

## COMPANION ANIMAL NUTRITION

# Effects of different carbohydrate sources on taurine status in healthy Beagle dogs

Julia Guazzelli Pezzali,<sup>\*</sup> Heather L. Acuff,<sup>\*</sup> Will Henry,<sup>†</sup> Celeste Alexander,<sup>‡</sup> Kelly S. Swanson,<sup>‡,§</sup> and Charles G Aldrich<sup>\*,1</sup>

<sup>\*</sup>Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, <sup>†</sup>Director of Product Innovation, Extru-Tech, Inc., Sabetha, KS 66534, <sup>‡</sup>Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801, <sup>§</sup>Department of Animal Sciences, University of Illinois, Urbana, IL 61801

<sup>1</sup>Corresponding author: [aldrich4@ksu.edu](mailto:aldrich4@ksu.edu)

ORCID numbers: 0000-0001-8162-7590 (C. Alexander); 0000-0001-5518-3076 (K. S. Swanson); 0000-0002-8983-3652 (C. G. Aldrich).

## Abstract

This study evaluated the effects of a grain-based (GB) and grain-free (GF) diet on protein utilization and taurine status in healthy Beagle dogs. Two practical dog diets sufficient in crude protein, sulfur amino acids, and taurine content were formulated with the same ingredients with exception of the carbohydrate sources. The GB contained sorghum, millet, and spelt while potatoes, peas, and tapioca starch were used in the GF. A total of 12 Beagle dogs were used in a completely randomized design with six replicates per treatment. The study consisted of an adaptation period of 2 wk followed by an experimental period of 28 d in which GB and GF were fed to the dogs. At the end of the adaptation period and every 2 wk after it (day 0, day 14, day 28), markers of taurine metabolism were analyzed in whole blood (taurine), plasma (taurine, methionine, and cystine), urine (taurine:creatinine), and fresh fecal samples (primary and secondary bile acids). Fecal samples were collected during the last 6 d of experimental period for digestibility assessment using titanium dioxide as an external marker. Taurine markers and digestibility data were analyzed in a repeated measures model and one-way ANOVA, respectively, using PROC GLIMMIX in SAS (version 9.4). Apparent crude protein digestibility was not affected by treatment, but dogs fed GF diet had lower apparent organic matter digestibility compared with those fed GB ( $P < 0.05$ ). Greater plasma taurine concentrations were observed at days 14 and 28 compared with day 0; wherein dogs fed GF exhibited greater increase compared to those fed GB ( $P < 0.05$ ). Whole blood taurine concentrations, plasma methionine concentrations, and urinary taurine:creatinine were also greater at days 14 and 28 compared with day 0 ( $P < 0.05$ ), but no effect of diet was observed. Total bile acid excretion was similar between GF and GB groups, but dogs fed GF excreted a higher proportion of primary bile acids compared with those fed GB (25.49% vs. 12.09% at day 28, respectively). In summary, overall taurine status was not affected by dietary treatments, however, our results suggest that the higher content of oligosaccharides and soluble fibers in the GF diet may alter the composition of the fecal bile acid pool.

**Keywords:** bile acid, grain-free, legumes, oligosaccharides, taurine

## Introduction

Pet humanization has driven the pet food industry toward products perceived as healthy and natural by pet owners. Considering that grains have been perceived as unhealthy

ingredients by some pet owners (LaFlamme, 2014), grain-free (GF) diets have become a major portion of the pet food industry. In 2017, this category represented more than 40% of the dog foods sold in the United States (Phillips-Donaldson, 2018). GF

diets are formulated using legumes and tubers as the major replacements for cereal grains. Although legumes and tubers have also been consumed by humans for centuries with little to no side effects, a recent warning letter from Food and Drug Administration (FDA; FDA, 2018) has raised concern about the safety of pet diets based on these ingredients.

The FDA has received a meaningful number of reports ( $n = 524$ ) concerning dilated cardiomyopathy (DCM) with a high incidence of dietary histories in which dogs were eating GF diets. This has led to an investigation of a possible link between these two factors. DCM is characterized by enlargement of the left ventricle and systolic dysfunction (Bélanger et al., 2005). Consequently, the heart loses its ability to pump blood to the body resulting in a life-threatening condition due to congestive heart failure. Causative factors for DCM include genetic factors, immune-mediated disorders, viral infections, toxins, and nutritional deficiencies (Sisson et al., 2000). Taurine deficiency has been recognized for years as an important nutritional factor involved in the development of this condition (Pion et al., 1987).

Taurine is an amino-sulfonic acid which is not currently considered a required amino acid in dog diets because they can synthesize sufficient amounts when the precursors methionine and/or cystine are available in adequate quantities in their diet (Torres et al., 2003). Taurine is a unique amino acid (sulfone) which is not incorporated into proteins, but rather found in free form in the tissue (interstitial fluid) with a variety of important roles in biological processes (Huxtable, 1992). There is evidence that taurine is essential for normal cardiac function (Takahara et al., 1986; Huxtable, 1987); wherein, dogs can develop DCM secondary to taurine deficiency. Furthermore, taurine plays an important role in bile acid (BA) metabolism (Enright et al., 2018). Dogs conjugate BA exclusively with taurine (Czuba and Vessey, 1981); thus, any factor impacting BA metabolism may affect taurine homeostasis.

It is well recognized that taurine deficiency can occur in dogs if methionine and/or cysteine are not provided in sufficient amounts in the diet. Initially, it was thought that the GF diets involved in the FDA report may have not met the maintenance requirement of sulfur amino acids for adult dogs, resulting in taurine deficiency and subsequent DCM. However, this was not the case (FDA, 2019), which has raised the possibility that other intrinsic factors inherent in legumes or other environmental factors may be impairing taurine status in dogs. It has been hypothesized that diets containing high levels of fermentable fiber could lead to gastrointestinal losses of taurine through the BA cycle (Mansilla et al., 2019). Ko and Fascetti (2016) reported that beet pulp, a moderately fermentable fiber can affect taurine status in dogs by decreasing protein digestibility and increasing excretion of BA. However, these previous results were observed in dogs fed purified diets, which are not representative of commercial diets available in the market. It is well known that legumes contain high concentrations of oligosaccharides, which are highly fermentable carbohydrates (Mohan et al., 2016). Thus, the increased content of fermentable substrate present in GF diets may lead to gastrointestinal losses of taurine even in a taurine supplemented diet. To date, there are no studies evaluating practical GF diets sufficient in protein and sulfur amino acids on their effect on taurine status in dogs. Thus, the aim of our study was to investigate the effects of a grain-based (GB) and a GF diet sufficient in crude protein (CP), taurine, and sulfur amino acids on protein digestibility and taurine status in healthy Beagle dogs. We hypothesized that 1) GF and GB diets would result in similar digestibility; and 2) GF diet would have higher concentrations of oligosaccharides which would result

in greater fecal losses of taurine and lower concentrations of taurine in whole blood and/or plasma.

