



Nutrition and Disease

The Pulse of It: Dietary Inclusion of Up to 45% Whole Pulse Ingredients with Chicken Meal and Pea Starch in a Complete and Balanced Diet Does Not Affect Cardiac Function, Fasted Sulfur Amino Acid Status, or Other Gross Measures of Health in Adult Dogs

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ABSTRACT

Background: Pulses are an attractive alternative protein source for all mammals; however, recent reports suggest that these ingredients may be related to developing dilated cardiomyopathy in dogs.

Objectives: The primary objective of this study was to quantify the effects of dietary pulse intake by adult dogs on cardiac function using echocardiographic measurements and cardiac biomarkers N-terminal pro-B-type natriuretic peptide and cardiac troponin I (cTnI). Second, to investigate the effects of pulse consumption on plasma sulfur amino acid (SAA) concentrations as pulses are generally low in SAA and may limit taurine synthesis. Last, to assess the general safety and efficacy of feeding pulse-containing diets on canine body composition and hematological and biochemical indices.

Methods: Twenty-eight privately-owned domestic Siberian Huskies (13 females; 4 intact, and 15 males; 6 intact) with a mean age of 5.3 ± 2.8 y ($\pm$ SD) were randomly assigned to 1 of 4 dietary treatments ($n = 7/\text{treatment}$), with equal micronutrient supplementation and increasing whole pulse ingredient inclusion (0%, 15%, 30%, and 45%) with pea starch used to balance protein and energy.

Results: After 20 wks of feeding, there were no differences ($P > 0.05$) in echocardiographic parameters, N-terminal pro-B-type natriuretic peptide, and cTnI concentrations among treatments or across time within treatment ($P > 0.05$), indicating no differences in cardiac function among treatments. Concentrations of cTnI remained below the safe upper limit of 0.2 ng/mL for all dogs. Plasma SAA status, body composition, and hematological and biochemical indices were similar among treatments and over time ($P > 0.05$).

Conclusions: The results from this study suggest that increasing the inclusion of pulses up to 45% with the removal of grains and equal micronutrient supplementation does not impact cardiac function concurrent with dilated cardiomyopathy, body composition, or SAA status and is safe for healthy adult dogs to consume when fed for 20 wks.

Keywords: dilated cardiomyopathy, canine, pulses, grain-free, echocardiography, taurine, methionine

Introduction

Pulse-containing diets for canines have been increasing in popularity over the past few decades and represent nearly half of

the pet food market in North America [1]. Grain-free (GF) diets often replace cereal grain ingredients with other carbohydrate sources such as tapioca and potatoes, but most commonly with pulse ingredients. By definition, pulses are dry legumes that are

Abbreviations: AA, amino acid; AAFCO, Association of American Feed Control Officials; CBC, complete blood count; Ctl, grain-inclusive control diet; cTnI, cardiac troponin I; Cys, cysteine; DCM, dilated cardiomyopathy; DXA, dual-energy x-ray absorptiometry; ECHO, echocardiogram; EF, ejection fraction; FS, fractional shortening; GF, grain-free; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVESV, left ventricular end-systolic volume; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; Met, methionine; NT-proBNP, N-terminal pro-B-type natriuretic peptide; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; SAA, sulfur amino acid; Tau, taurine; UPLC, ultra-performance LC.

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harvested for their seeds, including peas, lentils, and beans [2,3]. They are rich in protein and fiber [4] and low in dietary fat [5], making pulses a valuable source of protein in plant-based diets as well as diets intended for weight management in dogs [6,7]. In humans, pulses are reported to have pre-emptive cardiometabolic disease implications, given their nutritional composition and observed improvements in circulating lipid concentrations [8], postprandial glycemic response [9], and body weight (BW) management [10]. Similarly, in dogs, pulse ingredients reduce the postprandial glycemic response [11–14]. These attributes of pulse consumption are advantageous in preventing heart diseases like atherosclerosis and thrombosis in humans [15–17]. Given these positive metabolic effects, pulses have been increasingly sought-after in the formulation of diets for dogs and suggested to be included in human diets as well.

Although pulses are high in protein, they are often limited in their concentrations of sulfur amino acids (SAA), methionine (Met), and cysteine (Cys) [18]. Met is an indispensable proteogenic amino acid (AA) responsible for methyl donation and is essential for endogenous Cys production [19]. Dietary Cys can spare or reduce the Met requirement [20], and it is the direct precursor to taurine (Tau) [21]. Tau is a nonproteogenic AA that is found in high concentrations in cardiac muscle and modulates the contractility of the heart to maintain blood flow [22,23]. Insufficient or poor digestibility of ingredients supplying Met or Cys can lead to Tau deficiency, possibly resulting in cardiac dysfunction [24–26]. An insufficient supply of proteogenic AAs can also result in limited protein synthesis and an eventual reduction in lean mass [27]. Additionally, muscle-wasting diseases, like cachexia and sarcopenia, are common comorbidities related to congestive heart failure [28]. Loss of lean muscle mass can occur without a drastic change in BW; thus, assessment of lean mass through medical imaging tests like dual-energy x-ray absorptiometry (DXA) scans can aid in diagnosing sarcopenia for patients at risk of cardiac disease. A 2012 study observed a correlation between muscle wasting and reduced left ventricular (LV) ejection fraction (EF) in patients with chronic heart failure [29].

Recently there has been speculation that pulse-inclusive diets contribute to the development of nutritionally-mediated dilated cardiomyopathy (DCM) in dogs [30]. This followed the FDA statements regarding an increase in reported DCM cases in dog breeds that were consuming GF diets that were not known to be genetically susceptible to the disease. Previously, the supplementation of Tau reversed DCM in a family of Golden Retrievers [22]. Additionally, plasma concentrations of Tau were observed to be lower in large breed dogs, despite higher plasma Met concentrations, compared to medium or small breeds [31], suggesting potential metabolic differences among dog breeds. Deficiencies of other nutrients like Met and Cys that are necessary for synthesizing metabolites essential for cardiac function, like Tau and carnitine, could also play a role in developing nutritionally-mediated DCM.

Numerous studies have since aimed to investigate the impact of pulse-inclusive diets on canine cardiac health [32–35]. However, some of the limitations in these various studies include confounding variables such as the unknown etiology of DCM, varying use of medical intervention, or the absence of comprehensive diet analytics, all of which weaken the ability to make

conclusions about the effects of pulse ingredients alone. Therefore, controlled studies on the implications of pulse ingredients on canine cardiac health are required to investigate a causative effect.

Cardiac health is most reliably evaluated by an echocardiogram (ECHO) [36,37]. DCM is a disease that results in low systolic function of the heart, and primary assessments for DCM pertain to the left ventricle [38,39]. Echocardiographic developments for both humans [40] and dogs with DCM include increased LV end-diastolic and end-systolic volumes (LVEDV and LVESV, respectively), increased LV internal diameter in diastole and in systole (LVIDd and LVIDs) decreased EF percentage (EF%), fractional shortening percentage (FS%), and sphericity index (SI) [41,42]. Additionally, increases in cardiac biomarkers, serum cardiac troponin I (cTnI), and plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) are widely used to quantify cardiac damage in human and canine patients [42,43,44,45].

Given that pulses are limited in SAA, an upstream precursor of Tau, limited Met concentrations in pulse-containing diets may also be a contributing factor. However, the presence of pulse ingredients in canine diets does not contribute to low plasma or whole blood (WB) Tau in healthy dogs when fed extruded diets that exceed the Association of American Feed Control Officials (AAFCO) protein requirements [46,47]. Additionally, Reilly et al. [48] reported that dogs consuming diets with 45% green lentil inclusion for 90 d had no observable differences in plasma SAA status compared to dogs consuming a grain-inclusive, pulse-free diet. Despite this, the direct implications of pulse ingredients on canine cardiac health remain a knowledge gap.

Therefore, the present study aimed to quantify the effects of pulse ingredients on canine health and metabolism. Specifically, the focus was to investigate how increasing the inclusion of pulses from 0%–45% in micro, and macronutrient-balanced diets that are fed to maintain BW may affect ECHO measurements, cardiac biomarkers (NT-proBNP and cTnI), fasted SAA concentrations, body composition, and hematological and biochemical indices, after 20 wks of feeding. The range of pulse inclusion in the experimental diets is meant to be reflective of concentrations that exist within commercially available diets that exceed AAFCO guidelines [49]. We hypothesized that dogs consuming diets with greater concentrations of pulse ingredients over a 20-wk period would: experience changes in ECHO measurements concurrent with the development of DCM (increased LVIDd and LVIDs, LVEDV, LVESV, decreased EF%, FS%, and SI); have elevated serum cTnI and plasma NT-proBNP; have lower fasted plasma SAA but similar WB Tau concentrations; have lower lean soft tissue mass as measured by DXA; and have changes in hematological and biochemical parameters.

