient deficits that affect the health of animals in the long term.

In the formulation of grain-free pet foods, cereal grains are replaced with alternative ingredient(s). Animal-derived ingredients are expensive relative to plant-based ingredients. Thus, pulses, a subset of legumes, are often used as the replacement. In addition to containing substantial fiber, pulses also contain significant concentrations of protein and are used to partly meet indispensable AA requirements. Of interest, soybean meal and pulses contain 48% and 25% crude protein, respectively, which is substantially greater than the average protein concentration for grains (11%; [Table 1](#)). Although the high-protein content in soybean meal and pulses is indicative of higher concentration of AAs compared with grains, it does not imply AA balance. Soybean meal and pulses are high in lysine (mg/g protein) but low in sulfur AAs (mg/g protein), whereas the reverse is true for cereals. Plant-based ingredients tend to have lower ileal digestibility coefficients for protein compared with protein from animal sources ([FAO and WHO, 1991](#)). Thus, dog foods that contain substantial amounts of pulses, lower proportions of animal-based ingredients, and do not address AA imbalances through the addition of alternate ingredients or fortification, may risk AA deficiencies. To mitigate this risk across the pet food industry and ensure the final pet diets are nutritionally adequate and balanced, it is prudent that the digestibility coefficients of all final pet food products be calculated.

Considering the addition of high-fiber ingredients to dog foods. By definition, dietary fiber is carbohydrates that are resistant to digestion by endogenous enzymes in the gastrointestinal tract ([NRC, 2006](#)). Typical fibers include arabinoxylan, raffinose, inulin, β -glucan, cellulose, and pectin ([NRC, 2006](#)). Common ingredients to increase fiber content in companion animal diets include beet pulp, corn fiber, rice bran, whole grains, and pulse fibers ([de Godoy et al., 2013](#)). Achieving an optimal fiber concentration in canine diets has diverse positive physiological effects in the gastrointestinal tract; for example, higher fermentable fiber intake has been shown to slow the transit time of digesta, increasing satiety of the animal ([Haber et al., 1977](#)). Moreover, high-fiber diets generally have lower energy density making them an important nutritional strategy for controlling body weight ([Johnson et al., 2008](#)) and reducing the incidence of diarrhea ([Homann et al., 1994](#)). Gut health is also improved with higher consumption of fiber; fermentable fiber can act as a prebiotic and increase the population of health-promoting microbiota including lactobacilli and

bifidobacteria ([Roberfroid, 2005](#)). Although not required by AAFCO to fulfill the criteria of “complete and balanced,” fiber is an important component of the diet, and depending on the type of fiber and the amount consumed, fiber can increase the gut health status. Adding the necessary amount and type of fiber in the diet is crucial for optimal dog nutrition.

Despite the benefits of fiber in the diet, fiber can also affect enterohepatic recycling of taurine (discussed above). In monogastric species, including humans, high dietary fermentable fiber may also decrease digestibility and availability of dietary AAs ([Blackburn and Southgate, 1981](#); [Degen et al., 2007](#)) and, in some cases, increase the risk of DCM in dogs fed diets that marginally meet requirements for sulfur AAs. Moreover, higher concentrations of dietary fiber increase the size of the gastrointestinal tract in pigs and poultry ([Nyachoti et al., 2000](#)), increasing nutrient utilization in this organ. It has been determined in pigs that on average the gastrointestinal tract catabolizes 30% of dietary indispensable AAs during absorption, and this utilization represents ~50% for sulfur AAs ([Stoll et al., 1998](#); [Mansilla et al., 2018](#)), further reducing precursor availability for taurine synthesis and increasing the risk for taurine deficiency. For some high-fiber diets, fortification of specific nutrients, including taurine and other sulfur AAs, might be beneficial to avoid nutrient deficiencies.