## Materials and Methods

The study was completed at Kansas State University during the summer of 2018. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Kansas State University under protocol #3883.

### Animals and Diets

Twelve neutered Beagle dogs (males = 8; females = 4) of similar age ( $4.4 \text{ years} \pm 0.22 \text{ years}$ , mean  $\pm$  SD), and initial body weight ( $12.17 \text{ kg} \pm 1.33 \text{ kg}$ , mean  $\pm$  SD) were individually housed in cages ( $1.83 \text{ m} \times 1.20 \text{ m}$ ) in a temperature ( $22$  to  $23 \text{ }^\circ\text{C}$ ) and light-controlled (16 h light:8 h dark cycle) building. Food for all dogs was provided twice daily at 0800 and 1600 h, and water was provided ad libitum throughout the study. Daily metabolizable energy was calculated as an average for inactive dogs ( $\text{ME, kcal/d} = 95 \times \text{BW}^{0.75}$ ) according to the National Research Council (NRC, 2006). The amount of food initially offered was calculated by dividing the daily ME of each dog by the ME of the experimental diet provided. Weekly body condition score (BCS) and body weight were measured, and amount of food provided was adjusted to maintain body weight. The BCS was evaluated using a 9-point body condition score scale according to Laflamme (1997).

The study consisted of a 2-wk adaptation period followed by a 4-wk experimental period. During the adaptation period, dogs were fed a basal diet (BD) to obtain baseline values for blood, urine, and fresh fecal sample analysis. The BD was a complete and balanced commercial laboratory dog food (Lab Diet #5L18\*, LabDiet, Purina, St. Louis, MI). After the adaptation period, dogs were paired according to their body weight, from lowest to highest, totaling six experimental pairs. In each pair, dogs were randomly assigned to one of the two experimental diets: GB or GF. The experimental diets were formulated to contain the same proportion of carbohydrate sources (50.55% of the formula as fed basis) and to exceed all the dog's nutrient requirements for maintenance (NRC, 2006). The GB was formulated with sorghum, millet, and spelt, and the GF diet was formulated with peas, potatoes, and tapioca starch. All other ingredients were included in both diets at the same percentage (as fed basis). Ancient grains were chosen as the carbohydrate sources in the GB diet because they may represent an alternative to the GF claim in the pet food industry. In the GF diet, peas were added at a higher level at the expense of tapioca starch to accommodate the extrusion process (Pezzali and Aldrich, 2019). Experimental diets were formulated to exceed the requirements of CP and sulfur amino acids for adult dogs (NRC, 2006), and were taurine supplemented to: 1) represent commercial diets available in the market, 2) evaluate the effect of shifting grain to grain-free carbohydrate sources (in a protein, sulfur amino acid, and taurine sufficient matrix) on nutritional composition, protein digestibility, and taurine status in dogs.

### Chemical Analysis of Food and Fecal Samples

Food and fecal samples were analyzed for dry matter (DM; AOAC 930.15), organic matter (OM; AOAC 942.05), CP (AOAC 990.03), and fat by acid hydrolysis (AOAC 954.02) in a commercial laboratory (Midwest Laboratories, Omaha, NE). Total dietary fiber (TDF) and insoluble fiber were analyzed in experimental diets using a commercial kit (TDF-100A; Sigma-Aldrich; St. Louis, MO). Soluble fiber was computed by difference. Dietary

concentrations of amino acids were analyzed according to AOAC (2006; method 982.30E; University of Missouri Experiment Station Chemical Laboratories, Columbia, MO). Short-chain oligosaccharides (raffinose, stachyose, and verbascose) were measured in experimental diets using HPLC according to Smiricky et al. (2002).

### Sample Collection, Processing, and Analysis

At the end of the adaptation period, and every 2 wk from the beginning of the experimental period (days 0, 14, and 28), blood, urine, and fresh fecal samples were collected and frozen for later analysis of markers of taurine metabolism as described below.

### Plasma and Whole Blood Samples

Dogs were fasted for 15 h, and blood (~6 mL) was collected through cephalic venipuncture before the morning feeding at each sampling day. Immediately after being drawn, blood samples were transferred to a heparin vacutainer tube (#367886 BD). Tubes were chilled on ice until transfer to the laboratory. A whole blood sample (~2 mL) was transferred to 0.5 mL test tubes and stored frozen (-80 °C) until analysis. Samples in heparin tubes were centrifuged (~1,200 × *g* for 10 min at room temperature) to harvest plasma. Plasma samples were stored in 0.5 mL test tubes and kept frozen (-80 °C) until analysis. Concentrations of taurine, methionine, and cystine were analyzed in plasma samples (University of Missouri Experiment Station Chemical Laboratories, Columbia, MO) according to Holtrop et al. (2002).

Whole blood taurine concentrations were analyzed (Kansas State University, Manhattan, KS); wherein, whole blood samples (750 µL) were mixed with equal volume (750 µL) of SeraPrep solution (SP100, Pickering Laboratories, Mountain View, CA) to lyse red blood cells. After cooling on ice for 30 min, samples were vortexed and centrifuged (17,000 × *g*, 10 min, 4 °C). The supernatant was filtered through a 1.2 µm Nylon Membrane Filter (GVS, 1213797). The HPLC analysis was measured by cation exchange chromatography with post-column derivatization with *o*-phthalaldehyde. A 4 × 100 mm lithium ion-exchange column (Pickering Laboratories), a cation-exchange guard column (GARD Column, Pickering Laboratories), and lithium eluents (Pickering Laboratories) were used. Flow rate was 0.4 mL/min and the total run time was 30 min. The initial eluent was 100% mobile phase A (1700-1125) contained 0.7% lithium citrate, 0.6% lithium chloride, and 0.2% sulfolane and was pumped for 5 min. The eluent was then switched to 30% mobile phase A and 70% mobile phase B (RG003) containing 2.56% lithium chloride, 1.05% lithium citrate, and 0.18% lithium hydroxide for 10 min. The *o*-phthalaldehyde reagent, containing 0.3 g *o*-phthalaldehyde (Pickering Laboratories) dissolved in 3 mL methanol, 2 g *N,N*-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor; Pickering Laboratories), and 3 mL of 30% (wt/wt) Brij 35 (Ricca Chemical Co., Arlington, TX) dissolved in 950 mL of 5.4% potassium borate (OPA diluent; Pickering Laboratories), was mixed with column effluent at a rate of 0.4 mL/min and allowed to react for 10 s at 21 °C before fluorescence detection with excitation at 330 nm and emission at 465 nm (HP 1046A Fluorescence Detector; Agilent Technologies, Santa Clara, CA).

### Fresh Fecal and Urine Samples

Urine and fresh fecal sample collections were performed 2 d before blood collection. Fresh urine samples (~10 mL) were collected from pans located underneath each dogs' cage, transferred into screw cap containers, and stored at -20 °C.