Methods

Animals and housing

Twenty-eight privately-owned Siberian Huskies (13 females; 4 intact, and 15 males; 6 intact) between the ages of 1–10 y old (mean 5.29 ± 2.83 y, mean $\pm$ SD), and mean BW of 23.22 ± 3.73 kg were used in the study. Dogs were housed in an off-site facility (Rajenn Siberian Huskies, Ayr, Ontario, Canada; 43.31°N) in

TABLE 1

The ingredient list for dietary treatments with increasing concentrations of whole pulse ingredient inclusion [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)] on an as-fed basis

Ingredient	Ctl	Pulse15	Pulse30	Pulse45
Whole grain corn	33	-	-	-
Corn gluten meal	12	-	-	-
Chicken meal	25	33	27.25	25
Pea starch	2.20	24.20	14.94	2.20
Whole green and yellow peas flour	-	5	10	15
Whole pinto beans flour	-	5	10	15
Whole chickpeas and lentils (50:50) flour	-	5	10	15
Fresh chicken	10	10	10	10
Chicken fat	7.5	7.5	7.5	7.5
Ground miscanthus grass	2.00	2.00	2.00	2.00
Natural chicken flavor (dry)	1.50	1.50	1.50	1.50
Natural chicken flavor (liquid)	2.5	2.5	2.5	2.5
Salt	2.5	2.5	2.5	2.5
Potassium chloride	0.75	0.75	0.75	0.75
Kelp	0.25	0.25	0.25	0.25

All diets were formulated with equal inclusion of vitamin and mineral premixes; vitamin B premix canine (0.2%), vitamin A,D,E (0.2%), choline chloride (1.5%), zinc (0.1%), vitamin B5 (0.05%), selenium (0.05%), natural antioxidant liquid (0.04%), natural antioxidant dry (0.02%), copper (0.01%).

Technical additives per kg diet: choline chloride (0.4 ug), copper proteinate (copper chelate of amino acid hydrate; 11 mg), selenium yeast (0.3 mg), vitamin A (15,000 IU), vitamin D (2000 IU), vitamin E (200 IU), vitamin B1 (100 mg), vitamin B2 (40 mg), vitamin B3 (200 mg), vitamin B5 (57 mg), vitamin B6 (30 mg), vitamin B9 (3 mg), zinc (zinc chelate of amino acid hydrate; 160 mg).

Ctl, grain-inclusive control diet; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion.

outdoor free-run kennels that ranged from 3.5–80 m² in size. Dogs were pair or group housed with up to 8 dogs per kennel and were provided with individual shelters for rest and protection against the weather. The study was conducted through the summer into the fall season during the months of May–October 2021. A complete blood count (CBC) and biochemistry panel were performed on all dogs prior to the start of the study to confirm all dogs were healthy and eligible for study enrollment. The study was approved by the University of Guelph's Animal Care Committee (Animal Use Protocol #4553).

Diets and study design

Four weeks before the study period (week –4 to –1), dogs were acclimated to being fed once daily at 16:00 with a dry extruded diet (Acana Healthy Grains Dog Food Red Meat, Champion Petfoods; [Supplemental Table 1](#)). At feeding, all dogs were tethered and fed individually to ensure accurate monitoring of food consumption. Each dog was given 1 h to consume their food, and any remaining food was weighed and recorded. During this wash-in period, dogs were initially fed based on historical feeding records for maintenance energy requirements. Throughout the entire study period, dogs were weighed at the start of each week, and feed intake was adjusted to maintain baseline BW measured at week 0. Dogs always had ad libitum access to fresh water.

At baseline, dogs were assigned to 1 of 4 groups, blocked for ECHO EF, age, BW, and sex. These groups were then randomly allocated to 1 of 4 dry extruded experimental diets (n = 7/group) formulated by Champion Petfoods that were fed over 20 wks: 1) grain-inclusive diet with 33% corn inclusion and 0% whole pulse ingredient inclusion (Ctl); 2) GF diet with 15% inclusion of whole pulse ingredients (Pulse15); 3) GF diet with 30% inclusion of whole pulse ingredients (Pulse30); 4) GF diet with 45%

inclusion of whole pulse ingredients (Pulse45). [Table 1](#) outlines the ingredient composition of all experimental diets. A mixture of whole milled pulses, specifically green and yellow peas, pinto beans, and a 50:50 mix of chickpeas and lentils were added to diets at combined inclusion rates of 15%, 30%, and 45% in place of whole grain corn and corn gluten meal in the Ctl diet. All experimental diets were formulated to meet a similar macronutrient composition. These are also representative of macronutrient compositions that exist commonly in the marketplace. By ensuring similar nutrient delivery, this design allowed us to explore whether there are any inherent properties of pulses that adversely affect physiological outcomes. The present study was not designed to explore the effects of nutrient deficiencies. Chicken meal and pea starch were used in all diets to meet protein and energy targets. Pea starch used in the present study was derived from air-classified dehulled pea flour (~92% dry mass, 70% starch, 7%–10% crude protein, 1%–2% crude fiber, and 1%–2% ash). Given that whole pulses contain 30%–40% starch, pea starch progressively decreased as the proportion of whole pulses increased in the pulse diets. The addition of pea starch also provided small concentrations of crude protein, particularly in the Pulse15 and Pulse 30 diets, to assist with attaining protein equivalence across diets. A small amount of pea starch (2.2%) was also used in the grain-inclusive Ctl diet at a equivalent concentration to Pulse45. Pea starch was chosen as the starch ingredient in all diets for balancing macronutrients in order to prevent potential confounding effects related to introducing additional ingredients that differed between the Control and Pulse diets. No synthetic AAs were supplemented in the diets. From the ingredients used, the Met and Cys content in all diets exceeded AAFCO recommendations by >60%. All diets used the same vitamin/mineral premixes and were processed and manufactured with the same batches of ingredients and

TABLE 2

The proximate analysis, amino acid content, fiber, mineral, and vitamin analysis of experimental diets with increasing concentrations of whole pulse ingredient inclusion [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)] on an as-fed basis

Experimental diets	Ctl	Pulse15	Pulse30	Pulse45
Proximate analysis, (%)				
Moisture	9.58	10.6	11.2	10.2
Crude protein	31.92	32.72	31.52	32.42
Crude fat	15.46	13.5	13.59	14.28
Total dietary fiber	5.5	6.65	8.21	8.31
Nitrogen-free extract (NFE, calculated) ¹	33.83	31.5	31.71	30.83
Ash	7.09	8.62	8.7	8.84
Calculated metabolizable energy, (kcal/kg) ²	3615.31	3395.32	3368.05	3427.26
Amino acids, (%)				
Arginine	1.84	2.27	2.21	2.31
Lysine	1.43	1.98	1.94	1.98
Methionine	0.76	0.63	0.59	0.58
Phenylalanine	1.73	1.53	1.55	1.65
Cysteine	0.48	0.46	0.45	0.45
Histidine	0.89	0.91	0.89	0.91
Isoleucine	1.19	1.28	1.24	1.28
Leucine	3.21	2.57	2.33	2.43
Threonine	1.18	1.3	1.25	1.3
Tryptophan	0.21	0.3	0.31	0.29
Tyrosine	0.98	0.85	0.82	0.85
Valine	1.39	1.5	1.45	1.51
Taurine ³	0.08	0.13	0.12	0.11
Fiber				
Total insoluble fiber, (%)	4.75	5.78	6.19	6.36
Total soluble fiber, (%)	0.75	0.87	2.02	1.95
Oligosaccharides, (mg/g)				
Sucrose	4.9	10.87	12.42	12.09
Raffinose	0.46	3.12	3.91	2.49
Stachyose	0.81	8.31	7.95	10.3
Verbascose	0.333	4.62	3.51	2.96
Total starch, (%)	22.03	16.95	16.77	15.89
Minerals and vitamins				
Calcium, (%)	0.9	1.06	1.01	0.92
Phosphorus, (%)	0.73	0.94	0.79	0.79
Calcium : Phosphorus	1.11	1.01	1.14	1.05
Magnesium, (%)	0.07	0.1	0.11	0.12
EPA+DHA, (%)	0.02	0.04	0.02	0.02
LA, (%)	3.52	2.53	2.56	2.85
Potassium, (%)	0.75	1.08	1.18	1.23
Sodium, (%)	0.99	1.16	1.22	1.18
Chloride, (%)	1.84	2.82	2.1	2.17
Iron, (mg/kg)	303.81	170.75	62.96	140.99
Copper, (mg/kg)	24.14	20.03	18.12	18.5
Manganese, (mg/kg)	11.03	13.77	15.81	16.79
Zinc, (mg/kg)	268.55	210.98	193.58	211.93
Iodine, (mg/kg)	2.32	2.82	2.49	2.81
Selenium, (mg/kg)	1.33	0.93	1.14	1.13
Vitamin A, (IU/kg)	16,637.28	16,807.2	21,578.4	17,600.8
Vitamin D, (IU/kg)	1419.59	1501.92	1429.68	1517.62
Vitamin E, (IU/kg)	139.25	138.57	140.3	159.84
Thiamine (B1), (mg/kg)	70.71	109.96	85.96	90.7
Riboflavin (B2), (mg/kg)	40.24	59.45	48.48	47.41
Pantothenic acid (B5), (mg/kg)	53.44	65.8	55.68	57.47
Niacin (B3), (mg/kg)	205.25	289.66	239.76	245.15
PN (B6), (mg/kg)	24.5	34.6	27.17	28.29
Folic acid (B9), (mg/kg)	2.82	4.43	3.64	3.92
Vitamin B12, (ug/kg)	0.27	0.41	0.32	0.36

Method references for items as they appear in the table: moisture (AOAC 930.15), crude protein (AOAC 992.15), crude fat (AOAC 954.02), total dietary fiber (AOAC 991.43), ash (AOAC 942.05), arginine (USDA MSS2), lysine (USDA MSS2), methionine (USDA MSS2), phenylalanine (USDA MSS2), cysteine (USDA MSS2), histidine (USDA MSS2), isoleucine (USDA MSS2), leucine (USDA MSS2), threonine (USDA MSS2), tryptophan (USDA MSS2), tyrosine (USDA MSS2), valine (USDA MSS2), taurine (USDA 6.011), total insoluble fiber (AOAC 991.43), total soluble fiber (AOAC 991.43), oligosaccharides (mg/g), total starch (AOAC 979.10), calcium (AOAC 2015.01), phosphorus (AOAC 2015.01), magnesium (AOAC 2015.01), EPA+DHA (AOAC 996.06), LA (AOAC 996.06), potassium (AOAC 2015.01), sodium (AOAC 2015.01), chloride (AOAC 983.14), iron (AOAC 2015.01), copper (AOAC 2015.01), manganese (AOAC 2015.01), zinc (AOAC 2015.01), iodine (AOAC 2012.14), selenium (AOAC 2015.01), vitamin A [analyst (1984)109:489], vitamin D (AOAC 2016.05), vitamin E (AOAC 992.03), thiamine (AOAC 942.23), riboflavin (AOAC 970.65),

pantothenic acid (AOAC 960.46), niacin (AOAC 985.34), PN (AOAC 960.46), folic acid (AOAC 960.46), vitamin B12 (AOAC 960.46).