Compared with the pet food industry, in other industries where high-fiber ingredients (coproducts) are routinely used (e.g., swine industry), the effects of fiber on the absorption of nutrients have been given more attention when formulating diets ([NRC, 2012](#)). For example, highly fermentable fiber in swine diets increases the threonine requirement to compensate for the increase in mucus (mucin protein) production in the intestinal cell lining ([Lien et al., 1997](#); [Mathai et al., 2016](#)). This has underpinned the development of “requirement models” ([NRC, 2012](#)) to tailor nutrient requirements for pigs while accounting for the different nutrient interactions. In contrast, in the pet food industry, the only concentrations of nutrients used for comparison are those recommended by [AAFCO \(2018\)](#). Such recommendations are static and may not encompass all the effects of the different nutrient combinations in the final diet. There is a clear need in companion animal nutrition to improve the understanding of the interactions of different ingredients and how these alter nutrient requirements for different breeds, age, and physiological status of dogs.

Other recent publications highlight the need for careful nutrient formulation. Several recent papers, both original research and reviews, likewise highlight the unknowns surrounding grain-free diets (typically legume or pulse-based, but sometimes also with “exotic” ingredients such as kangaroo, bison, or wild boar) and DCM. For example, [Adin et al. \(2019\)](#) examined 48 dogs of many breeds with diagnosed DCM and having a known diet history. Among grain-free diets being consumed in this study, 1 dog was particularly associated with DCM, possibly underscoring the importance of specific diet formulation. Furthermore, 2 dogs switched from that diet to other grain-free diets showed improvement in their DCM; it is unclear if those dogs were taurine deficient or if they also received taurine and/or carnitine supplementation. This suggests that grain-free composition per se may not be the root cause of DCM. Another recently published case series of 24 Golden Retrievers with DCM and known diet histories were evaluated, and an association between grain-free diets and DCM was suggested ([Kaplan et al., 2018](#)). Most dogs (15 of 24) were fed a single diet which was significantly associated with low blood taurine concentrations, again suggesting that specific diet formulation may play an important role. However, as in the previous study, soluble vs. insoluble fiber concentrations were not available for the diets, nor were taurine, methionine, or cysteine concentrations, meaning that the true nutrient profiles of the diets could not be assessed and reinforcing the point that diet formulation for nutrients—not ingredients—is essential. It also suggests that nutrient requirements may vary widely based on breed, diet, and other phenotypic data. Indeed, most of the dogs with DCM in the previously described study were consuming less energy compared with their predicted requirements ([Kaplan et al., 2018](#)). It also bears pointing out that the numbers in both studies were very low (representing less than 100 DCM-affected dogs between them), which surely represents a fraction of the dogs consuming grain-free, pulse-based diets. A recent thoughtful review supports these conclusions by reiterating the crucial need for plant-based diets for dogs to be formulated with sufficient quantities of bioavailable methionine and cysteine to support adequate taurine synthesis ([Dodd et al., 2018](#)). This can be achieved with the addition of purified AAs and other sources that are readily available ([Gloaguen et al., 2014](#)). Finally, a recent commentary carefully concludes that a true cause-and-effect relationship

between grain-free diets and DCM has not been proven, and other factors may ultimately be more important ([Freeman et al., 2018](#)). Taken together, these recent publications may point to faulty nutrient formulation in some, but not all, grain-free diets.

CONCLUSIONS

Recently, it has been suggested that pulse ingredients in commercial dog foods are associated with a limited number of cases of DCM. Although pulse ingredients have been implicated for having negative effects on the taurine status in dogs (deficiency of which is a known cause of canine DCM) based on the available evidence, the relationship between pulses and canine DCM remains undefined. However, the FDA statement may harm consideration of protein alternatives, such as pulses, as quality ingredients in pet foods and undermine attempts to diversify ingredients used across the food chain as the global population continues to grow. Ingredients do not represent the nutritional composition of the diet, and therefore, nutrient deficiencies should not be attributed to individual ingredients. The authors of this commentary recognize the important role of endogenous, and perhaps exogenous, taurine in the prevention of DCM in some dogs. The assurance of appropriate concentrations of all indispensable sulfur AAs, including methionine and cysteine, is crucial for ensuring adequate endogenous synthesis of taurine and to meet the metabolic demands of dogs. Additional dietary factors, such as methyl donors required for sulfur AA metabolism, carnitine for energy production in muscle, and dietary fiber, as well as animal factors, such as breed, size, and health status, should also be investigated when nutrient deficiency-related DCM is suspected.