Urinary taurine concentrations were measured (Kansas State University) according to the same procedure used for determination of taurine concentrations in whole blood samples, but with an additional dilution step. In this procedure, urinary supernatant was diluted 1:20 with lithium diluent (Li220, Pickering Laboratories) before injecting into the HPLC. To normalize urinary taurine concentrations, a separate aliquot of urine (~1 mL) was analyzed for creatinine.

Fresh fecal samples were collected within 15 min of defecation, placed in 2 mL cryovials, and stored frozen (-80 °C) until further analysis. Fecal BA concentrations (cholic acid, chenodeoxycholic acid, lithocholic acid, and deoxycholic acid) were analyzed (University of Illinois, Urbana, IL) using a HPLC procedure according to Kakiyama et al. (2014).

### Digestibility Assessment and Calculation

During the last 6 d of the experimental period, fecal samples were collected for digestibility assessment using the same experimental units described previously (*N* = 12 Beagle dogs; *N* = 6 dogs per treatment). Sample collection was begun at 0800 on d 22 and extended for the next 144 h. Digestibility was determined using titanium dioxide (high purity grade titanium dioxide; Sensient, St. Louis, MO) as an external marker. Titanium dioxide was diluted into dry digest (0.25:0.75, respectively), and the blend was applied as top dressing on each dog's food at the morning feeding. The daily amount of blend was calculated to reach an inclusion rate of titanium dioxide at 0.4% of each meal. Fecal samples were collected, placed in whirl-pak bags, and stored in the freezer at -15 °C until further analysis. Fecal samples from each dog were combined, placed in an aluminum pan, and dried at 55 °C until constant weight was achieved. Feces were then ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Chemical composition of experimental diets and fecal samples were performed as described previously. Titanium concentrations in food and fecal samples were analyzed as described by Myers et al. (2004). The following equation was used to calculate apparent total tract nutrient digestibility (ATTD):

$$\text{Nutrient digestibility} = 1 - \frac{(\% \text{ Ti in food} \times \% \text{ nutrient in feces})}{(\% \text{ Ti in feces} \times \% \text{ nutrient in food})}$$

### Statistical Analysis

The experiment was conducted as a completely randomized design with six experimental units (dog) per treatment. Taurine markers data were analyzed as change from baseline and as measured values in a repeated measures model with the exception of fecal BA, which were analyzed as total amount and also as proportional change from baseline relative to total BA to decrease individual variability. Baseline differences between dietary treatments were tested. Each dog served as its own control; thus, baseline values from each dog were used to assess changes over time for the same animal. In the statistical model, the effect of diet, time, and their interaction were tested. Dog was used as a random effect and diet as a fixed effect. When presented as change from baseline, data from day 0 is reported as least square means, and data from days 14 and 28 are expressed as the difference from day 0.

Digestibility data was analyzed by one-way ANOVA. Diet was used as a fixed effect and dog was used as random effect. All data were analyzed using the PROC GLIMMIX procedure in SAS (SAS version 9.4, SAS Inst., Inc., Cary, NC). Means were separated using Fisher's LSD, and results were significant at *P* ≤ 0.05.

## Results

The CP (Table 1) and sulfur amino acid concentrations (Table 2) of the BD and experimental diets exceeded the recommended allowance of adult dogs for maintenance (10% and 0.65% respectively; NRC, 2006). Taurine concentrations of experimental diets were slightly higher compared with BD because both diets were taurine supplemented (Table 2). Although TDF content in GF was not much higher compared with the other diets, soluble fiber concentrations in GF were 2.31 and 1.88 times higher compared with BD and GB, respectively. A similar outcome was observed for oligosaccharides; concentrations of oligosaccharides were 2.61 and 4.89 times higher in GF compared with BD and GB, respectively.

During the experimental period, food intake was similar ( $P > 0.05$ ) between dogs fed GB and GF (143 vs. 146 g/d, respectively;

**Table 1.** Ingredient and chemical composition of basal diet and experimental treatments

Item	BD	Experimental treatment	
		Grain-based	Grain-free
Ingredient composition (% on as-fed basis)			
Hydrolyzed pork protein	—	42.17	42.17
Potato, white	—	—	16.85
Peas, green field	—	—	26.35
Tapioca starch	—	—	7.35
Spelt	—	16.85	—
Millet	—	16.85	—
Sorghum	—	16.86	—
Chicken fat	—	4.00	4.00
Dry digest	—	1.00	1.00
Salt	—	0.48	0.48
Potassium chloride	—	0.30	0.30
Choline chlorine, 60% dry	—	0.24	0.24
Vitamin premix	—	0.24	0.24
Dicalcium phosphate	—	0.24	0.24
Calcium carbonate	—	0.24	0.24
Trace mineral premix	—	0.17	0.17
Fish oil, menhaden	—	0.12	0.12
Taurine	—	0.12	0.12
Natural antioxidant	—	0.12	0.12
Chemical composition			
Moisture (%)	7.40	6.51	7.43
Crude protein (%), DMB	32.10	37.30	37.70
Crude fat (%), DMB	18.50	12.10	10.40
Nitrogen-free extract (calculated; %, DMB)	—	44.46	44.86
Ash (%), DMB	—	3.85	3.79
Total dietary fiber (%), DMB	10.17	10.39	12.57
Insoluble fiber	8.47	8.30	8.65
Soluble fiber	1.70	2.09	3.92
Soluble:insoluble	0.20	0.25	0.45
Oligosaccharides ( $\mu\text{g/g}$ on DMB)			
Raffinose	1586.00	746.00	2379.00
Stachyose	2393.00	1509.00	6334.00
Verbascose	1068.00	458.00	4464.00
Total	5047.00	2713.00	13178.00
Calculated metabolizable energy* (kcal/g DMB)	4.09	3.89	3.77

BS, basal diet; DMB, dry matter basis.

\*Metabolizable energy = 8.5 kcal ME/g fat + 3.5 kcal ME/g crude protein + 3.5 kcal ME/g nitrogen-free extract.

Table 3). No differences were observed ( $P > 0.05$ ) in ATTD of CP and crude fat between treatments. However, ATTD of organic matter was slightly higher ( $P < 0.05$ ) for dogs fed GB compared with those fed GF (Table 3).

