

**A Multimodal Investigation into the Suitability of Plant-based Diets for
Companion Dogs and Cats**

by

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ABSTRACT

A MULTIMODAL INVESTIGATION INTO THE SUITABILITY OF PLANT-BASED DIETS FOR COMPANION DOGS AND CATS

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Dogs and cats were domesticated from wild carnivores, though some owners feed plant-based diets. The impact of excluding animal-derived ingredients from the canine or feline diet is not currently well known. The research presented here included three studies investigating the sufficiency of plant-based diets for dogs and cats: a survey of owner perception of health and wellbeing in dogs and cats fed plant-based or animal-containing diets, analysis of nutrients in commercial plant-based diets available in Ontario, and a diet trial comparing an experimental plant-based diet to a conventional commercial animal-containing diet in client-owned dogs.

Responses from 1,413 dog owners and 1,325 cat owners across Canada and the USA revealed similar results for both dogs and cats. Owners reported fewer perceived health disorders, specifically with respect to gastrointestinal and hepatic conditions. More owners of cats fed plant-based diets believed their cats to be in ideal body condition and very good health. Lifespan of dogs fed plant-based diets appeared to be longer, no difference in lifespan was reported for cats.

Analyses of 26 commercial plant-based diets revealed sulfur amino acids, taurine, arachidonic acid, EPA + DHA, calcium, phosphorus and vitamin D to be consistently below industry recommendations. Four products labelled for adult dogs met minimum recommendations for canine maintenance, no diet met minimum recommendations for feline maintenance or growth of puppies or kittens.

All 61 dogs completing the diet trial maintained body weight and composition, and measured indices of health and wellness. Reductions in platelet count, branched-chain amino acids, creatinine, blood urea nitrogen, cholesterol and ratio of omega-6 to omega-3 fatty acids were detected in dogs fed the plant-based diet, though values were maintained within the normal reference ranges. A shift from vitamin D₃ to D₂ metabolites occurred, though total vitamin D analogues, ionized calcium and bone mineralization were unaffected.

The findings of this research provide veterinarians with insight into the perspectives of pet owners to improve communications regarding pet health and nutrition, and demonstrate to nutritionists areas requiring improvement in this industry niche. Finally, further investigations of impacts of plant-based diets on canine health are proposed.

DEDICATION

I dedicate this thesis to my husband, Richard Dodd, for his patience and support, without which I would have been unable to complete this work, and to my family for nurturing my dreams and giving me the opportunity to pursue my passion. Grannie, I wish you were here to see me finally finish this. I hope I made you proud.

ACKNOWLEDGEMENTS

My deepest gratitude to Dr. Adronie Verbrugge for bringing my research ideas to life and guiding me through both my academic studies and clinical training. I brought my early thoughts to you before I had even finished my veterinary degree, and you cultivated these into full projects and programs spanning over five years, teaching me how to build my studies from the ground up. I am grateful for your expertise and patience that allowed me to learn so much more than I expected. I cannot imagine any other PhD or residency supervisor could have brought to my studies what you have, and there's no one else I would have rather worked with than you. Thank you.

A sincere thank you is deserved as well by Dr. Sarah Abood for her mentorship and guidance starting during the middle of my studies and continuing long after you moved on from the Ontario Veterinary College. Your sincere enthusiasm and infectious positivity were truly appreciated, as you stepped into an advisory role and took me on during some of the most challenging periods of my studies. Together, we forged our way from personal crises to a global pandemic, and I'm still not sure which was worse. I know I will continue to learn and grow with your support for years to come. Thank you.

I would be remiss not to acknowledge my first research supervisor, Dr. Chris Riley, who introduced me to the idea of pursuing post-graduate education when I was still in my second year of vet school. Following from this, Dr. Becca Leung and Dr. Nick Cave gave me the confidence to pursue my passion in the field of nutrition and pushed me to pursue a combination of clinical training and academic study.

My advisory committee have guided my work from the first day of my PhD, and for their assistance, oversight and advise I am grateful. Dr. Cate Dewey acted as co-advisor with Dr. Adronie Verbrugghe and brought essential epidemiological experience to the team. Dr. Deep Khosa kindly guided the projects, especially the survey-based ones, and provided much insight and access to resources. Dr Jennifer Adolphe was a key figure in securing our relationship with Petcurean, producing the trial diets and acquiring post-production nutrient analyses of the trial diets. I have such appreciation for each of these advisors for their contributions to my thesis and development as a young researcher. Thank you.

Throughout these research projects presented in this thesis, partnerships between the OVC Nutrition Team and pet food industry were made, including Vecado and Petcurean, who, in conjunction with MITACS and NSERC provided the financial support for my research. The OVC Pet Trust also provided generous financial support. Without these partnerships and organizations, this research would not have been able to take place.