It is the responsibility of animal nutritionists to formulate balanced diets for dogs, and other animals, by looking beyond the goal of meeting AAFCO recommendations or satisfying unsubstantiated market trends. Pulses and other plant-based ingredients can be used to formulate nutritionally adequate dog foods, and final product formulations should be assessed for nutrient balance and bioavailability, especially when using a limited number of ingredients. Although dietary factors are important in the prevention of sulfur AA deficiency and development of DCM, empirical data and mechanistic studies are required to better understand the indispensable AA requirements of dogs and preventing DCM. In diets that contain high concentrations of dietary fiber, compensative inclusion

of dietary indispensable sulfur AAs, including exogenous taurine, might be required to offset the possibility of increased fecal excretion or microbial assimilation of taurine in the large intestine. Processing conditions may also require adjustments to ensure the presence or effects of antinutritional factors are minimized and nutrient bioavailability is not compromised. Greater awareness of AA balance is crucial for ensuring that AA requirements are met for dogs consuming static diets.

LITERATURE CITED

- AAFCO. 2018. Association of American feed control officials. Official Publication Association of American Feed Control Inc., Oxford.
- Adin, D., T. C. DeFrancesco, B. Keene, S. Tou, K. Meurs, C. Atkins, B. Aona, K. Kurtz, L. Barron, and K. Saker. 2019. Echocardiographic phenotype of canine dilated cardiomyopathy differs based on diet type. *J. Vet. Card.* 21:1–9. doi: 10.1016/j.jvc.2018.11.002.
- Alroy, J., J. E. Rush, L. Freeman, M. S. Amarendhra Kumar, A. Karuri, K. Chase, and S. Sarkar. 2000. Inherited infantile dilated cardiomyopathy in dogs: genetic, clinical, biochemical, and morphologic findings. *Am. J. Med. Genet.* 95:57–66. doi:10.1002/1096-8628(20001106)95:1<57::AID-AJMG12>3.0.CO;2-O
- Arslan, C. 2006. L-Carnitine and its use as a feed additive in poultry feeding a review. *Revue Med Vet.* 157:134–142.
- Avilés-Gaxiola, S., C. Chuck-Hernández, and S. O. Serna Saldívar. 2018. Inactivation methods of trypsin inhibitor in legumes: a review. *J. Food Sci.* 83:17–29. doi: 10.1111/1750-3841.13985
- Backus, R. C., K. S. Ko, A. J. Fascetti, M. D. Kittleson, K. A. Macdonald, D. J. Maggs, J. R. Berg, and Q. R. Rogers. 2006. Low plasma taurine concentration in Newfoundland dogs is associated with low plasma methionine and cyst(e)ine concentrations and low taurine synthesis. *J. Nutr.* 136:2525–2533. doi: 10.1093/jn/136.10.2525
- Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. *J. Nutr.* 116:2339–2349. doi: 10.1093/jn/116.12.2339
- Baker, D. H. 1991. Comparative nutrition of cats and dogs. *Annu. Rev. Nutr.* 11:239–263. doi: 10.1146/annurev.nu.11.070191.001323
- Bakker, A. J., and H. M. Berg. 2002. Effect of taurine on sarcoplasmic reticulum function and force in skinned fast-twitch skeletal muscle fibres of the rat. *J. Physiol.* 538:185–194. doi: 10.1113/jphysiol.2001.012872
- Bélanger, M. C., M. Ouellet, G. Queney, and M. Moreau. 2005. Taurine-deficient dilated cardiomyopathy in a family of golden retrievers. *J. Am. Anim. Hosp. Assoc.* 41:284–291. doi: 10.5326/0410284