Taurine markers data are presented as measured values and as change from baseline in table 4 and 5, respectively. For the sake of this article, only the change from baseline data will be discussed. Baseline values for all variables were similar between dietary treatments ( $P < 0.05$ ). All taurine markers were within reference ranges for adult dogs throughout the study, with the exception of plasma taurine concentrations which were above the reference range (41 to 97 nmol/mL) from healthy adult Beagles dogs according to Sanderson (2006). Plasma methionine concentrations were affected by time ( $P < 0.05$ ), with an increase on days 14 and 28 compared with day 0 regardless of the dietary treatment (Table 5). On day 28, the mean plasma methionine concentrations were 65.68 and 62.59 nmol/mL for the GB and GF group, respectively. No effects of diet and time were observed for plasma cystine concentrations ( $P > 0.05$ ). A significant diet and time effect was observed for plasma taurine concentrations ( $P < 0.05$ ); an increase was observed on days 14 and 28 compared with baseline with a greater increase in dogs fed GF compared with those fed GB. On day 28, the mean plasma taurine concentrations were 138.68 and 170.62 nmol/mL for the GB and GF group, respectively. A similar effect of time was observed on whole blood taurine concentrations ( $P < 0.05$ ), but this variable was not different between dietary treatments ( $P > 0.05$ ). On day 28, the mean whole blood taurine concentrations were 253.76 and 271.47 nmol/mL for the GB and GF group.

**Table 2.** Amino acid composition of basal diet and experimental treatments

Item	BD	Experimental treatment	
		Grain-based	Grain-free
EAA* (% on as-DMB)			
Arginine	1.55	2.17	2.40
Histidine	0.67	0.75	0.77
Isoleucine	1.14	1.54	1.59
Leucine	3.02	3.14	3.03
Lysine	2.21	1.99	2.31
Methionine	0.64	0.64	0.57
Methionine + Cysteine	1.04	1.28	1.18
Phenylalanine	1.43	1.79	1.80
Threonine	1.08	1.44	1.49
Tryptophan	0.20	0.35	0.36
Valine	1.36	2.03	2.10
NEAA* (% on as-DMB)			
Alanine	2.10	2.27	2.13
Asparagine + aspartate	2.22	3.06	3.55
Glutamate + glutamine	4.65	5.60	4.95
Glycine	1.92	2.44	2.42
Hydroxylysine	0.10	0.11	0.15
Lanthionine	0.01	-	-
Ornithine	0.02	0.02	0.02
Proline	2.20	2.42	2.1
Serine	1.22	1.47	1.39
Taurine	0.25	0.33	0.35
Tyrosine	1.12	1.35	1.37
EAA:NEAA	0.93:1	0.93:1	1:1

BS, basal diet; DMB, dry matter basis.

\*Analyzed.

Urinary taurine:creatinine increased on days 14 and 28 ( $P = 0.05$ ) compared with day 0 with no effect of diet ( $P > 0.05$ ).

Total excretion of BA was similar between GB and GF groups (Table 6) and was not affected by time ( $P > 0.05$ ). However, excretion of cholic acid, and consequently total primary BA (cholic acid + chenodeoxycholic acid), was higher in dogs fed GF ( $P < 0.05$ ). Excretion of primary and secondary BA as a proportion of total BA was also significantly different between dietary treatments ( $P < 0.05$ ; Table 7). Excretion of cholic acid as proportion of total fecal BA was affected by diet; wherein dogs fed GB exhibited a lower ( $P < 0.05$ ) proportion compared with the ones fed GF. Consequently, dogs fed GF had a higher excretion of primary BA as a proportion of total BA compared with those fed GB. On day 28, primary BA represented 25.49% and 12.09% of total BA in dogs fed GF and GB, respectively. Excretion of lithocholic acid as a proportion of total BA was also affected by diet, with dogs fed GB excreting a higher proportion compared with the dogs fed GF. As a result, a lower excretion of secondary BA as a proportion of total BA was observed in dogs fed GF compared to those fed GB. On day 28, secondary BA represented 74.51% and 87.91% of total BA in dogs fed GF and GB, respectively.

## Discussion

This experiment was performed to examine whether GB or GF carbohydrate sources influenced overall taurine status in Beagle dogs. The first aspect to consider was their impact on the chemical composition of the diets and on nutrient utilization by dogs. The replacement of grain with grain-free carbohydrate sources did not result in a significant change of overall nutrient

composition between dietary treatments. Although it is known that legumes have a greater TDF content compared with cereal grains due to a greater concentration of soluble fibers (Bednar et al. 2001; de-Oliveira et al., 2012), we did not observe an important difference in TDF content between dietary treatments. However, we observed a more prominent difference in the composition (soluble and insoluble fiber) of TDF between dietary treatments; soluble fiber concentrations were higher in the GF diet. It is noteworthy that peas possess greater concentrations of fermentable oligosaccharides (Tosh and Yada, 2010), which may not be recovered during the TDF procedure. As expected, the GF had greater concentrations of oligosaccharides compared with the GB. This finding agrees with Alvarenga and Aldrich (2019) who also reported a higher content of oligosaccharides in a legume-based diet (faba beans) compared with a GB control.

We observed a slight difference in lysine and sulfur amino acid concentrations between dietary treatments. Cereal grains and legumes are considered nutritionally complementary ingredients regarding their amino acid profile. While legumes are rich in lysine and deficient in sulfur amino acids, cereal grains present the opposite profile (Marquardt and Bell, 1988). Although a greater difference of lysine and sulfur amino acid concentrations between GF and GB were expected, we observed a higher lysine and lower sulfur amino acid content in the GF compared with the GB. The high inclusion of hydrolyzed pork, and moderate inclusion of peas may have influenced these results. Unfortunately, we do not have the chemical and amino acid profile of each ingredient used in this study to help better explain the nutritional composition of experimental diets.

All dogs remained healthy throughout the study. Food intake was similar between dietary treatments, and no meal refusals were observed. While the ATTD of CP and crude fat were not affected by carbohydrate sources, a lower ATTD of OM was observed in dogs fed the GF diet. This result is probably due to the greater TDF and oligosaccharide content in the GF diet. However, in another study conducted by our laboratory (Pezzali and Aldrich, 2019), we did not detect differences in ATTD of OM in dogs fed GB and GF diets. Differences in the extrusion processing condition, digestibility methodology, and experimental design may explain the different results observed. High inclusion of peas may affect ATTD of CP as reported by Carciofi et al. (2008), however the inclusion rate (26.35%) used in our study did not affect this parameter. Thus, we do not believe protein digestibility had a prominent impact on taurine status of dogs in the current study.

Both GB and GF diets were taurine supplemented which explains the increase in plasma taurine concentrations during

**Table 3.** Apparent total tract digestibility and feed intake of dogs fed grain-based and grain-free diets

Item	Experimental treatment		SEM	P value
	Grain-based	Grain-free		
	N = 6	N = 6		
Feed intake (as fed-basis), g/d	143.00	146.00	2.265	0.3599
ATTD, %				
Organic matter	87.27	84.98	0.568	0.0170
Crude protein	86.86	85.17	0.628	0.0864
Crude fat	90.59	91.43	0.465	0.2290

ATTD, apparent total tract digestibility.