AOAC, Association of Official Analytical Chemists; Ctl, grain-inclusive control diet; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; NFE, nitrogen-free extract; PN, pyridoxine; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; USDA, United States Department of Agriculture.

¹ NFE = 100 - (moisture + protein + fat + fiber + ash).

² Metabolizable energy = [(8.5 kcal metabolizable energy (ME) x g crude fat) + (3.5 kcal ME x g crude protein) + (3.5 kcal ME x g NFE) x 10].

³ Taurine was analyzed separately, 7 mo later.

under similar conditions. Final formulated and processed diets were analyzed to confirm the nutritional equivalency described in Table 2.

Echocardiographic examination

ECHOs were performed by a board-certified veterinary cardiologist, who was blinded to the groups and dietary treatments, using a Vivid E90 echocardiographic system (General Electric) as previously described [50]. Dogs were transported to Ontario Veterinary College Health Sciences Centre (OVC HSC, Guelph, ON) for ECHO assessment during the week -2 (pre-treatment) and week 20 (post-treatment). Cardiac assessment during the week -2 was used for treatment group allocation. Prior to ECHO, dogs received 0.2–0.4 mg/kg IV (10 mg maximum) butorphanol (Butorphanol Tartrate, 2 mg/mL, Torbugesic, Zoetis Inc.).

LVIDd and LVIDs were obtained from right parasternal short-axis M-mode; volume measurements (LVEDV and LVESV) using single plane Simpson's method of discs from a right parasternal long-axis view or left parasternal, apical 4-chamber view; and LV length was measured from the right parasternal long-axis view [41,51]. These measurements were used to calculate FS%, EF%, LVIDd, and LVIDs normalized to BW (LVIDdN and LVIDsN, respectively), LVEDV, and LVESV indexed to body surface area (LVEDVi and LVESVi, respectively), and SI [52]. In addition, the left atrial (LA) size was assessed by the left atrium to aorta ratio from a right parasternal short-axis view and the LA major dimension from a right parasternal long-axis view [53]. All measurements were obtained in triplicate with the mean value used for analysis. Simultaneous single-lead electrocardiography was performed, in addition to 9-lead electrocardiography, to

evaluate for arrhythmias and for waveform analysis (GE MAC 5500; General Electric). All formulas used to calculate respective echocardiographic parameters can be found in Table 3.

DXA

DXA was used to assess body composition and bone mineralization. The DXA technology is considered the golden standard for assessing body composition and has been validated against chemical analysis in dogs [54]. Scans were completed at the OVC HSC during weeks -2 and 20. Following their ECHO, dogs were sedated with intramuscular dexmedetomidine (2–15 ug/kg) (dexmedetomidine hydrochloride, 0.5 mg/mL; DEXDOMITOR, Zoetis Inc.). Dogs were placed in dorsal recumbency and scanned in duplicate on a fan-beam DXA device (Lunar PRODIGY Advance GE Healthcare). Total Body scanning mode was utilized with a thick setting, and estimates for lean soft tissue mass (g), fat mass (g), BMC (kg), and BMD (g/cm²) were obtained from the system software (encore version 16; GE Healthcare). Scans were completed in 10–20 min. Following the scan, dogs were administered the sedation reversal agent, atipamezole (Atipamezole hydrochloride, 5 mg/mL; ANTISEDAN, Zoetis Inc.) by intramuscular injection (0.4–1.2 ug/kg).

Blood sample collection and CBC and biochemistry analysis

Pre-study hematology CBC and biochemistry assessments were conducted on week -3, whereas baseline cTnI and NT-proBNP were assessed on week 0. After dogs were allocated to treatments, 2.5 mL of blood was collected for CBC, biochemistry, and cTnI on weeks 3, 6, 10, 14, and 18. An additional 1 mL was collected on weeks 10, 14, and 18 for NT-proBNP analysis. All blood samples were collected after a 15-h fast through cephalic venipuncture with a Vacutainer system (Becton, Dickinson and Company).

For CBC, 1 mL of blood was collected into EDTA tubes and kept on ice until submission to the Animal Health Laboratory (AHL; Animal Health Laboratory, University of Guelph, Guelph, ON). A separate 1.5 mL of blood was collected into a serum blood tube and kept on ice until centrifuged (Thermo Scientific Sorvall ST 16 Centrifuge, Thermo Fisher Scientific) at 2500 × g for 15 min at 4°C. One aliquot of 0.5 mL of serum was submitted to AHL for biochemistry analysis [calcium; phosphorus; magnesium; sodium (Na); potassium (K); Na:K ratio; albumin (A); globulin (G); A:G ratio; urea; creatinine; glucose; cholesterol; bilirubin [total (TB), direct (DB), and indirect (IB)]; alkaline phosphatase (ALP); γ-glutamyltransferase (GGT); alanine aminotransferase (ALT); aspartate aminotransferase (AST); creatine kinase; amylase; and lipase]. The remaining 0.5 mL of serum was sent to IDEXX Laboratory Inc. (IDEXX Laboratories Inc., Markham, ON, Canada) for cTnI analysis. Detectable concentrations of cTnI in serum were concentrations >0.2 ng/mL. Concentrations below the detection limit were reported as <0.2 ng/mL. For NT-proBNP analysis, an

TABLE 3

Echocardiographic parameters that are calculated after initial measurements are taken

Parameter	Calculation
LVIDdN, (cm/kg BW ^{0.294})	(LVIDd/10)/(BW ^{0.294})
LVIDsN, (cm/kg BW ^{0.315})	(LVIDs/10)/(BW ^{0.315})
FS, (%)	[(LVIDd-LVIDs)/LVIDd] x 100%
SI	LVlength/LVIDd
LVEDVi, (mL/m ²)	LVEDV/BSA ¹
LVESVi, (mL/m ²)	LVESV/BSA ¹
EF, (%)	[(LVEDV - LVESV)/LVEDV] x 100%

BSA, body surface area; BW, body weight; EF, ejection fraction; FS, fractional shortening; LVEDS, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVESVi, left ventricular end-systolic volume indexed to body surface area; LVIDd, left ventricular internal diameter in diastole; LVIDdN, left ventricular internal diameter in diastole normalized to body size; LVIDs, left ventricular internal diameter in systole; LVIDsN, left ventricular internal diameter in systole normalized to body size; SI, sphericity index.

¹ BSA = 0.101 x BW^(2/3)

additional 1 mL of blood was collected into EDTA tubes and kept on ice until centrifuged at $1200 \times g$ for 15 min at 4°C. Plasma was separated (0.5–0.75 mL) and analyzed with a canine-specific sandwich ELISA by IDEXX Laboratory Inc. NT-proBNP concentrations were reported within a 250–10,000 pmol/L range. Values <250 pmol/L were reported as <250 pmol/L. Cardiac biomarkers were always analyzed within their respective week of sample collection.

Plasma SAA, WB Tau, and glutathione analysis

During weeks –1, 2, 4, 8, 16, and 20, fasted plasma and WB were collected for analysis of the SAA; Met, Cys, homocysteine (Hcy), glutathione (GSH), and Tau as part of a larger meal response (not assessed in this paper). A fasted 5 mL of blood sample was collected via cephalic catheter from each dog into a sodium heparin vacutainer (Becton, Dickinson and Company) using a winged infusion set (Terumo Surflo Winged Infusion Set, 21 G x .75; Terumo Medical Corporation). Before centrifuging, 0.5 mL of WB was separated and placed on ice, then kept in a –80°C freezer until analysis. The remaining sample (4 mL) was centrifuged within 30 min of collection at $1200 \times g$ for 15 min at 4°C using a Beckman J6-MI centrifuge (Beckman Coulter). The plasma was separated, placed on ice, and stored in a –80°C freezer until analysis.

Plasma Met and Tau, and WB Tau concentrations were analyzed using ultra-performance LC (UPLC) [adapted from Bidlingmeyer et al. [55]; Waters Corporation]. In brief, 100 µL of 10% sulfosalicylic acid (Sigma-Aldrich) was utilized to deproteinize 100 µL plasma or WB. Prior to deproteinization, WB samples were frozen at –80°C and thawed twice to release Tau through lysis of RBC. Next, samples were centrifuged at $14,000 \times g$ for 5 min. AA standards and deproteinized samples were derivatized using an AccQ-Tag Ultra derivatization kit (Waters Corporation). The derivatized AAs 1 µL were separated in an AccQ-Tag Ultra RP Column (2.1 × 100 mm, 1.7 µm; Waters Corporation) and maintained at 55°C through UPLC with UV detection (260 nm). Met and Tau peak areas were compared with known standards, then analyzed with Waters Empower 2 Software (Waters Corporation).