- Blackburn, N. A., and Southgate D. A. T. 1981. Protein digestibility and absorption: effects of fibre and the extent of individual variation. Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements Rome; October 5–17.
- Borgarelli, M., R. A. Santilli, D. Chiavegato, G. D’Agnolo, R. Zanatta, A. Mannelli, and A. Tarducci. 2006. Prognostic indicators for dogs with dilated cardiomyopathy. *J. Vet. Intern. Med.* 20:104–110. doi: 10.1111/j.1939-1676.2006.tb02829.x
- Butterwick, R. F., P. J. Markwell, and C. J. Thorne. 1994. Effect of level and source of dietary fiber on food intake in the dog. *J. Nutr.* 124(12 Suppl):2695S–2700S. doi: 10.1093/jn/124.suppl_12.2695S.
- Cattanach, B. M., J. Dukes-McEwan, P. R. Wotton, H. M. Stephenson, and R. M. Hamilton. 2015. A pedigree-based genetic appraisal of boxer ARVC and the role of the striatin mutation. *Vet. Rec.* 176:492. doi: 10.1136/vr.102821.
- Columbus, D., and C. F. de Lange. 2012. Evidence for validity of ileal digestibility coefficients in monogastrics. *Br. J. Nutr.* 108 (Suppl 2):S264–S272. doi: 10.1017/S0007114512002334.
- Degen, L., V. Halas, and L. Babinszky. 2007. Effect of dietary fibre on protein and fat digestibility and its consequences on diet formulation for growing and fattening pigs: a review. *Act. Agr. Scand. A-AN.* 57:1–9. doi: 10.1080/09064700701372038
- Dodd, S. A. S., J. L. Adolphe, and A. Verbrughe. 2018. Plant-based diets for dogs. *J. Am. Vet. Med. Assoc.* 253:1425–1432. doi: 10.2460/javma.253.11.1425
- Dutton, E., and J. López-Alvarez. 2018. An update on canine cardiomyopathies – is it all in the genes? *J. Small. Anim. Pract.* 59:455–464. doi: 10.1111/jsap.12841
- FAO. 1991. Food and agriculture organization of the United Nations. Protein quality evaluation. Report of Joint FAO/WHO, Expert Consultation, Rome, Italy.
- Fascetti, A. J., J. R. Reed, Q. R. Rogers, and R. C. Backus. 2003. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997-2001). *J. Am. Vet. Med. Assoc.* 223:1137–1141. doi: 10.2460/javma.2003.223.1137
- FDA, Center for Veterinary Medicine. 2018. FDA investigating potential connection between diet and cases of canine heart disease. <https://www.fda.gov/animalveterinary/new-sevents/cvmupdates/ucm613305.htm> (Accessed 12 July 2018.)
- Flanagan, J. L., P. A. Simmons, J. Vehige, M. D. Willcox, and Q. Garrett. 2010. Role of carnitine in disease. *Nutr. Metab. (Lond).* 7:30. doi: 10.1186/1743-7075-7-30
- Freeman, L. M., and J. E. Rush. 2006. Cardiovascular diseases: nutritional modulation. In: P. Pibot, V. Biourge, and D. Elliott, editors, *Encyclopedia of canine clinical nutrition*. Aniwa SAS, Aimargues. p. 316–347.
- Freeman, L. M., K. E. Michel, D. J. Brown, P. M. Kaplan, M. E. Stamoulis, S. L. Rosenthal, B. W. Keene, and J. E. Rush. 1996. Idiopathic dilated cardiomyopathy in dalmatians: nine cases (1990-1995). *J. Am. Vet. Med. Assoc.* 209:1592–1596.
- Freeman, L. M., J. A. Stern, R. Fries, D. B. Adin, and J. E. Rush. 2018. Diet-associated dilated cardiomyopathy in dogs: what do we know? *J. Am. Vet. Med. Assoc.* 253:1390–1394. doi: 10.2460/javma.253.11.1390
- Gloaguen, M., N. Le Floc’h, E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. *J. Anim. Sci.* 92:637–644. doi: 10.2527/jas.2013-6514
- de Godoy, M. R., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. *Nutrients* 5:3099–3117. doi: 10.3390/nu5083099.