**Table 4.** Taurine markers in Beagle dogs fed diets formulated with different carbohydrate sources

Item	Grain-based				Grain-free				Probability*, P<			
	N <sup>†</sup>	Baseline	14 d	28 d	N <sup>†</sup>	Baseline	14 d	28 d	SEM	T	D	D × T
Plasma, nmol/mL												
Methionine	6	57.72	65.66	65.70	5	55.53	62.66	62.62	3.18	0.028	0.279	0.987
Cystine	6	27.79	28.58	27.96	5	27.33	29.61	28.86	1.74	0.658	0.723	0.886
Taurine	6	121.89 <sup>c</sup>	138.30 <sup>bc</sup>	138.57 <sup>bc</sup>	5	110.51 <sup>c</sup>	192.73 <sup>a</sup>	169.13 <sup>ab</sup>	13.30	0.002	0.026	0.047
Whole blood, nmol/mL												
Taurine	6	228.87	235.19	253.63	6	240.11	255.35	271.58	13.68	0.134	0.151	0.944
Urine, M:M												
Taurine:creatinine	6	0.35	0.52	0.58	6	0.35	0.56	0.54	0.09	0.037	0.853	0.916

\*Effect of time (T), diet (D), and their interaction (D × T;  $P < 0.05$ ).

<sup>†</sup>N = number of observations.

**Table 5.** Change from baseline of taurine markers in Beagle dogs fed diets formulated with different carbohydrate sources

Item	Grain-based				Grain-free				SEM <sup>¶</sup>	Probability,* P<		
	N <sup>†</sup>	Baseline <sup>‡</sup>	14 d <sup>  </sup>	28 d <sup>§</sup>	N <sup>†</sup>	Baseline <sup>‡</sup>	14 d <sup>  </sup>	28 d <sup>§</sup>		T	D	D* <sup>¶</sup> T
Plasma, nmol/mL												
Methionine	6	57.72	+7.94	+7.98	5	55.53	+7.13	+7.09	2.79	0.017	0.807	0.985
Cystine	6	27.79	+0.79	+0.17	5	27.33	+2.28	+1.52	0.94	0.285	0.232	0.689
Taurine	6	121.89	+16.41	+16.68	5	110.51	+82.22	+58.62	10.03	0.003	<b>0.003</b>	0.068
Whole blood, nmol/mL												
Taurine	6	228.87	+6.32	+24.76	6	240.11	+15.23	+31.47	13.60	0.029	0.529	0.899
Urine, M:M												
Taurine:creatinine	6	0.35	+0.17	+0.23	6	0.35	+0.21	+0.19	0.09	0.051	0.982	0.916

\*Effect of time (T), diet (D), and their interaction (D × T; P < 0.05).

<sup>†</sup>N = number of observations.

<sup>‡</sup>Baseline = variable mean prior feeding experimental diets.

<sup>||</sup>14 d = mean from day 14—baseline mean.

<sup>§</sup>28 d = mean from day 28—baseline mean.

<sup>¶</sup>Standard error of the mean associated with change from baseline (14 and 28 d).

**Table 6.** Bile acid excretion in dogs fed diets formulated with different carbohydrate sources

Item, μmol/g stool	Grain-based				Grain-free				SEM	Probability,* P<		
	N <sup>†</sup>	Baseline	14 d	28 d	N <sup>†</sup>	Baseline	14 d	28 d		T	D	D × T
Primary bile acids												
CA	6	0.2639	0.2681	0.2523	6	0.3261	0.4990	0.4769	0.086	0.565	0.021	0.549
CDCA	6	0.0174	0.0306	0.0234	6	0.0153	0.0381	0.0305	0.001	0.201	0.606	0.859
CA + CDCA	6	0.2813	0.2987	0.2757	6	0.3414	0.5371	0.5074	0.092	0.495	0.026	0.557
Secondary bile acids												
DCA	6	0.7372	0.8517	1.1537	6	0.6309	1.0278	1.2821	0.409	0.437	0.845	0.934
LCA	6	0.7213	0.8426	1.3110	6	0.7518	0.8775	1.2400	0.347	0.281	0.995	0.985
DCA + LCA	6	1.4586	1.6943	2.4647	6	1.3827	1.9053	2.5220	0.751	0.363	0.917	0.982
Total bile acids	6	1.7399	1.9930	2.7404	6	1.7241	2.4423	3.0295	0.810	0.372	0.718	0.958

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

\*Effect of time (T), diet (D), and their interaction (D × T; P < 0.05).

<sup>†</sup>N = number of observations.

the experimental period compared with the baseline. Dietary supplementation of taurine and methionine has been previously correlated with higher plasma concentrations of these amino acids in cats (Heinze et al., 2009). Although methionine content of experimental diets was not greater than the BD diet, the total sulfur amino acid content (methionine + cysteine) was slightly higher. Thus, cysteine may have spared methionine resulting in increased plasma values during the experimental period. In the current study, average plasma concentrations of taurine for both groups were above the reference range (97 nm/mL) reported for dogs by Sanderson (2006). Two main factors may be leading to the high plasma concentrations of taurine observed in the present study. The first is the anticoagulant effect. In our study, plasma samples were collected in lithium heparin. It was reported previously that plasma concentrations of taurine are higher when plasma is collected in lithium heparin compared with those collected in sodium citrate (Kramer et al., 1995). Furthermore, it is known that taurine can be released from formed elements of the blood into plasma during clotting (Sanderson, 2006). Difficulties during venipuncture in this study may have caused partial hemolysis of samples and greater release of taurine from platelets or other blood elements resulting in high plasma taurine concentrations. We also believe this resulted in the higher increase of plasma taurine concentrations in dogs fed GF compared with those fed GB rather than a diet effect.

Whole blood taurine concentrations also increased during the experimental period which agrees with the results of Delaney et al. (2003) who also observed higher concentrations of taurine in whole blood of dogs consuming a taurine-supplemented diet. However, whole blood taurine concentrations were not affected by diet as observed in plasma samples. Accuracy of whole blood taurine concentrations are considered higher than those assessed in plasma (Heinze et al., 2009). They also likely reflect better the concentrations of taurine in skeletal muscle (Pacioretty et al., 2001). Thus, one should not rely only on plasma results to investigate taurine status in dogs because this fraction is more labile due to influences of external factors.