Lastly, as found in the Dedication, I have utmost appreciation and gratitude to my husband and family for their constant support and patience over the last thirteen years of my post-secondary education. My parents instilled in me my love for all living beings, leading to my pursuit of veterinary medicine, as well as my drive to pursue the answers to all my questions - and to find more questions when the first ones are answered. Your belief in me and constant support allowed me to find my way to where I am today. Thank you. Richard: you've pushed me further than anyone else to continue to grow and develop as a person. I barely recognize myself today from who I was back in my undergrad when we first met. Thank you for always inspiring

me to better myself. You have patiently waited for my education to finish for about a decade now and I can finally say I'm done... for now.

STATEMENT OF WORK

Study design

All research ideas were my original conceptions, shaped into projects by Dr. Adronie Verbrugghe. With Dr. Verbrugghe's assistance, I designed and submitted all Research proposals, research ethics applications and animal use protocols. The projects described in Chapters Three and Four were approved by the Research Ethics Board under Research Ethics Approval number 18-07-039. The projects described in Chapters Six and Seven were approved by the Research Ethics Board under Research Ethics Approval number 19-02-036 and by the Animal Care Committee under Animal Utilization Protocol number 4129. In addition, Dr. Deep Khosa assisted with design of the qualitative study (Chapters Three and Four), while Dr. Jennifer Adolphe of Petcurean Pet Nutrition was heavily involved in design of the feeding trial (Chapters Six and Seven). Dr Adolphe formulated the experimental diet and oversaw manufacturing of the diets. Both trial diets, the experimental and the commercial control, were donated by Petcurean Pet Nutrition as in-kind contribution.

Data collection

I designed the survey for Chapters Three and Four, with feedback from Dr. Verbrugghe and Dr. Khosa, I promoted and distributed the survey to pet owners across Canada and the USA by utilizing retailers and social media groups. The plant-based pet food distributor Vecado helped to promote and distribute the survey to their customers within Canada. I collected all survey data. I contacted companies and acquired diet samples for nutrient analyses (Chapter Five). I prepared samples and analyzed fatty acids by gas chromatography in the Human Health and Nutritional Sciences laboratory under the guidance of Dr. David Ma. With Dr. Verbrugghe's

guidance, I identified laboratories to conduct analyses that could not be performed in house and prepared and submitted samples to them. I designed the enrolment survey for the diet trial (Chapters Six and Seven) with assistance from Dr. Verbrugghe. I met with participants, examined dogs, collected samples and conducted the feeding trial.

Data analysis

I conducted all data entry, cleaning, organization, analysis and storage. Input was sought from Dr. Khosa specifically for analysis of the survey data (Chapters Three and Four), and from Dr. Cate Dewey for analysis of the survey data (Chapters Three and Four) and trial data (Chapters Six and Seven). All data was organized into Excel spreadsheets (Microsoft® Excel for Mac, 2019) and analyzed in Stata (Stata/IC 15.1 for Mac, 2018), with input from Drs. Verbrugghe, Khosa and Dewey.

Presentation of results

I prepared all chapters presented in this thesis. Initial manuscripts were edited with feedback from Dr. Verbrugghe, following which each was sent to my advisory committee: Drs. Dewey, Khosa and Adolphe for their feedback. Three chapters were submitted as manuscripts for publication prior to submission of the thesis. Chapter Three was undergoing peer review and Chapters Four and Five published at the time of thesis submission. Chapter Five received additional feedback from additional co-authors, Drs. Anna Shoveller, Andrea Fascetti, Zengshou Yu and Ma, prior to publication.

Data were also presented as posters at international conferences. Findings from Chapters Three and Four were presented at the European Society for Veterinary and Comparative

Nutrition in 2020 and 2019, respectively. Results from Chapter Five were presented at the American College of Veterinary Internal Medicine Forum in 2020.

Financial support

Funding opportunities were sought out by Dr. Verbrugghe and I. I wrote all grant and scholarship applications with guidance and feedback from Dr. Verbrugghe. My stipend was supported by the OVC PhD Fellowship and NSERC Post Graduate Scholarship. Chapters Three and Four were supported by a MITACS Accelerate grant (#IT11731) in partnership with Vecado. Chapter Five was funded by the OVC Pet Trust. Chapters Six and Seven were supported by a Collaborative Research Development grant (#CRD 530333-18) from NSERC in conjunction with Petcurean Pet Nutrition.