- Haber, G. B., K. W. Heaton, D. Murphy, and L. F. Burroughs. 1977. Depletion and disruption of dietary fibre. Effects on satiety, plasma-glucose, and serum-insulin. *Lancet* 2:679–682. doi: 10.1016/S0140-6736(77)90494-9
- Homann, H. H., M. Kemen, C. Fuessenich, M. Senkal, and V. Zumtobel. 1994. Reduction in diarrhea incidence by soluble fiber in patients receiving total or supplemental enteral nutrition. *JPEN. J. Parenter. Enteral Nutr.* 18:486–490. doi: 10.1177/0148607194018006486
- Hoppel, C. 2003. The role of carnitine in normal and altered fatty acid metabolism. *Am. J. Kidney Dis.* 41:S4–12. doi: 10.1016/S0272-6386(03)00112-4
- Hou, Y., Y. Yin, and G. Wu. 2015. Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Exp. Biol. Med. (Maywood).* 240:997–1007. doi: 10.1177/1535370215587913
- Hurrell, R. F., P. A. Finot, and J. E. Ford. 1983. Storage of milk powders under adverse conditions. I. Losses of lysine and of other essential amino acids as determined by chemical and microbiological methods. *Br. J. Nutr.* 49:343–354. doi: 10.1079/BJN19830043
- Huxtable, R. J. 1992. Physiological actions of taurine. *Physiol. Rev.* 72:101–163. doi: 10.1152/physrev.1992.72.1.101
- Ito, T., S. W. Schaffer, and J. Azuma. 2012. The potential usefulness of taurine on diabetes mellitus and its complications. *Amino Acids* 42:1529–1539. doi: 10.1007/s00726-011-0883-5
- Johnson, L., A. P. Mander, L. R. Jones, P. M. Emmett, and S. A. Jebb. 2008. Energy-dense, low-fiber, high-fat dietary pattern is associated with increased fatness in childhood. *Am. J. Clin. Nutr.* 87:846–854. doi: 10.1093/ajcn/87.4.846
- Johnson, M. L., C. M. Parsons, G. C. Fahey, Jr, N. R. Merchen, and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *J. Anim. Sci.* 76:1112–1122. doi: 10.2527/1998.7641112x
- Kaplan, J. L., J. A. Stern, A. J. Fascetti, J. A. Larsen, H. Skolnik, G. D. Peddle, R. D. Kienle, A. Waxman, M. Cocchiario, C. T. Gunther-Harrington, et al. 2018. Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. *PLoS One* 13:e0209112. doi: 10.1371/journal.pone.0209112
- Keene, B. W., D. P. Panciera, C. E. Atkins, V. Regitz, M. J. Schmidt, and A. L. Shug. 1991. Myocardial L-carnitine deficiency in a family of dogs with dilated cardiomyopathy. *J. Am. Vet. Med. Assoc.* 198:647–650.
- Kim, S. W., Q. R. Rogers, and J. G. Morris. 1996a. Dietary antibiotics decrease taurine loss in cats fed a canned heat-processed diet. *J. Nutr.* 126:509–515. doi: 10.1093/jn/126.2.509
- Kim, S. W., Q. R. Rogers, and J. G. Morris. 1996b. Maillard reaction products in purified diets induce taurine depletion in cats which is reversed by antibiotics. *J. Nutr.* 126:195–201. doi: 10.1093/jn/126.1.195
- Kittleson, M. D., B. Keene, P. D. Pion, and C. G. Loyer. 1997. Results of the multicenter spaniel trial (MUST): taurine- and carnitine-responsive dilated cardiomyopathy in american cocker spaniels with decreased plasma taurine concentration. *J. Vet. Intern. Med.* 11:204–211. doi: 10.1111/j.1939-1676.1997.tb00092.x