Total plasma Cys, Hcy, and total GSH were analyzed using a UPLC method modified by Banton et al. [56]. First, 2 mM EDTA solution (Sigma-Aldrich) + 50 µL 8-aminonaphthalene-1,3,6-trisulfonic acid, disodium salt (Thermo Fisher Scientific) in 0.1 M K-borate (pH 9.5), and 30 µL of the reducing agent, tris(2-carboxyethyl)phosphine (Sigma-Aldrich) in 1X phosphate-buffered saline (Thermo Fisher Scientific) were added to 75 µL plasma and 75 µL the internal standard, *N*-acetyl-L-cysteine (Sigma-Aldrich) in 0.1 M K-borate (pH 9.5) + 2 mM EDTA. Samples were immediately refrigerated for 30 min. Samples were then deproteinized with 125 µL 70% perchloric acid (Sigma-Aldrich). After 10 min at room temperature, samples were centrifuged at $12,000 \times g$ for 1 min. The supernatant was removed and mixed with 100 µL of 2 M K-borate (pH 10.5) + 5 mM EDTA and 50 µL of the fluorescent thiol-specific dye, 7-fluorobenzofurazan-4-sulfonic acid ammonium salt (Sigma-Aldrich) in 0.1 M K-borate (pH 9.5) + 2 mM EDTA. Samples were then placed in a water bath maintained at 60°C for 60 min. Once this was complete, samples were immediately put on ice for 5 min before being centrifuged again in the same manner as before. Protected from the light, 100 µL was transferred to a UPLC vial

for analysis. The derivatized thiols (1 L injection) were separated in a Waters Corporation Acquity UPLC BEH C18 column (2.1 50 mm, 1.7 m) that was kept at 28°C using UPLC with fluorescence detection at 515 nm emission and 385 nm excitation. Finally, peak areas were compared with known standards and analyzed with Waters Empower 2 Software (Waters Corporation).

Statistical analysis

Fasted blood samples (NT-proBNP, SAA, hematology, and biochemistry), feed intake, and BW were analyzed as repeated measures using the PROC GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc.). Echocardiographic and DXA data were analyzed as a mixed model using the PROC GLIMMIX procedure of SAS version 9.4. Respectively, prestudy and baseline measurements for NT-proBNP, SAA, CBC, biochemistry, ECHO, and DXA data were used as a covariate in the model. Echocardiographic and DXA week 20 (post-treatment) measurements were used as the observation points to assess the effects of treatment on cardiac health and body composition, respectively. Additionally, for ECHO, NT-proBNP, SAA, and DXA data, the strength of linear, cubic, or quadratic relationships among treatments was analyzed using pre-planned orthogonal contrast statements. In all models, the dog was considered a random effect, whereas week and treatment were treated as fixed effects. The effect of treatment, week, and their interaction were evaluated for all parameters. All means were separated using the Tukey-Kramer adjustment and considered to be significant at $P \leq 0.05$.

Results

Exclusions

One dog (male, age 4, Ctl) was excluded from the ECHO assessment and DXA analysis due to behavior that prevented safe restraint for the procedures. The dog remained on study for all other outcomes except SAA analysis.

On weeks 3, 14, and 18, 3 different dogs (1 male, age 10, Pulse45; 1 female, age 7, Pulse15; 1 female, age 10, Pulse15, respectively) were omitted from blood collections for CBC and biochemistry analysis because of being prescribed antibiotics by the dog's regular veterinarian because of an open wound and infected nails, unrelated to the study. On week 10, there was insufficient blood collection for biochemistry analysis for 2 dogs (1 female, age 7, Pulse15; 1 female, age 10, Pulse45) and CBC analysis for 3 dogs (1 female, age 7, Pulse15; 1 female, age 6, Pulse30; 1 male, age 3, Pulse45). In addition, on week 10, there was no CBC analysis because of an error in sample handling.

Feed intake and BW

Feed intake and BW were not different among treatments or over time ($P > 0.05$). Mean BW (kg) for each treatment group was; Ctl = 23.70 ± 1.39 , Pulse15 = 22.65 ± 1.39 , Pulse30 = 22.41 ± 1.39 , and Pulse45 = 23.15 ± 1.39 . Mean feed intake in grams for each treatment group was; Ctl = 270.95 ± 23.72 , Pulse15 = 327.98 ± 23.72 , Pulse30 = 328.3 ± 23.72 , and Pulse45 = 291.51 ± 23.72 .

ECHO and electrocardiography

Echocardiographic and electrocardiographic parameters were not different among treatments or across time (Table 4, $P > 0.05$).

TABLE 4

Echocardiographic and electrocardiographic measurements presented as least square means $\pm$ SEM at baseline (week 0) and final (week 20) for all experimental groups [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)]

Echo parameter	Treatments					P values			
	Week ²	Ctl	Pulse15	Pulse30	Pulse45	Trt	L	Q	C
EF, (%)	0	51.83 $\pm$ 2.40	51.57 $\pm$ 2.23	51 $\pm$ 2.23	51.42 $\pm$ 2.23	0.38	0.86	0.14	0.36
	20	50.16 $\pm$ 2.40	48.57 $\pm$ 2.23	46.28 $\pm$ 2.23	50.28 $\pm$ 2.23				
FS, (%)	0	30.76 $\pm$ 1.95	29.92 $\pm$ 1.81	30.17 $\pm$ 1.81	31.68 $\pm$ 1.81	0.69	0.32	0.60	0.73
	20	29.25 $\pm$ 1.95	29.3 $\pm$ 1.81	29.41 $\pm$ 1.81	32.21 $\pm$ 1.81				
LVIDd, (mm)	0	37.18 $\pm$ 1.58	37.04 $\pm$ 1.46	38.14 $\pm$ 1.46	33.95 $\pm$ 1.46	0.64	0.86	0.21	0.91
	20	37.4 $\pm$ 1.58	36.02 $\pm$ 1.46	36.88 $\pm$ 1.46	34.8 $\pm$ 1.46				
LVIDs, (mm)	0	25.85 $\pm$ 1.44	25.78 $\pm$ 1.34	26.61 $\pm$ 1.34	23.15 $\pm$ 1.34	0.81	0.53	0.47	0.93
	20	26.48 $\pm$ 1.44	25.52 $\pm$ 1.34	26.02 $\pm$ 1.34	23.65 $\pm$ 1.34				
LVEDV, (mL)	0	59.45 $\pm$ 4.33	58.42 $\pm$ 4.00	56.72 $\pm$ 4.00	51.15 $\pm$ 4.00	0.24	0.56	0.06	0.77
	20	61.56 $\pm$ 4.33	56.12 $\pm$ 4.00	53.22 $\pm$ 4.00	52.61 $\pm$ 4.00				
LVESV, (mL)	0	28.13 $\pm$ 2.76	28.62 $\pm$ 2.55	27.98 $\pm$ 2.55	24.84 $\pm$ 2.55	0.92	0.59	0.71	0.76
	20	30.45 $\pm$ 2.76	29.32 $\pm$ 2.55	28.68 $\pm$ 2.55	26.17 $\pm$ 2.55				
LVlength, (mm)	0	68.68 $\pm$ 1.82	68.88 $\pm$ 1.69	66.41 $\pm$ 1.69	68.27 $\pm$ 1.69	0.70	0.60	0.49	0.40
	20	69.5 $\pm$ 1.82	69.34 $\pm$ 1.69	67.48 $\pm$ 1.69	68.71 $\pm$ 1.69				
SI	0	1.87 $\pm$ 0.08	1.87 $\pm$ 0.07	1.74 $\pm$ 0.07	2.03 $\pm$ 0.07	0.67	0.77	0.53	0.36
	20	1.87 $\pm$ 0.08	1.94 $\pm$ 0.07	1.78 $\pm$ 0.07	1.98 $\pm$ 0.07				
LVIDdN ¹	0	1.45 $\pm$ 0.06	1.49 $\pm$ 0.06	1.52 $\pm$ 0.06	1.33 $\pm$ 0.06	0.27	0.93	0.05	0.82
	20	1.47 $\pm$ 0.06	1.43 $\pm$ 0.06	1.46 $\pm$ 0.06	1.39 $\pm$ 0.06				
LVIDsN ¹	0	0.94 $\pm$ 0.05	0.97 $\pm$ 0.05	1 $\pm$ 0.05	0.85 $\pm$ 0.05	0.55	0.71	0.17	0.91
	20	0.97 $\pm$ 0.05	0.95 $\pm$ 0.05	0.97 $\pm$ 0.05	0.88 $\pm$ 0.05				
LVEDVi, (mL/m ²)	0	69.29 $\pm$ 4.7	73.86 $\pm$ 4.4	70.58 $\pm$ 4.4	60.61 $\pm$ 4.4	0.04	0.64	0.01	0.99
	20	73.66 $\pm$ 4.7	68.54 $\pm$ 4.4	65.14 $\pm$ 4.4	64.67 $\pm$ 4.4				
LVESVi, (mL/m ²)	0	33.15 $\pm$ 3.6	36 $\pm$ 3.11	34.27 $\pm$ 3.11	29.5 $\pm$ 3.11	0.68	0.76	0.27	0.68
	20	36.79 $\pm$ 3.6	35.82 $\pm$ 3.11	35.15 $\pm$ 3.11	32.35 $\pm$ 3.11				
LAAo	0	1.23 $\pm$ 0.04	1.28 $\pm$ 0.04	1.27 $\pm$ 0.04	1.12 $\pm$ 0.04	0.43	0.22	0.32	0.95
	20	1.15 $\pm$ 0.04	1.22 $\pm$ 0.04	1.24 $\pm$ 0.04	1.18 $\pm$ 0.04				
LAMajor (mm)	0	37 $\pm$ 1.05	36.57 $\pm$ 0.97	38 $\pm$ 0.97	38.85 $\pm$ 0.97	0.59	0.28	0.41	0.73
	20	37 $\pm$ 1.05	36 $\pm$ 0.97	38 $\pm$ 0.97	36.57 $\pm$ 0.97				
HR, (bpm)	0	111.67 $\pm$ 9.12	112.68 $\pm$ 8.44	108.57 $\pm$ 8.44	104.29 $\pm$ 8.44	0.58	0.27	0.49	0.55
	20	113.33 $\pm$ 8.70	101.43 $\pm$ 8.05	101.43 $\pm$ 8.05	97.14 $\pm$ 8.05				
QRSdur, (ms)	0	41.66 $\pm$ 2.76	42.85 $\pm$ 2.55	47.14 $\pm$ 2.55	41.42 $\pm$ 2.55	0.22	0.06	0.91	0.44
	20	40 $\pm$ 3.01	45.71 $\pm$ 2.79	48.57 $\pm$ 2.79	40 $\pm$ 2.79				
PR interval, (ms)	0	108.33 $\pm$ 5.08	114.29 $\pm$ 4.7	124.29 $\pm$ 4.7	112.86 $\pm$ 4.7	0.70	0.78	0.37	0.49
	20	105 $\pm$ 7.00	114.29 $\pm$ 6.48	115.71 $\pm$ 6.48	108.57 $\pm$ 6.48				