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LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCALTURE

AA	Amino acid
AAFCO	Association of American Feed Control Officials
ALA	Alpha-linolenic acid
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ARA	Arachidonic acid
Arg	Arginine
BCAA	Branched-chain amino acid
BCS	Body condition score
BUN	Blood urea nitrogen
C	Carnitine or carbon
CLA	Conjugated linolenic acid
COOH	Carboxyl group
CK	Creatine kinase
Cys	Cyst(e)ine
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FEDIAF	European Pet Food Industry Federation
FS	Faecal score
GLA	Gamma-linolenic acid
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
His	Histidine
HPLC	High-performance liquid chromatography
Ile	Isoleucine
LA	Linoleic acid
Leu	Leucine
LPC	Lysophosphatidylcholine
Lys	Lysine
MB	Meat-based
MBD	Meat-based diet
MCV	Mean corpuscular volume
Met	Methionine
MPV	Mean platelet volume
MS	Mass spectroscopy
NH ₂	Amino group
PB	Plant-based
PBD	Plant-based diet
PB+MB	Plant-based main diet with meat-based snacks or treats, or animal-derived supplements

PB+MB/H	Plant-based main diet with meat-based snacks or treats, or animal-derived supplements and/or plant-based diet with access to outdoors or the ability to hunt
PC	Phosphatidylcholine
Phe	Phenylalanine
PUFA	Polyunsaturated fatty acid
SAA	Sulfur amino acid
SM	Sphingomyelins
SM(OH)	Hydroxysphingomyelins
Thr	Threonine
Try	Tryptophan
Tyr	Tyrosine
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
Val	Valine

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1 CHAPTER ONE: Introduction and Literature Review

1.1 Introduction

Though domestic dogs and cats are facultative and obligate carnivores, respectively, around 1% may be fed entirely plant-based diets (PBD), meaning diets devoid of any animal-derived ingredient (Dodd et al., 2019b). The conventional formulation of commercial dog and cat foods utilizes a combination of animal- and plant-derived ingredients to provide a diet containing each of the nutrients known to be essential for the species and lifestage for which the product is intended. In theory, it is possible to provide each essential nutrient required by dogs, cats, puppies and kittens without including animal products. In practice, however, there is little data to support or refute this practice.

Pet owners feeding a PBD to their pet do so largely out of ethical motivations, with concerns regarding the rights and welfare of other animals (Dodd et al., 2019b). There is also a concern amongst nearly half of vegan pet owners that animal products may be unhealthy, and a perception that a PBD is neither unnatural nor unhealthy for dogs and/or cats, though over half recognize a risk of the diet providing incomplete nutrition (Dodd et al., 2019b). Within human nutrition and dietary habits, there is a link between eating a PBD and a greater perceived awareness of health and nutrition (Pilař et al., 2021, Bedford and Barr, 2005, Davey et al., 2002, Hoek et al., 2004, Waldmann et al., 2003). Benefits of PBD for humans have been widely reported, predominantly with respect to lower body mass index, reduced obesity, inflammatory

and chronic disease conditions, and protection against cardiovascular disease and cancer (Berkow and Barnard, 2006, Bradbury et al., 2014, Dinu et al., 2017, Hafström et al., 2001, Kaartinen et al., 2009, Kahleova et al., 2018, Le and Sabaté, 2014, McDougall, 1999, Trap and Barnard, 2010, Turner-McGrievy et al., 2017). These benefits are largely attributed to the reduced intake of cholesterol, saturated fatty acids, heme iron, and sulfur amino acids, as well as increased intake of antioxidants, polyphenols, and fibres (Allès et al., 2017, Berkow and Barnard, 2006, Bradbury et al., 2014, Craig, 2010, Davey et al., 2002, Etemadi et al., 2017, Dinu et al., 2017, McCarty et al., 2009, Melchert et al., 1987). People eating a PBD themselves have more concern regarding the healthiness or unhealthiness of different foods, which may translate into concerns for their pets (Lea and Worsley, 2001). However, some nutritional concerns have also been reported in humans consuming unsupplemented PBD, including risk of deficiency of some essential nutrients, such as vitamins B12 and D (Allès et al., 2017, Craig, 2010, Weikert et al., 2020). Furthermore, two considerations must be borne in mind when attempting to extrapolate from human nutrition to pet nutrition. Firstly, dietary-associated or dietary-responsive disorders suffered by humans, such as atherosclerosis associated with cholesterol intake, may not be shared by dogs or cats (Hoenig, 2012). Secondly, many of the proposed health benefits of PBD for humans are, as mentioned, with respect to nutrients which are either avoided or enhanced in a PBD. At least with respect to the nutrients currently recognized as essential for dogs and cats, the nutrient profiles of commercial pet foods are more consistent regardless of the ingredients used. Thus, for example, the proposed mechanism of slowed aging in humans eating low-methionine PBD is not comparable to dogs and cats fed a PBD, as the methionine content

within the pet's food must be within certain concentration parameters in order to meet industry recommendations and animal requirements (McCarty, 2003, McCarty et al., 2009).