- Ko, K. S., R. C. Backus, J. R. Berg, M. W. Lame, and Q. R. Rogers. 2007. Differences in taurine synthesis rate among dogs relate to differences in their maintenance energy requirement. *J. Nutr.* 137:1171–1175. doi: 10.1093/jn/137.5.1171
- Kramer, G. A., M. D. Kittleson, P. R. Fox, J. Lewis, and P. D. Pion. 1995. Plasma taurine concentrations in normal dogs and in dogs with heart disease. *J. Vet. Intern. Med.* 9:253–258. doi: 10.1111/j.1939-1676.1995.tb01076.x
- Lien, K. A., W. C. Sauer, and M. Fenton. 1997. Mucin output in ileal digesta of pigs fed a protein-free diet. *Z. Ernährungswiss.* 36:182–190. doi: 10.1007/BF01611398
- Linder, D., and M. Mueller. 2014. Pet obesity management: beyond nutrition. *Vet. Clin. North Am. Small Anim. Pract.* 44:789–806, vii. doi: 10.1016/j.cvsm.2014.03.004
- Mansilla, W. D., K. E. Silva, C. Zhu, C. M. Nyachoti, J. K. Htoo, J. P. Cant, and C. F. M. de Lange. 2018. Ammonia-nitrogen added to low-crude-protein diets deficient in dispensable amino acid-nitrogen increases the net release of alanine, citrulline, and glutamate post-splanchnic organ metabolism in growing pigs. *J. Nutr.* 148:1081–1087. doi: 10.1093/jn/nxy076
- Marinangeli, C. P. F., J. Curran, S. I. Barr, J. Slavin, S. Puri, S. Swaminathan, L. Tapsell, and C. A. Patterson. 2017. Enhancing nutrition with pulses: defining a recommended serving size for adults. *Nutr. Rev.* 75:990–1006. doi: 10.1093/nutrit/nux058
- Maron, B. J., J. A. Towbin, G. Thiene, C. Antzelevitch, D. Corrado, D. Arnett, A. J. Moss, C. E. Seidman, J. B. Young. 2006. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation.* 113:1807–1816. doi: 10.1161/CIRCULATIONAHA.106.174287
- Marshall, H. F., K. C. Chang, K. S. Miller, and L. D. Satterlee. 1982. Sulfur amino acid stability: effects of processing on legume proteins. *J. Food Sci.* 47:1170–4. doi: 10.1111/j.1365-2621.1982.tb07642.x
- Martin, M. W., M. J. Stafford Johnson, and B. Celona. 2009. Canine dilated cardiomyopathy: a retrospective study of signalment, presentation and clinical findings in 369 cases. *J. Small Anim. Pract.* 50:23–29. doi: 10.1111/j.1748-5827.2008.00659.x
- Mathai, J. K., J. K. Htoo, J. E. Thomson, K. J. Touchette, and H. H. Stein. 2016. Effects of dietary fiber on the ideal standardized ileal digestible threonine:lysine ratio for twenty-five to fifty kilogram growing gilts. *J. Anim. Sci.* 94:4217–4230. doi: 10.2527/jas.2016-0680
- Menniti, M. F., G. M. Davenport, A. K. Shoveller, J. P. Cant, and V. R. Osborne. 2014. Effect of graded inclusion of dietary soybean meal on nutrient digestibility, health, and metabolic indices of adult dogs. *J. Anim. Sci.* 92:2094–2104. doi: 10.2527/jas.2013-7226
- Merheb, M., R. T. Daher, M. Nasrallah, R. Sabra, F. N. Ziyadeh, and K. Barada. 2007. Taurine intestinal absorption and renal excretion test in diabetic patients: a pilot study. *Diabetes Care* 30:2652–2654. doi: 10.2337/dc07-0872
- Meurs, K. M., S. Lahmers, B. W. Keene, S. N. White, M. A. Oyama, E. Mauceli, and K. Lindblad-Toh. 2012. A splice site mutation in a gene encoding for PDK4, a mitochondrial protein, is associated with