Ctl ($n = 6$), Pulse15 ($n = 7$), Pulse30 ($n = 7$), Pulse45 ($n = 7$).

Orthogonal contrasts; L = linear, Q = quadratic, C = cubic.

BW, body weight; Ctl, grain-inclusive control diet; ECG, electrocardiogram; EF, ejection fraction; FS, fractional shortening; HR, heart rate in beats per min (bpm); LAAO, left atrial dimension to aortic diameter ratio; LA major, left atrial dimension; LVD, left ventricle dimension length; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed to body surface area; LVIDd, left ventricular internal diameter in end-diastole; LVIDdN, diastolic dimension normalized to body size; LVIDs, left ventricular internal diameter in end-systole; LVIDsN, systolic dimension normalized to body size; PR interval, distance from onset of P wave (atrial depolarization) to onset of QRS complex; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; QRSdur, duration of the QRS complex (ventricular depolarization) on ECG; SI, sphericity index; Trt, treatment.

¹ LVIDdN is reported as cm/kg BW^{0.294} and LVIDsN is reported as cm/kg BW^{0.315}

² All week 0 measurements were used as a covariate in the statistical model.

Although the null hypothesis was rejected ($P < 0.05$) in the F-test for LVEDVi, there were no changes observed between treatment groups or weeks when pairwise comparisons were conducted using the Tukey-Kramer adjustment. Moreover, LVIDdN and LVEDVi were observed to have a quadratic trend ($P < 0.05$) across dietary pulse inclusion. All other ECHO and electrocardiographic parameters did not have any linear, quadratic, or cubic trends ($P > 0.05$) across the experimental groups.

Cardiac biomarkers

Serum cTnI concentrations remained below the detection limit of 0.2 ng/mL for all dogs throughout the duration of the study and, as such, could not be statistically analyzed. The mean NT-proBNP

concentration for all dogs was 532 ± 297 pmol/L for all time points, with concentrations ranging from the lower detectable limit of <250 to 1820 pmol/L (Table 5). Plasma NT-proBNP concentrations were not different among treatments across time ($P > 0.05$). However, there was an effect of the week, where NT-proBNP concentrations were significantly higher ($P < 0.05$; Table 5) in week 10 than in weeks 0, 14, and 18 for all treatment groups.

DXA

DXA analysis showed no differences in body composition for total body less head fat mass (kg), fat percentage, lean soft tissue mass (kg), BMC (kg), or BMD (g/cm²) between treatment groups or over time ($P > 0.05$) (Table 6).

TABLE 5
Plasma N-terminal pro-B-type natriuretic peptide concentrations (nmol/L) for all experimental diets [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)] in weeks 0, 10, 14, and 18, presented as least square means ± SEM

n	Treatment				Treatment x wk				Wk				P values				Contrasts			
	Mean ± SEM				Wk 0 ¹				Wk 10				Wk 14				Wk 18			
	21				7				7				7				7			
Ctl	567 ± 61				407 ± 68	827 ± 109	426 ± 61		373 ± 34	807 ± 55 ^A	484 ± 30 ^B	472 ± 26 ^B	0.914	<.0001	0.779	0.9216	0.5177			0.7837
Pulse15	595 ± 63				420 ± 68	801 ± 116	470 ± 61													
Pulse30	620 ± 61				339 ± 68	837 ± 109	541 ± 61													
Pulse45	568 ± 61				324 ± 68	762 ± 109	498 ± 61													

Ctl (n = 7), Pulse15 (n = 7), Pulse30 (n = 7), Pulse45 (n = 7).
Orthogonal contrasts; L = linear, Q = quadratic, C = cubic.
Ctl, grain-inclusive control diet; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; Trt, treatment.
^{A,B}Values that do not share a common letter significantly differ at *P* < 0.05.
¹ Week 0 observations were used as covariate data in the statistical model.

Fasted plasma SAA, Tau, GSH, and WB Tau

There were no treatment effects observed for Met, Cys, Hcy, Tau, or WB Tau concentrations (*P* > 0.05; Table 7). Additionally, there were no treatment-by-week interaction effects for any SAA measured (*P* > 0.05). GSH was observed to have a treatment effect by which concentrations were greater in the Pulse15 group compared to Ctl (*P* < 0.05); however, both were similar to Pulse30 and Pulse45 (*P* > 0.05). Moreover, there was an effect of time observed for Cys, GSH, Tau, and WB Tau (*P* < 0.05; Table 7). Concentrations of Cys were greatest across all groups on week 8 compared to all other weeks (*P* < 0.05), whereas week 2 had the lowest concentration but was comparable to week 4. Similarly, concentrations of GSH were greater on week 8 compared to weeks 2 and 4 (*P* < 0.05) but were similar to weeks 16 and 20 (*P* > 0.05). Plasma Tau was greatest at week 20 compared to weeks 2, 4, and 8 (*P* < 0.05) but was similar to concentrations observed on week 16 (*P* > 0.05). WB Tau was similar across all weeks, except concentrations on week 4 were different from week 2 (*P* < 0.05). Based on orthogonal contrasts, fasted plasma Met concentrations displayed a negative linear trend as pulse inclusion in diets increased (*P* < 0.05). There were no other trends observed for SAA.

Hematology

Dogs consuming Pulse15 had lower concentrations of mean corpuscular hemoglobin (MCH) compared to dogs on Pulse30 and Pulse45 (*P* < 0.05; Supplemental Table 2), but all pulse groups were similar to dogs on the Ctl diet (*P* > 0.05), and all concentrations remained within the defined reference range. There were no other treatment or interaction effects observed (*P* > 0.05; Supplemental Table 2). However, the concentrations for hemoglobin, hematocrit, mean corpuscular volume, MCH, red cell distribution width, platelets, mean platelet volume, Plateletcrit, and total solids protein differed among weeks but remained within the outlined reference ranges (AHL; Supplemental Table 2). Although the null hypothesis was rejected for weekly monocyte concentrations (*P* < 0.05; Supplemental Table 2), no significant differences between weeks were observed when pairwise comparisons were performed using the Tukey-Kramer adjustment.

Biochemistry

Biochemical analytes phosphorus, Na:K ratio, urea, creatinine, and glucose differed between the treatment groups (*P* < 0.05; Supplemental Table 3), but all values remained within the defined reference range for healthy dogs by AHL. Phosphorus concentrations were observed to be greater for dogs consuming Pulse30 and Pulse45 compared to Ctl (*P* < 0.05; Supplemental Table 3); however, all were similar to dogs eating Pulse15 (*P* > 0.05). Concentrations for urea were greater for dogs consuming Pulse15 compared to dogs fed Pulse45 (*P* < 0.05); however, both groups were similar to Ctl and Pulse30 (*P* > 0.05; Supplemental Table 3). Creatinine concentrations were greater in Ctl compared to Pulse45 (*P* < 0.05), whereas both were comparable to the other groups (*P* > 0.05; Supplemental Table 3). Lastly, glucose concentrations were observed to be greater in Ctl compared to Pulse30 (*P* < 0.05), but all were similar to Pulse15 and Pulse45 (*P* > 0.05; Supplemental Table 3). Although Na:K ratio concentrations rejected the null hypothesis (*P* < 0.05), no significant differences between treatment groups were observed when

TABLE 6

Mean body composition measurements at baseline (week 0) and final assessment (week 20) for all experimental groups [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)] presented as least square means $\pm$ SEM