In the current study, we also investigated urinary and gastrointestinal losses of taurine. Because a single fresh sample of urine (~10 mL) was collected rather than performing full 24 h urine collection, urinary taurine concentration was corrected for creatinine and expressed as taurine:creatinine (molar) ratio to account for the effect of collection at different time points. Our results indicate that dogs demonstrated an adaptive renal response to taurine due to dietary factors, and because they were not taurine deficient, tubular reabsorption of taurine was decreased. It is known that the kidney is major site for regulation of taurine homeostasis in cats (Park et al., 1989), and similar mechanism has been reported in dogs by Torres et al. (2003). Although both GF and GB groups increased urinary

**Table 7.** Change from baseline as proportion of bile acids in Beagle dogs fed diets formulated with different carbohydrate sources

Item	Grain-based				Grain-free				SEM <sup>¶</sup>	Probability,* P<		
	N <sup>†</sup>	Baseline <sup>‡</sup>	14 d <sup>§</sup>	28 d <sup>§</sup>	N <sup>†</sup>	Baseline <sup>‡</sup>	14 d <sup>§</sup>	28 d <sup>§</sup>		T	D	D × T
Primary, % of total												
CA	6	26.03	-12.03	-14.98	6	21.12	+4.64	+2.83	6.82	0.672	0.048	0.356
CDCA	6	1.10	+0.40	-0.06	6	0.86	+0.83	+0.68	0.49	0.468	0.337	0.753
CA + CDCA	6	27.13	-11.63	-15.04	6	21.98	+5.47	+3.51	6.78	0.699	0.040	0.328
Secondary, % of total												
DCA	6	36.73	+6.07	+3.73	6	36.30	1.65	-0.17	3.25	0.502	0.305	0.761
LCA	6	36.14	+5.57	+11.31	6	41.72	-7.13	-3.35	4.42	0.519	0.017	0.215
DCA + LCA	6	72.87	+11.63	+15.04	6	78.02	-5.47	-3.51	6.78	0.699	0.040	0.328
Total, μmol/g stool	6	1.74	+0.25	+1.00	6	1.72	+0.72	+1.31	0.86	0.412	0.716	0.963

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

\*Effect of time (T), diet (D), and their interaction (D × T; P < 0.05).

<sup>†</sup>N = number of observations.

<sup>‡</sup>Baseline = variable mean prior feeding experimental diets.

<sup>§</sup>14 d = mean from day 14—baseline mean.

<sup>§</sup>28 d = mean from day 28—baseline mean.

<sup>¶</sup>Standard error of the mean associated with change from baseline (14 and 28 d).

excretion of taurine during the experimental period, the whole blood and plasma taurine concentrations still increased over time. A previous study also observed that kidney reabsorption of taurine did not affect plasma taurine concentrations. However, the authors of that work stated that urinary taurine measurements were highly variable and may have influenced the results (Gray et al., 2016). Even though taurine homeostasis is controlled by the renal system alone, taurine status can be affected by gastrointestinal losses through BA metabolism.

In this study, we attempted to use fecal BA as a possible marker for gastrointestinal losses of taurine rather than measuring fecal taurine concentrations because intestinal bacteria can degrade it in the colon (Hickman et al., 1992). This will ostensibly underestimate fecal taurine concentrations. The primary BA, cholic acid and chenodeoxycholic acid, are synthesized in the liver and conjugated with taurine before release into the small intestine. In the distal ileum, the majority of conjugated BA (95%) are reabsorbed and transported back to the liver through the enterohepatic circulation (Ajouz et al., 2014). Any disruption in this recycling process may lead to fecal losses of BA and taurine. It has been suggested that some fibers may bind BA in the intestinal lumen resulting in higher excretion of these metabolites (Garcia-Diez et al., 1996; Stratton-Phelps et al., 2002). Furthermore, luminal pH can be affected by end-products of fermentation of nondigestible carbohydrates, which can impact BA solubility and excretion. Thus, our hypothesis was that the GF diet would culminate in a greater excretion of BA due to greater TDF and oligosaccharide concentrations, resulting in increased gastrointestinal losses of taurine. However, we did not observe a significant difference in total fecal excretion of BA between the GB and GF groups. Similar fecal concentrations of BA between the GB and GF groups in this study suggests that the higher concentrations of soluble fiber and/or oligosaccharides in the GF diet did not play a major role in increasing excretion of BA. However, it is worth noting that we observed large variability in this analysis within dogs in the same group. This may suggest that a higher number of samples would be needed to observe statistical difference in future work.

Although total excretion of BA was similar between dietary treatments, excretion of primary and secondary BA as a proportion of total BA was affected by dietary treatment; with higher proportion of primary BA and lower proportion of

secondary BA were observed in the GF group. In the terminal ileum and colon, deconjugation of primary BA can occur through the action of the commensal microbial enzyme bile salt hydrolase (BSH). This deconjugation step serves as a gateway reaction for the dehydroxylation of primary BA in the colon through the bacterial enzyme 7-alpha dehydroxylase (7AD) to form secondary BA (Long et al., 2017). Thus, perturbations to the gut microbial community may impact BA composition. Thereby, microbiota should be considered as an essential factor in BA metabolism and taurine homeostasis. It has already been reported that dietary components can affect fecal BA profile in dogs (Herstad et al., 2018). Some fibers may impact gastrointestinal losses through fermentation. Dogs lack the enzymes necessary to digest legume oligosaccharides which are delivered to the lower intestine and are fermented by the colonic bacteria (Jezierny et al., 2010). Increased microbial fermentation may result in greater catabolism of taurine similar to that previously reported in cats (Kim et al., 1996). Furthermore, production of short-chain fatty acids as a result of fiber fermentation may decrease luminal pH leading to a decrease in BA solubility, and therefore to an increase in BA excretion. The higher proportion of primary BA present in dogs fed the GF diet may suggest that: 1) deconjugation of primary BA may not have been efficiently performed by the gut microbiota—either due to a decreased population of bacteria capable of producing BSH or to a decreased expression of this enzyme, consequently blocking the 7AD step; and/or 2) some dietary component caused a drastic reduction in the abundance or activity of 7AD. We believe that the higher content of nondigestible oligosaccharides and/or soluble fiber in the GF diet may have increased microbial fermentation resulting in a change in the fecal BA profile, although changes in the microbiota due to a GF diet have yet to be investigated. The ideal proportion and concentration of primary and secondary BA to maximize animal health have not been fully explored to date. However, it has been observed that excessive concentration of secondary BA, mainly lithocholic acid, can lead to DNA damage, oxidative/nitrosative stress, mutation, and apoptosis (Payne et al., 2008). More studies are necessary to investigate the effects of these compounds on dog health.

Although different proportions of primary and secondary BA were observed between GF and GB groups, whole blood taurine

concentrations were not affected by dietary treatment. It is important to point out that Beagle dogs are not predisposed to DCM or taurine deficiency. Thus, the change in BA profile observed in our study may lead to greater impact in large dogs that have higher taurine requirements (Ko et al., 2007). Moreover, many other factors besides breed can influence taurine synthesis, and thus requirements. For example, in humans and rats it has already been reported that gender (Guertin et al., 1991), age (Rigo and Senter, 1977; Zelikovic et al., 1990), stress (Lorenço and Camilo, 2002), and some pathologies, such as obesity (Nardelli et al., 2011) and diabetes (Merheb et al., 2007) might affect taurine requirement. Unfortunately, most of these conditions have not been evaluated in canines to date. Therefore, not only dietary factors but also other physiological mechanisms should be considered during investigation of taurine status in dogs with DCM. We hypothesized that if there were intrinsic factors inherent in legumes impacting taurine status in dogs, we would have observed lower concentrations of taurine in whole blood and/or plasma even in a taurine sufficient scenario; however, this was not the case. In our study, we demonstrated a complete and balanced taurine supplemented grain-free dog food did not result in lower concentrations of taurine in whole blood and plasma compared with a GB diet. There is still no scientific evidence showing that grain-free diets are a causative factor for DCM; however, there is a necessity to evaluate in depth the relationship between these two factors. Furthermore, we would offer that the short duration of our study is also a limiting factor that should be factored into the interpretation. Although 28 days is sufficient time to observe adaptive changes in taurine markers (Torres et al., 2003), a longer study would be ideal to capture the long-term effects of GF diets on dogs. Our study still provides valuable information and serves as important direction to future studies in this area of research.