- the development of dilated cardiomyopathy in the doberman pinscher. *Hum. Genet.* 131:1319–1325. doi: 10.1007/s00439-012-1158-2
- Meurs, K. M., J. A. Stern, D. D. Sisson, M. D. Kittleson, S. M. Cunningham, M. K. Ames, C. E. Atkins, T. DeFrancesco, T. E. Hodge, B. W. Keene, et al. 2013. Association of dilated cardiomyopathy with the striatin mutation genotype in boxer dogs. *J. Vet. Intern. Med.* 27:1437–1440. doi: 10.1111/jvim.12163
- Moehn, S., R. F. Bertolo, P. B. Pencharz, and R. O. Ball. 2005. Development of the indicator amino acid oxidation technique to determine the availability of amino acids from dietary protein in pigs. *J. Nutr.* 135:2866–2870. doi: 10.1093/jn/135.12.2866
- Moise, N. S., L. M. Paciorety, F. A. Kallfelz, M. H. Stipanuk, J. M. King, and R. F. Gilmour, Jr. 1991. Dietary taurine deficiency and dilated cardiomyopathy in the fox. *Am. Heart J.* 121:541–547. doi: 10.1016/0002-8703(91)90724-V
- Monnet, E., E. C. Orton, M. Salman, and J. Boon. 1995. Idiopathic dilated cardiomyopathy in dogs: survival and prognostic indicators. *J. Vet. Intern. Med.* 9:12–17. doi: 10.1111/j.1939-1676.1995.tb03266.x
- Nardelli, T. R., R. A. Ribeiro, S. L. Balbo, E. C. Vanzela, E. M. Carneiro, A. C. Boschero, and M. L. Bonfleur. 2011. Taurine prevents fat deposition and ameliorates plasma lipid profile in monosodium glutamate-obese rats. *Amino Acids* 41:901–908. doi: 10.1007/s00726-010-0789-7
- NRC, National Research Council. 2006. Nutrient requirements of dogs and cats. 10th ed. Natl. Acad. Press, Washington, DC.
- NRC, National Research Council. 2012. Nutrient requirements of swine. 11th ed. Natl. Acad. Press, Washington, DC.
- Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, S. Leeson, and H. Schulze. 2000. Dietary influence on organ size and in vitro oxygen consumption by visceral organs of growing pigs. *Livest Prod Sci.* 65:229–237. doi: 10.1016/S0301-6226(00)00157-3
- O'Máille, E. R., T. G. Richards, and A. H. Short. 1965. Acute taurine depletion and maximal rates of hepatic conjugation and secretion of cholic acid in the dog. *J. Physiol.* 180:67–79.
- Owczarek-Lipska, M., T. B. Mausberg, H. Stephenson, J. Dukes-McEwan, G. Wess, and T. Leeb. 2013. A 16-bp deletion in the canine PDK4 gene is not associated with dilated cardiomyopathy in a European cohort of doberman pinschers. *Anim. Genet.* 44:239. doi: 10.1111/j.1365-2052.2012.02396.x
- Patterson, C. A., J. Curran, and T. Der. 2017. Effect of processing on antinutrient compounds in pulses. *Cereal Chemistry.* 94:2–10. doi: 10.1094/CCHEM-05-16-0144-FI
- Pion, P. D., M. D. Kittleson, Q. R. Rogers, and J. G. Morris. 1987. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. *Science* 237:764–768. doi: 10.1126/science.3616607
- Pion, P. D., S. L. Sanderson, and M. D. Kittleson. 1998. The effectiveness of taurine and levocarnitine in dogs with heart disease. *Vet. Clin. North Am. Small Anim. Pract.* 28:1495–514, ix. doi: 10.1016/S0195-5616(98)50134-9
- Rășanu, T., M. Mehedinti-Hâncu, M. Alexianu, T. Mehedinti, E. Gheorghe, and I. Damian. 2012. Carnitine deficiency. *Rom. J. Morphol. Embryol.* 53:203–206.
- Rice, J. E., and S. L. Ihle. 1994. Effects of diet on fecal occult blood testing in healthy dogs. *Can. J. Vet. Res.* 58:134–137.
- Roberfroid, M. B. 2005. Introducing inulin-type fructans. *Br. J. Nutr.* 93 (Suppl 1):S13–S25. doi: 10.1079/BJN20041350