	Week ¹	Treatments				P values			
		Ctl	Pulse15	Pulse30	Pulse45	Trt	L	Q	C
Fat, (kg)	0	6.04 $\pm$ 1.03	5.20 $\pm$ 0.95	4.96 $\pm$ 0.95	4.84 $\pm$ 0.95	0.4432	0.604	0.2749	0.4441
	20	5.46 $\pm$ 0.92	4.84 $\pm$ 0.86	5.62 $\pm$ 0.86	4.84 $\pm$ 0.86				
Lean, (kg)	0	16.85 $\pm$ 1.07	15.85 $\pm$ 0.99	15.83 $\pm$ 0.99	16.46 $\pm$ 0.99	0.5662	0.9025	0.3482	0.5127
	20	16.09 $\pm$ 1.07	15.49 $\pm$ 0.99	15.80 $\pm$ 0.99	15.72 $\pm$ 0.99				
BMC, (kg)	0	0.76 $\pm$ 0.07	0.67 $\pm$ 0.06	0.66 $\pm$ 0.06	0.75 $\pm$ 0.06	0.3739	0.1046	0.4457	0.8132
	20	0.78 $\pm$ 0.04	0.68 $\pm$ 0.04	0.67 $\pm$ 0.04	0.68 $\pm$ 0.04				
BMD, (g/cm ²)	0	0.71 $\pm$ 0.04	0.65 $\pm$ 0.04	0.63 $\pm$ 0.04	0.69 $\pm$ 0.04	0.3078	0.0849	0.5315	0.6057
	20	0.71 $\pm$ 0.02	0.68 $\pm$ 0.02	0.65 $\pm$ 0.02	0.67 $\pm$ 0.02				

Ctl ($n = 6$), Pulse15 ($n = 7$), Pulse30 ($n = 7$), Pulse45 ($n = 7$).

Orthogonal contrasts; L = linear, Q = quadratic, C = cubic.

BMC, bone mineral content; BMD, bone mineral density; Ctl, grain-inclusive control diet; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; Trt, treatment.

¹ All week 0 measurements were used as a covariate in the statistical model.

pairwise comparisons were performed using the Tukey-Kramer adjustment. There were no treatment-by-week interaction effects observed for any biochemical analyte ($P > 0.05$; [Supplemental Table 3](#)). However, the concentrations for calcium, sodium, potassium, chloride, carbon dioxide, anion gap, Na:K ratio, total protein, albumin, globulin, A:G ratio, creatinine, glucose, cholesterol, total, conjugated and free bilirubin, GGT, creatine kinase, and lipase differed among weeks ($P < 0.05$; [Supplemental Table 3](#)). Similar to CBC results, the mean concentrations of all analytes remained within the established reference ranges for healthy animals.

Discussion

The results from this study suggest that moderate protein diets containing up to 45% whole pulse ingredients, specifically the combination of green and yellow peas, pinto beans, chickpeas, and lentils, have no adverse effects on cardiac health, fasted SAA concentrations, body composition, and gross health indicators in healthy adult dogs as per the assessment methods used in this study after 20 wks of feeding. In addition, regardless of the amount of pulses consumed, none of the dogs in the current study revealed echocardiographic or electrocardiographic changes that could indicate the development of DCM after 20 wks.

The pea starch used in the present study was from dry fractionated dehulled pea flour, compared to pea starch from wet fractionation, which can contain higher protein concentrations. In this study, in addition to assisting with meeting energy requirements and based on the proximal analysis of pea starch outlined in the Methods section, this ingredient provided ~ 0.6 , 6%, 4%, and 0.6% crude protein to the Ctl, Pulse15, Pulse30, and Pulse45 diets, respectively. This demonstrates that using dry-fractionated pea starch assisted in providing protein equivalency, particularly for the Pulse15 and Pulse30 diets ([Table 1](#)). It is also relevant to note that pulse starches generally have higher amylose-to-amylopectin ratios compared to cereal starch [57], which may also facilitate the presence of resistant starch, which can evade digestion [58]. However, despite differences in amylose content, pea, and cereal starch are both $>98\%$ digestible by dogs [11]. Again, although pea starch was also added to the control diet, the amount of inclusion was very low (2.2%)

compared to the starch provided by whole grain corn and corn gluten meal, which, as ingredients, contain $>65\%$ starch and $>15\%$ starch, respectively.

Despite the use of pea starch across diets, given the low inclusion rates, it is improbable that pea-derived starch or protein concentrations would have been a dietary confounder for metabolic processes that would facilitate or mitigate the development of DCM. This is supported by results in the present study demonstrating no dose-related effects on metabolic outcomes, regardless of the concentration of the pea starch ingredient included in the diets. Dry fractionated pea starch is a common ingredient in commercial grain-containing and GF dog foods. Proposed hypotheses around pulses and associated metabolic perturbations leading to the development of DCM have been related to the presence of whole pulse ingredients that concurrently contain protein, fiber, and other minor constituents [30]. Nevertheless, further investigation is warranted to confirm the effect of pea starch on metabolic outcomes under the context of cardiac function.

Previous clinical studies were unable to investigate whole pulse ingredients as the single variable causative factor; however, current data suggest that including pulse ingredients in dog food is not a causative factor of DCM in dogs who are not genetically predisposed. Adin et al. [33] retrospectively compared echocardiographic measurements in multiple dog breeds diagnosed with DCM and reported that those consuming pulse-inclusive diets had greater LVIDs and LVIDd, along with a lower SI compared to those on grain-inclusive diets. Similarly, Kaplan et al. [32] reported improvements in echocardiographic FS, EF, LVIDd, and LVIDs in Golden Retrievers with Tau deficiency and concurrent DCM when switched onto a pulse-devoid and grain-inclusive diet with Tau supplementation. Freeman et al. [35] put dogs with DCM originally consuming either a legume/potato inclusive diet or a traditional grain-inclusive diet onto an option of grain-inclusive intervention diets and found a greater improvement after 9 mo in echocardiographic FS, LVIDdN, and LVIDsN for dogs who started with a legume inclusive diet. The Freeman et al. [35] study also included breeds highly susceptible to DCM, such as Dobermans, who have their own cardiac reference ranges for DCM [59]. Dogs in the current study were consuming a grain-inclusive diet for 4 wks prior to

TABLE 7

Concentration (nmol/mL) of fasted plasma methionine, homocysteine, cysteine, glutathione, taurine, and whole blood taurine for all experimental groups [Ctl (0% inclusion), Pulse15 (15% inclusion), Pulse30 (30% inclusion), Pulse45 (45% inclusion)] at prestudy and weeks 2, 4, 8, 16, and 20 presented as least square means $\pm$ SEM