## Conclusions

In summary, overall taurine status in dogs was not affected by dietary treatments with exception of plasma taurine concentrations and excretion of primary BA as a proportion of total BA, which were both higher in dogs fed GF. The higher excretion of primary BA in the GF group may be due to changes in the microbiota community caused by the higher content of oligosaccharides and soluble fiber. Interactions between the gut microbiota, fecal BA, and taurine losses should be further investigated to address how oligosaccharides and soluble fibers affect fecal losses of taurine.

## Conflict of interest statement

None declared.

## Literature Cited

Ajouz, H., D. Mukherji, and A. Shamseddine. 2014. Secondary bile acids: an underrecognized cause of colon cancer. *World J. Surg. Oncol.* 12:164. doi:10.1186/1477-7819-12-164

Alvarenga, I. C., and C. G. Aldrich. 2019. The effect of increasing levels of dehulled faba beans (*Vicia faba* L.) on extrusion and product parameters for dry expanded dog food. *Foods* 8:26. doi:10.3390/foods8010026

Bednar, G. E., A. R. Patil, S. M. Murray, C. M. Grieshop, N. R. Merchen, and G. C. Fahey Jr. 2001. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their

large bowel fermentability in vitro in a canine model. *J. Nutr.* 131:276–286. doi:10.1093/jn/131.2.276

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

Carciofi, A. C., F. S. Takakura, L. D. de-Oliveira, E. Teshima, J. T. Jeremias, M. A. Brunetto, and F. Prada. 2008. Effects of six carbohydrate sources on dog diet digestibility and post-prandial glucose and insulin response. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 92:326–336. doi:10.1111/j.1439-0396.2007.00794.x

Czuba, B., and D. A. Vessey. 1981. Identification of a unique mammalian species of choyl-CoA: amino acid N-acyltransferase. *Biochim. Biophys. Acta* 665:612–614. doi:10.1016/0005-2760(81)90278-2

Delaney, S. J., P. H. Kass, Q. R. Rogers, and A. J. Fascetti. 2003. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 87:236–244. doi:10.1046/j.1439-0396.2003.00433.x

Enright, E. F., B. T. Griffin, C. G. M. Gahan, and S. A. Joyce. 2018. Microbiome-mediated bile acid modification: role in intestinal drug absorption and metabolism. *Pharmacol. Res.* 133:170–186. doi:10.1016/j.phrs.2018.04.009

FDA. 2018. FDA investigating potential connection between diet and cases of canine heart disease. [July 12, 2018; accessed August 15, 2019]. Available from <https://www.fda.gov/animal-veterinary/cvm-updates/fda-investigating-potential-connection-between-diet-and-cases-canine-heart-disease>.

FDA. 2019. FDA investigation into potential link between certain diets and canine dilated cardiomyopathy. [updated June 27, 2019; accessed August 15, 2019]. Available from <https://www.fda.gov/animal-veterinary/news-events/fda-investigation-potential-link-between-certain-diets-and-canine-dilated-cardiomyopathy>.

Garcia-Diez, F., V. Garcia-Mediavilla, J. E. Bayon, and J. Gonzalez-Gallego. 1996. Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. *J. Nutr.* 126:1766–1771. doi:10.1093/jn/126.7.1766

Gray, K., L. G. Alexander, R. Staunton, A. Colyer, A. Watson, and A. J. Fascetti. 2016. The effect of 48-hour fasting on taurine status in healthy adult dogs. *J. Anim. Physiol. Anim. Nutr.* 100(3):532–536. doi:10.1111/jpn.12378

Guertin, F., C. C. Roy, G. Lepage, A. Perea, R. Giguère, I. Yousef, and B. Tuchweber. 1991. Effect of taurine on total parenteral nutrition-associated cholestasis. *J. Parenter. Enteral Nutr.* 15:247–251. doi:10.1177/0148607191015003247

Heinze, C. R., J. A. Larsen, P. H. Kass, and A. J. Fascetti. 2009. Plasma amino acid and whole blood taurine concentrations in cats eating commercially prepared diets. *Am. J. Vet. Res.* 70:1374–1382. doi:10.2460/ajvr.70.11.1374

Herstad, K. M. V., H. T. Rønning, A. M. Bakke, L. Moe, and E. Skancke. 2018. Changes in the faecal bile acid profile in dogs fed dry food vs high content of beef: a pilot study. *Acta Vet. Scand.* 60:29. doi:10.1186/s13028-018-0383-7

Hickman, M. A., M. L. Bruss, J. G. Morris, and Q. R. Rogers. 1992. Dietary protein source (soybean vs. casein) and taurine status affect kinetics of the enterohepatic circulation of taurocholic acid in cats. *J. Nutr.* 122:1019–1028. doi:10.1093/jn/122.4.1019

Holtrop S., N. G. G. M. Abeling, and A. H. van Gennip. 2002. Recommendations to improve the quality of diagnostic quantitative analysis of amino acids in plasma and urine using cation-exchange liquid chromatography with post column ninhydrin reaction and detection. [accessed December 17, 2018]. Available from [https://www.erndim.org/store/docs/amino-EDECCATE128918-10-4-2011.pdf?fbclid=IwAR0PwieZyvkw7HUyAYoJ-puEbc6pz0af\\_7y8knLEajDenG-3EkXmyTz6zMI](https://www.erndim.org/store/docs/amino-EDECCATE128918-10-4-2011.pdf?fbclid=IwAR0PwieZyvkw7HUyAYoJ-puEbc6pz0af_7y8knLEajDenG-3EkXmyTz6zMI).