- Robinson, J. L., S. V. Harding, J. A. Brunton, and R. F. Bertolo. 2016a. Dietary methyl donors contribute to whole-body protein turnover and protein synthesis in skeletal muscle and the jejunum in neonatal piglets. *J. Nutr.* 146:2007–2012. doi: 10.3945/jn.115.226035
- Robinson, J. L., L. E. McBreaity, E. W. Randell, J. A. Brunton, and R. F. Bertolo. 2016b. Restriction of dietary methyl donors limits methionine availability and affects the partitioning of dietary methionine for creatine and phosphatidylcholine synthesis in the neonatal piglet. *J. Nutr. Biochem.* 35:81–86. doi: 10.1016/j.jnutbio.2016.07.001
- Sanderson, S. L., K. L. Gross, P. N. Ogburn, C. Calvert, G. Jacobs, S. R. Lowry, K. A. Bird, L. A. Koehler, and L. L. Swanson. 2001. Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. *Am. J. Vet. Res.* 62:1616–1623. doi:10.2460/ajvr.2001.62.1616
- Schaffer, S. W., C. J. Jong, K. C. Ramila, and J. Azuma. 2010. Physiological roles of taurine in heart and muscle. *J. Biomed. Sci.* 17 (Suppl 1):S2. doi: 10.1186/1423-0127-17-S1-S2
- Sisson, D. D., W. P. Thomas, and B. W. Keene. 2000. Primary myocardial disease in the dog. In: S. J. Ettinger, and E. C. Feldman, editors, *Textbook of veterinary internal medicine. Diseases of the dog and cat.* 5th ed. WB Saunders Co., Philadelphia. p. 874–895.
- Spitze, A. R., D. L. Wong, Q. R. Rogers, and A. J. Fascetti. 2003. Taurine concentrations in animal feed ingredients; cooking influences taurine content. *J. Anim. Physiol. Anim. Nutr. (Berl).* 87:251–262. doi: 10.1046/j.1439-0396.2003.00434.x
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr.* 128:606–614. doi: 10.1093/jn/128.3.606
- Story, J. A., and D. Kritchevsky. 1978. Bile acid metabolism and fiber. *Am. J. Clin. Nutr.* 31 (10 Suppl):S199–S202. doi: 10.1093/ajcn/31.10.S199
- Swanson, K. S., R. A. Carter, T. P. Yount, J. Aretz, and P. R. Buff. 2013. Nutritional sustainability of pet foods. *Adv. Nutr.* 4:141–150. doi: 10.3945/an.112.003335
- Teodorowicz, M., J. van Neerven, and H. Savelkoul. 2017. Food processing: the influence of the Maillard reaction on immunogenicity and allergenicity of food proteins. *Nutrition* 9:835. doi: 10.3390/nu9080835
- Tôrres, C. L., R. C. Backus, A. J. Fascetti, and Q. R. Rogers. 2003. Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy. *J. Anim. Physiol. Anim. Nutr. (Berl).* 87:359–372. doi:10.1046/j.1439-0396.2003.00446.x
- Tosh, S.M., and S. Yada. 2010. Dietary fibres in pulse seeds and fractions: characterization, functional attributes, and applications. *Food Res. Int.* 43:450–460. doi: 10.1016/j.foodres.2009.09.005
- Werner, P., M. G. Raducha, U. Prociuk, M. M. Sleeper, T. J. Van Winkle, and P. S. Henthorn. 2008. A novel locus for dilated cardiomyopathy maps to canine

- chromosome 8. *Genomics* 91:517–521. doi: 10.1016/j.ygeno.2008.03.007
- Worden, J. A., and M. H. Stipanuk. 1985. A comparison by species, age and sex of cysteinesulfinatase decarboxylase activity and taurine concentration in liver and brain of animals. *Comp. Biochem. Physiol.* 82B:233–239. doi: 10.1016/0305-0491(85)90232-9
- Yamka, R. M., U. Jamikorn, A. D. True, and D. L. Harmon. 2003. Evaluation of soybean meal as a protein source in canine foods. *Anim. Feed Sci. Technol.* 109:121–132. doi: 10.1016/S0377-8401(03)00203-7
- Žilić, S., I. Bozović, and V. H. T. Šukalović. 2012. Thermal inactivation of soybean bioactive proteins. *Int. J. Food Eng.* 8:1556–3758. doi:10.1515/1556-3758.2521