	Fasted plasma SAA (nmol/mL)	Treatment	Treatment x wk						Wk						P values			Contrasts		
		Mean	Prestudy ¹	Wk 2	Wk 4	Wk 8	Wk 16	Wk 20	Prestudy ¹	Wk 2	Wk 4	Wk 8	Wk 16	Wk 20	Trt	Wk	Trt x Wk	L	Q	C
Met									47 $\pm$ 1.9	42 $\pm$ 1.6	43 $\pm$ 1.6	40 $\pm$ 1.6	43 $\pm$ 1.7	47 $\pm$ 1.7	0.1067	0.0567	0.6806	0.0337	0.2693	0.426
Ctl		47 $\pm$ 2.1	48 $\pm$ 4.0	47 $\pm$ 3.5	49 $\pm$ 4.6	43 $\pm$ 3.5	49 $\pm$ 3.7	49 $\pm$ 3.4												
Pulse15		41 $\pm$ 2.1	47 $\pm$ 3.9	39 $\pm$ 3.4	43 $\pm$ 3.5	37 $\pm$ 3.4	41 $\pm$ 3.5	47 $\pm$ 4.1												
Pulse30		42 $\pm$ 1.9	48 $\pm$ 3.7	43 $\pm$ 3.2	40 $\pm$ 3.3	41 $\pm$ 3.2	42 $\pm$ 3.4	43 $\pm$ 3.2												
Pulse45		40 $\pm$ 2.0	44 $\pm$ 3.7	39 $\pm$ 3.5	39 $\pm$ 3.4	37 $\pm$ 3.2	40 $\pm$ 3.2	47 $\pm$ 3.2												
Hcy									38 $\pm$ 2.5	31 $\pm$ 2.4	32 $\pm$ 2.4	34 $\pm$ 2.4	32 $\pm$ 2.5	35 $\pm$ 2.5	0.8715	0.7092	0.7748	0.444	0.7383	0.8329
Ctl		35 $\pm$ 2.9	46 $\pm$ 5.2	32 $\pm$ 5.1	38 $\pm$ 5.1	37 $\pm$ 5.1	29 $\pm$ 5.0	38 $\pm$ 5.1												
Pulse15		33 $\pm$ 2.8	39 $\pm$ 5.2	31 $\pm$ 5.0	34 $\pm$ 5.0	35 $\pm$ 5.0	31 $\pm$ 5.0	35 $\pm$ 6.1												
Pulse30		32 $\pm$ 2.7	27 $\pm$ 4.8	31 $\pm$ 4.7	31 $\pm$ 4.7	36 $\pm$ 4.7	29 $\pm$ 5.1	30 $\pm$ 4.7												
Pulse45		32 $\pm$ 2.6	39 $\pm$ 4.8	28 $\pm$ 5.0	27 $\pm$ 5.0	29 $\pm$ 4.6	40 $\pm$ 4.6	35 $\pm$ 4.6												
Cys									519 $\pm$ 16.7	592 $\pm$ 16.7 ^D	651 $\pm$ 16.7 ^{CD}	828 $\pm$ 16.4 ^A	707 $\pm$ 17.2 ^{BC}	732 $\pm$ 17.5 ^B	0.4772	<0.0001	0.4895	0.2057	0.4317	0.6202
Ctl		685 $\pm$ 15.2	501 $\pm$ 34.8	561 $\pm$ 34.2	590 $\pm$ 34.2	861 $\pm$ 34.2	694 $\pm$ 37.5	717 $\pm$ 34.2												
Pulse15		698 $\pm$ 16.4	465 $\pm$ 34.8	594 $\pm$ 34.5	681 $\pm$ 34.5	797 $\pm$ 34.5	727 $\pm$ 34.5	693 $\pm$ 42.2												
Pulse30		717 $\pm$ 14.2	554 $\pm$ 32.2	609 $\pm$ 31.7	693 $\pm$ 31.7	807 $\pm$ 31.7	693 $\pm$ 34.2	785 $\pm$ 31.7												
Pulse45		708 $\pm$ 14.4	555 $\pm$ 32.2	604 $\pm$ 34.3	639 $\pm$ 34.3	846 $\pm$ 31.8	715 $\pm$ 31.8	734 $\pm$ 31.8												
GSH									6 $\pm$ 0.3	6 $\pm$ 0.3 ^B	7 $\pm$ 0.3 ^B	8 $\pm$ 0.3 ^A	7 $\pm$ 0.3 ^{AB}	7 $\pm$ 0.3 ^{AB}	0.0257	0.0019	0.4453	0.0896	0.0505	0.0516
Ctl		6 $\pm$ 0.4 ^B	5 $\pm$ 0.7	5 $\pm$ 0.6	5 $\pm$ 0.6	6 $\pm$ 0.6	6 $\pm$ 0.7	6 $\pm$ 0.6												
Pulse15		8 $\pm$ 0.4 ^A	6 $\pm$ 0.6	7 $\pm$ 0.6	7 $\pm$ 0.6	8 $\pm$ 0.6	9 $\pm$ 0.6	9 $\pm$ 0.7												
Pulse30		7 $\pm$ 0.4 ^{AB}	5 $\pm$ 0.6	7 $\pm$ 0.6	6 $\pm$ 0.6	8 $\pm$ 0.6	7 $\pm$ 0.6	7 $\pm$ 0.6												
Pulse45		7 $\pm$ 0.4 ^{AB}	6 $\pm$ 0.6	6 $\pm$ 0.6	8 $\pm$ 0.6	8 $\pm$ 0.6	7 $\pm$ 0.6	7 $\pm$ 0.6												
Tau									106 $\pm$ 6.7	108 $\pm$ 7.1 ^{BC}	101 $\pm$ 7.1 ^C	94 $\pm$ 7.0 ^C	129 $\pm$ 7.2 ^{AB}	140 $\pm$ 7.3 ^A	0.9543	0.0003	0.8025	0.9429	0.8131	0.631
Ctl		115 $\pm$ 9.6	111 $\pm$ 14.0	98 $\pm$ 14.6	107 $\pm$ 14.6	88 $\pm$ 14.6	129 $\pm$ 15.6	152 $\pm$ 14.6												
Pulse15		113 $\pm$ 9.8	99 $\pm$ 13.6	113 $\pm$ 14.6	98 $\pm$ 14.6	85 $\pm$ 14.6	128 $\pm$ 14.6	138 $\pm$ 17.2												
Pulse30		118 $\pm$ 8.9	106 $\pm$ 13.0	109 $\pm$ 13.5	106 $\pm$ 13.5	101 $\pm$ 13.5	133 $\pm$ 14.2	143 $\pm$ 13.5												
Pulse45		112 $\pm$ 9.0	107 $\pm$ 13.0	112 $\pm$ 14.4	94 $\pm$ 14.3	102 $\pm$ 13.5	123 $\pm$ 13.5	127 $\pm$ 13.5												
WB Tau									191 $\pm$ 8.7	188 $\pm$ 9.4 ^{AC}	157 $\pm$ 9.2 ^B	169 $\pm$ 9.0 ^{AB}	192 $\pm$ 9.5 ^{AB}	188 $\pm$ 9.9 ^{AB}	0.6774	0.0211	0.2327	0.844	0.7415	0.2385
Ctl		173 $\pm$ 10.2	196 $\pm$ 19.3	203 $\pm$ 20.0	118 $\pm$ 20.0	163 $\pm$ 20.0	191 $\pm$ 22.4	191 $\pm$ 22.5												
Pulse15		188 $\pm$ 9.7	167 $\pm$ 17.6	218 $\pm$ 20.0	159 $\pm$ 8.3	179 $\pm$ 18.5	199 $\pm$ 18.5	185 $\pm$ 22.7												
Pulse30		173 $\pm$ 9.0	222 $\pm$ 16.3	169 $\pm$ 17.2	167 $\pm$ 17.1	160 $\pm$ 17.2	186 $\pm$ 18.7	181 $\pm$ 17.2												
Pulse45		181 $\pm$ 8.7	177 $\pm$ 16.3	163 $\pm$ 18.4	183 $\pm$ 18.4	173 $\pm$ 17.0	193 $\pm$ 17.0	193 $\pm$ 17.0												

Ctl ($n = 6$ at each week), Pulse15 ($n = 7$ for all weeks except week 20, $n = 5$), Pulse30 ($n = 7$ for all weeks, except week 16, $n = 6$), Pulse45 ($n = 6$ on weeks 2, 4, $n = 7$ on weeks 8, 16, 20). Orthogonal contrasts; L = linear, Q = quadratic, C = cubic.

Ctl, grain-inclusive control diet; Cys, cysteine; GSH, reduced glutathione; Hcy, homocysteine; Met, methionine; Pulse15, diet with 15% pulse inclusion; Pulse30, diet with 30% pulse inclusion; Pulse45, diet with 45% pulse inclusion; SAA, sulfur amino acid; Tau, taurine; WB, whole blood.

^{A-D} Values with A, B, C, D superscripts within the same category and row are significantly different ($P < 0.05$)

¹ Prestudy observations were used as covariate data in the statistical model.

being put on 1 of 4 experiment diets, and regardless of the switch from pulse-free to pulse-inclusive diets, dogs did not experience changes in echocardiographic measurements after 20 wks of feeding. Although quadratic trends were observed for LVEDVi and LVIDdN, there were no significant changes between treatment groups or over the 20-wk period that would suggest the development of DCM. In the case of DCM, dilation of the left ventricle would be observed; however, both Pulse15 and Pulse30 groups had smaller measurements than baseline, whereas Ctl and Pulse45 were larger. Minimal changes that were observed in all groups for LVEDVi and LVIDdN are not clinically relevant, especially considering that all other echocardiographic parameters were similar across all treatment groups and displayed no significant changes or trends.

Previous studies also had a number of confounding variables making it difficult to identify if the changes observed through echocardiographic assessments in the dogs were solely linked to the pulse inclusion in the diets. During these studies [32,33,35], Tau supplementation and/or cardiac medication (e.g., pimobendan, ACE inhibitors, or furosemide) were utilized, which are expected to improve cardiac function regardless of nutritional intervention [60,61]. Additionally, the nutrient composition of diets consumed by the dogs in these studies was not analyzed or controlled. Because of the differences in the formulation of GF diets evaluated by Kaplan et al. [32] and Adin et al. [33], it is difficult to establish a cause-and-effect relationship that is reflective of pulse ingredients alone on cardiac health in retrospective analyses. The criteria for grain-inclusive diet intervention, as explored by Freeman et al. [35], could also impact outcomes. Although not reported, owners were allowed to select diets with varying ingredient compositions and macro and micronutrient densities. The current study controlled for macro and micronutrient composition among treatments with the exception of total dietary fiber and aimed to ensure that food intake was adjusted to maintain BW and observed no effect of pulse ingredient or lack of grain on cardiac health. Future research should consider investigating individual dietary nutrients that may differ among pulse and grain-based dog foods.

In addition to observed cardiac function, biomarkers were normal among all dietary treatments in the current study. Serum cTnI did not exceed the healthy upper reference limit of 0.2 ng/mL for any dog throughout the duration of this study. As a marker of myocardial injury, serum cTnI is more sensitive in detecting myocardial abnormalities than other forms of troponin or other cardiac biomarkers [62–64]. A positive correlation between cTnI and the prevalence of DCM exists [64,65,66] and would suggest that cardiac dysfunction would be reflected through elevated cTnI concentrations. Healthy canine serum cTnI concentrations can range from 0.10–1.9 ng/mL, with a median concentration of 0.2 ng/mL, which is similar to reference ranges utilized in this study [62,67,68]. However, it is possible that minor fluctuations or differences in the serum cTnI concentrations occurred but were not detected because of the limited sensitivity of the assay used. In the future, high-sensitivity cTnI assays may be able to capture smaller fluctuations. The second cardiac biomarker, NT-proBNP, is secreted by the atrial and ventricular myocardium in response to increased myocardial strain or pressure or ventricular dilatation, and as such, it is a well-established biomarker for chronic cardiac dysfunction [69–72]. In the present study, plasma NT-proBNP

did not differ between treatment groups over the 20-wk feeding period, suggesting that varying inclusions of pulses did not induce strain on the myocardial muscle. There was no observed difference in concentrations between the treatment groups; however, there was an increase in NT-proBNP during week 10 in all dogs regardless of dietary treatment compared to concentrations during weeks 0, 14, and 18. This elevation during week 10 coincided with a heatwave during which dogs likely increased their water intake to maintain hydration as they were housed outdoors and had ad libitum access to drinking water. Increases in blood volume through increased water intake can cause the cardiac muscles to stretch and consequently excrete more NT-proBNP [73,74]. Individual dogs that experienced concentrations above the reference limit were monitored for clinical signs, and cTnI measurements were used as a supporting biomarker to assure dogs were not experiencing a cardiac injury. With the absence of clinical symptoms and the normal cTnI concentrations, it is possible that NT-proBNP fluctuations were in response to normal behavioral changes for heat regulation. Plasma NT-proBNP assessed during weeks 14 and 18 were similar to week 0, further suggesting that there was no interaction effect on cardiac function. Although NT-proBNP may be beneficial to complement echocardiographic or cTnI measurements, it may not be a reliable standalone marker for cardiac stress because of inter-breed variations [65,69,75,76] and transient fluctuations from environmental conditions [75,77]. Research is warranted to develop a standard NT-proBNP reference range for various breeds, including Siberian Huskies, dogs housed outdoors, and performance dogs. When using NT-proBNP as a marker of cardiac function, consideration should also be given to the environment as a confounding variable.