Huxtable, R. J. 1987. From heart to hypothesis: a mechanism for the calcium modulatory actions of taurine. In: Huxtable R. J.,



- F. Franconi, and A. Giotti, editors. *The biology of taurine*. MA: Springer. (Advances in experimental medicine and biology; vol. 217). doi:10.1007/978-1-4899-0405-8\_39
- Huxtable, R. J. 1992. Physiological actions of taurine. *Physiol. Rev.* 72:101–163. doi:10.1152/physrev.1992.72.1.101
- Jezierny, D., R. Mosenthin, and E. Bauer. 2010. The use of grain legumes as a protein source in pig nutrition: a review. *Anim. Feed Sci. Technol.* 157:111–128. doi:10.1016/j.anifeedsci.2010.03.001
- Kakiyama, G., A. Muto, H. Takei, H. Nittono, T. Murai, T. Kurosawa, A. F. Hofmann, W. M. Pandak, and J. S. Bajaj. 2014. A simple and accurate HPLC method for fecal bile acid profile in healthy and cirrhotic subjects: validation by GC-MS and LC-MS. *J. Lipid Res.* 55:978–990. doi:10.1194/jlr.D047506
- Kim, S. W., Q. R. Rogers, and J. G. Morris. 1996. Maillard reaction products in purified diets induce taurine depletion in cats which is reversed by antibiotics. *J. Nutr.* 126(1):195–201. doi:10.1093/jn/126.1.195
- 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
- Ko, K. S., and A. J. Fascetti. 2016. Dietary beet pulp decreases taurine status in dogs fed low protein diet. *J. Anim. Sci. Technol.* 58:29. doi:10.1186/s40781-016-0112-6
- 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
- Laflamme, D. P. 1997. Development and validation of a body condition score system for dogs. *Canine Pract.* 22:10–15.
- Laflamme, D., O. Izquierdo, L. Eirmann, and S. Binder. 2014. Myths and misperceptions about ingredients used in commercial pet foods. *Vet. Clin. North Am. Small Anim. Pract.* 44:689–698. v. doi:10.1016/j.cvsm.2014.03.002
- Long, S. L., C. G. M. Gahan, and S. A. Joyce. 2017. Interactions between gut bacteria and bile in health and disease. *Mol. Aspects Med.* 56:54–65. doi:10.1016/j.mam.2017.06.002
- Lourenço, R., and M. E. Camilo. 2002. Taurine: a conditionally essential amino acid in humans? An overview in health and disease. *Nutr. Hosp.* 17:262–270.
- Mansilla, W. D., C. P. F. Marinangeli, K. J. Ekenstedt, J. A. Larsen, G. Aldrich, D. A. Columbus, L. Weber, S. K. Abood, and A. K. Shoveller. 2019. Special topic: the association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation. *J. Anim. Sci.* 97:983–997. doi:10.1093/jas/sky488
- Marquardt, R. R., and J. M. Bell. 1988. Future potential of pulses for use in animal feeds. In: Summerfield, R. J., editor. *World crops: cool season food legumes*. Dordrecht: Kluwer Academic; p. 421–444.
- 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
- Mohan, V. R., P. S. Tresina, and E. D. Daffodil. 2016. Antinutritional factors in legume seeds: characteristics and determination. In: Cabaleiro, B., P. Finglas, and F. Toldra, editors. *The encyclopedia of food and health*. Oxford: Academic Press (Elsevier); p. 211–220.
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183. doi:10.2527/2004.821179x
- 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.
- National Research Council (NRC). 2006. *Nutrient requirements of dogs and cats*. 10th ed. Washington, DC: The National Academy Press.
- de-Oliveira, L. D., F. S. Takakura, E. Kienzle, M. A. Brunetto, E. Teshima, G. T. Pereira, R. S. Vasconcellos, and A. C. Carciofi. 2012. Fibre analysis and fibre digestibility in pet foods—a comparison of total dietary fibre, neutral and acid detergent fibre and crude fibre. *J. Anim. Physiol. Anim. Nutr.* 96:895–906. doi:10.1111/j.1439-0396.2011.01203.x
- Pacioretty, L., M. A. Hickman, J. G. Morris, and Q. R. Rogers. 2001. Kinetics of taurine depletion and repletion in plasma, serum, whole blood and skeletal muscle in cats. *Amino Acids* 21:417–427. doi:10.1007/s007260170006
- Park, T., Q. R. Rogers, J. G. Morris, and R. W. Chesney. 1989. Effect of dietary taurine on renal taurine transport by proximal tubule brush border membrane vesicles in the kitten. *J. Nutr.* 119:1452–1460. doi:10.1093/jn/119.10.1452
- Payne, C. M., C. Bernstein, K. Dvorak, and H. Bernstein. 2008. Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. *Clin. Exp. Gastroenterol.* 1:19–47. doi:10.2147/ceg.s4343
- Pezzali, J. G., and C. G. Aldrich. 2019. Effect of ancient grains and grain-free carbohydrate sources on extrusion parameters and nutrient utilization by dogs. *J. Anim. Sci.* 97:3758–3767. doi:10.1093/jas/skz237
- Phillips-Donaldson, D. 2018. Pet food ingredients: what's hot and getting hotter? [accessed August 15, 2019]. Available from <https://www.petfoodindustry.com/blogs/7-adventures-in-pet-food/post/7161-pet-food-ingredients-whats-hot-and-getting-hotter>.
- 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
- Rigo, J., and J. Senterre. 1977. Is taurine essential for the neonates? *Biol. Neonate* 32:73–76. doi:10.1159/000240997
- Sanderson, S. L. 2006. Taurine and carnitine in canine cardiomyopathy. *Vet. Clin. North Am. Small Anim. Pract.* 36:1325–43. vii. doi:10.1016/j.cvsm.2006.08.010
- Sisson, D. D., W. P. Thomas, and B. W. Keene. 2000. Primary myocardial disease in the dog. In: Ettinger, S. J., and E. C. Feldman, editors. *Textbook of veterinary internal medicine. Diseases of the dog and cat*. 5th ed. Philadelphia: WB Saunders Co.
- Smiricky, M. R., C. M. Grieshop, D. M. Albin, J. E. Wubben, V. M. Gabert, and G. C. Fahey Jr. 2002. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. *J. Anim. Sci.* 80:2433–2441. doi:10.2527/2002.8092433x
- Stratton-Phelps, M., R. C. Backus, Q. R. Rogers, and A. J. Fascetti. 2002. Dietary rice bran decreases plasma and whole-blood taurine in cats. *J. Nutr.* 132(6 Suppl. 2):1745S–1747S. doi:10.1093/jn/132.6.1745S
- Takahara, K., J. Azuma, N. Awata, H. Ohta, T. Hamaguchi, A. Sawamura, Y. Tanaka, S. Kishimoto, and N. Sperelakis. 1986. Beneficial effect of taurine in rabbits with chronic congestive heart failure. *Am. Heart J.* 112:1278–1284. doi:10.1016/0002-8703(86)90360-1
- Törres, 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
- Zelikovic, I., R. W. Chesney, A. L. Friedman, and C. E. Ahlfors. 1990. Taurine depletion in very low birth weight infants receiving prolonged total parenteral nutrition: role of renal immaturity. *J. Pediatr.* 116:301–306. doi:10.1016/s0022-3476(05)82898-7