As nutritionally-mediated DCM is a concern in seemingly healthy breeds, the low Met content of pulses [7,78] has been suggested as a potential mechanism for Met and subsequent Tau deficiency previously linked to DCM [25,79,80]. A diet lacking adequate protein can elevate the risk of low Tau concentrations and result in the development of DCM. Previously, Tau deficiency related to DCM was reported in dogs who were consuming diets containing 0.03%–0.09% Tau [81]. As aforementioned, the protein content of all experimental diets and, consequently, SAA concentrations in the current study exceeded AAFCO recommendations, whereas the Tau content for all diets was 0.08%–0.13% on a dry-matter basis. In the current study, after 20 wks of feeding, the fasted plasma Met, Hcy, and Tau concentrations were similar between the treatment groups, and all dog's Tau concentrations were well above concentrations related to a deficiency (<40 nmol/mL; [35]). Moreover, although not statistically significant, it is important to note that Cys concentrations are not as stable as other SAA upon collection; thus, these results should be interpreted with caution as they may overestimate the concentrations [80]. In a recent study, Beagles supplemented with double the AAFCO requirement of Met were observed to have similar plasma Tau concentrations as dogs fed a commercially available GF diet [56]. In contrast, in the current study, Met was not supplemented in experimental diets but still exceeded the AAFCO minimum requirement of 0.33%. However, because of the inherent nutrient composition of pulse ingredients, Met concentrations in the diets linearly decreased as pulse inclusion increased. Despite this, similar to Banton et al. [56], no differences in Tau concentrations were observed across

treatment groups, suggesting that Met is not the only factor directly affecting Tau status in the body.

Pulses have been used in the pet food industry for years; therefore, another focus of this study was to investigate the holistic implications of dietary pulses on canine health. Loss of lean body mass (LBM) and sarcopenia are processes that occur naturally in dogs with aging, but also because of an AA deficiency or the presence of anti-nutritional factors. Insufficient protein synthesis because of the bioavailability of proteogenic AAs, such as Met or Cys [82], and muscle wasting has been associated with DCM [83,84]. Kaplan et al. [32] noted that Golden Retrievers described as having ideal body weights were often underfed based on the author's calculations for metabolizable energy requirements. Most dogs were fed GF diets not intended for weight loss [32], and the restriction of feed can contribute to nutrient deficiencies, including some AAs over time [85,86]. Inadequate amounts of indispensable AA reduces protein synthesis and can result in muscle degradation or loss of LBM [87,88]. This is a considerable focus for future research as dogs who are prone to obesity, such as Golden Retrievers [89], maybe underfed with a diet not intended for weight loss and subsequently be susceptible to comorbidities related to nutrient inadequacy. In some cases, dogs do not lose weight when sarcopenia occurs; thus, utilization of DXA tools that can detect changes in lean mass with just a 0.3% coefficient of variation [90] can assist in the early diagnosis of such comorbidities. Overall, the body composition between the treatment groups and over time in this study which altered <0.1% from baseline, suggests that dogs maintain LBM when fed pulse-containing diets that are provided in amounts to maintain BW for 20 wks. This further supports the results of the SAA explored earlier, suggesting that pulses in a micro and macronutrient-balanced diet do not result in low plasma SAA concentrations and do not inhibit protein turnover when fed for 20 wks.

Moreover, CBC indices were within defined healthy reference ranges across all treatment groups throughout the study, elucidating that pulse inclusion does not have adverse health effects. Red cell indices such as red blood cell (RBC) count, hemoglobin, and MCH are indicators of hematological disorders, specifically different types of anemias in humans and dogs [91,92]. Contradictory to the findings from the current study, Bakke et al. [93] found that dogs eating a GF, pulse-inclusive diet consisting of 60% pulses (split peas and red and green lentils) had reduced RBC count and hemoglobin compared to dogs on a pulse-free diet. In contrast to the current study, where all treatment diets were balanced for macronutrients, Bakke et al. [93] described the treatment diet as having higher protein, fat, and double the crude fiber content of their control diet. Simultaneous modification of various nutrients induces significant challenges in identifying the dietary factors causing changes in hematological measurements. In the present study in which treatment diets were balanced for macronutrients, there were no changes in RBC count and hemoglobin. However, dogs consuming Pulse30 and Pulse45 had greater concentrations of MCH than dogs consuming Pulse15, but all pulse groups had similar MCH concentrations to the dogs on the Ctl pulse-devoid diet. Human research suggests that decreased MCH, a measure of mean hemoglobin capacity in red cells, accompanied by increased red cell distribution width, a measurement of red cell size, is an indication of myocardial infarction [94,95]. Although dogs

consuming greater concentrations of pulses had higher MCH than dogs on Pulse15 because all groups had comparable concentrations to Ctl and because all values were within the reference range, the small differences are not likely biologically relevant in regard to the implications of pulses on red cell indices.

Concentrations of creatinine and urea are affected by dietary protein intake and can be reflective of renal function [96]. Beagles who consumed a commercial high protein (41% crude protein) diet for 7 d had greater concentrations of blood urea and creatinine than their counterparts consuming a moderate protein (24% crude protein) diet [97]. Although all diets in the current study met similar crude protein targets, there were still differences observed among creatinine and urea concentrations. Both metabolites are involved and affected by myriad pathways in the body, and although it is possible that plasma concentrations of these indices can be used to assess kidney function [98,99] because all values were within their respective reference ranges, the differences seen in the present study are likely not biologically relevant. Overall, all biochemistry indices remained within the outlined reference ranges for healthy dogs (AHL); thus, differences observed among any analyte are likely not biologically significant.

The data from the present study are limited to assessing the combination of green and yellow peas, pinto beans, chickpeas, and lentils up to 45% inclusion in macronutrient-balanced diets that exceed AAFCO nutrient recommendations on cardiac health and therefore are not indicative of any other pulse ingredients or pulse ingredient combinations. To our knowledge, this is the longest controlled feeding study to date that assesses cardiac health in healthy adult dogs fed pulse-inclusive diets. Utilizing Siberian Huskies as the experimental group allowed for assessing pulse ingredients in healthy medium-large breed dogs while controlling for confounding effects of genetic predisposition. However, because of their genetic selection for performance, sled dogs constitute a genetically unique population [100,101], which may limit the results from being applied to the average sedentary household dog or breeds susceptible to cardiac disease.

This research focused on the effects of up to 45% whole pulse inclusion in canine extruded diets on cardiometabolic health and when no essential nutrients are potentially in deficit. Ensuring nutrient sufficiency in all diets allowed us to specifically ask the question of whether whole pulse ingredients, specifically the combination of green and yellow peas, pinto beans, chickpeas, and lentils, have inherent characteristics not related to their nutritional density and nutrient digestibility that may be a causative factor in the development of adverse cardiometabolic changes. Although not the objective of the current study, future research could also investigate the effects of pulse ingredients in diets formulated just to meet AAFCO recommendations rather than exceed them. Specifically, investigate how pulse ingredients play a role in SAA content in diets when protein is not formulated in excess. Indeed, research from our laboratory suggests that AA requirements are greater than the NRC-recommended allowance and AAFCO recommendations [31,102,103]. It is well established that Tau deficiencies could contribute to nutritionally-mediated DCM in dogs; therefore, the role of metabolic intermediates such as methyl donors folate and betaine, methyl receivers, carnitine, creatine, or cofactors, and vitamins B6 and B12 should be investigated to

understand their potential roles in DCM development better. Exploring diets that do not include animal-based protein, such as chicken, should also be explored in subsequent experiments. Replicating this study with other large breed dogs could further contribute to our understanding of these diets among phenotypes. Finally, investigating how processing methods affect the bioavailability of nutrients for pulse ingredients, considering individual dog genetics, lifestyle, age, and sex, are potential future avenues of research.

The current study suggests that dietary inclusion of up to 45% whole pulse ingredients, specifically green and yellow peas, pinto beans, chickpeas, and lentils in macro and micronutrient-balanced diets, do not have detrimental effects on cardiac health, body composition, SAA status or CBC and biochemistry analytes in healthy adult dogs after 20 wks of feeding. Singular ingredients will not provide every nutrient required to maintain health; as such, it is important to focus on comprehensive diet formulations that can satisfy nutritional requirements. Like humans, dogs need a mixture of ingredients to achieve all necessary macronutrient and micronutrient needs. Ultimately, pulses are a dependable protein alternative in the food industry, and this study emphasizes that they do not have adverse cardiac effects in healthy adult dogs when incorporated at concentrations as high as 45% in diets that exceed AAFCO requirements.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tjnut.2023.03.027>.

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