Investigation into PBD for dogs and cats has been limited. The first studies of PBD for dogs and cats were published in the early 2000s. Since then, only a handful of research articles focusing on the topic have been published. The purpose of this research is to build upon the current literature and investigate the nutritional sufficiency of PBD for companion dogs and cats.

1.2 Plant-based ingredients and pet nutritional requirements

There are limitations when attempting to provide all dietary essential nutrients without including animal products in a pet food formulation. The nutrients as recognized as essential by the Association of American Feed Control Officials (AAFCO) nutrient profiles are discussed here, with a focus on nutrients recognized to be of greatest concern in PBD (Dodd et al., 2018).

1.2.1 Protein and amino acids

Proteins are the foundation for cellular structures, enzymes, and other biologically active compounds (NRC, 2006). These macronutrients are composed of amino acids organized into primary (amino acid sequence), secondary (arrangement of sequences into folded sheets or helices), tertiary (folding of sheets and helices into 3D structures), and sometimes quaternary (binding of 3D subunits into a single larger form) structures. Each amino acid is composed of a carbon skeleton, a nitrogen-containing amino group (NH₂) and a carboxyl group (COOH). The identity and characteristics of individual amino acids are determined by their unique side chain.

Amino acids may exist freely, in small peptides, or incorporated into proteins, and when energy is scarce or amino acids plentiful, they may be catabolized for energy via gluconeogenesis or ketogenesis, depending on their configuration.

Animals are constantly creating proteins from a combination of 20 different proteinogenic amino acids either provided in the diet or synthesized *de novo* from carbon, hydrogen, oxygen and nitrogen precursors. Carbon skeletons obtained via catabolic pathways can be shunted into biosynthetic pathways to generate amino acids. Pyruvate, generated during glycolysis, and oxaloacetate and alpha-ketoglutarate from the citric acid cycle are the main precursors of amino acid synthesis (Wu et al., 2014a). The amino acids that can be synthesized this way by animals are termed dispensable amino acids, meaning they are not required in the diet as they can be generated from other nutrients. In dogs and most other mammals, alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, proline, and serine are classified as dispensable amino acids, leaving Arg, histidine (His), isoleucine (Ile), Leu, Lys, Met, Met+Cys, phenylalanine (Phe), Phe + tyrosine (Phe+Tyr), Trp and valine (Val) as indispensable (NRC, 2006, Milner, 1979a, Milner, 1979b, Milner, 1981, Burns et al., 1981, Cianciaruso et al., 1981). In cats, Tau, an amino sulfonic acid derivative of Met, is also indispensable (Pion et al., 1987). This concept of dispensable and indispensable may be oversimplified, and it has been suggested that, under certain conditions, otherwise dispensable amino acids may be conditionally indispensable (Reeds, 2000). The dietary essentiality of protein is more accurately an essentiality of amino acids, with a requirement for provision of adequate quantities of both dispensable and

indispensable amino acids to maintain nitrogen balance, lean body mass, growth, or other metabolic or physiological variables (NRC, 2006, Hou et al., 2015). In addition to their role as building blocks for protein and as catabolic substrates for energy production via gluconeogenesis and/or ketogenesis.

The amount of protein an animal requires in their diet is determined by their rate of protein turnover and losses (Yamamoto et al., 2019). Cats have a higher requirement for protein than dogs, due at least in part to an increased rate of amino acid catabolism for gluconeogenesis (Eisert, 2011, Verbrugge and Bakovic, 2013). In addition to quantity, quality of proteins is an important consideration. Protein quality is determined by the proportion of different amino acids it contains compared to requirements, as well as the digestibility and bioavailability of those amino acids (Moore and Soeters, 2015).

Dietary proteins are ubiquitous in all cells, though the amount of protein in animal or plant tissues can vary greatly (Millward, 1999). High-protein plant materials, such as legumes, nuts and seeds, contain as much protein as some animal tissues (Dodd et al., 2018). Even in plants with a high protein content, there may be limitations with respect to the amino acid profile of the proteins and availability of the amino acids present. While animal tissues are often considered sources of high-quality protein, meaning they contain all the essential amino acids an animal requires in quantities proportional to the animal's requirements, plant proteins tend to have insufficiencies in amino acids. In particular, the most commonly used plant-based protein source, legumes, have, in comparison to animal proteins, poor provision of sulfur amino acids (Mansilla

et al., 2019). Indeed, research teams have been working to increase the expression of Met in legumes for the purpose of increasing the biological value for human consumption (Müntz et al., 1998, Saalbach et al., 1994, Guo et al., 2020). In addition to comparatively low SAA content, interference with amino acid digestion and bioavailability due to anti-nutritional factors may further impair SAA intake from legumes (Yamka et al., 2003, Hill, 2004). For example, digestibility of Met and Cys were lower than other amino acids in plant-based proteins (Reilly et al., 2020). It has been postulated that the high fibre content of legumes further antagonises protein and amino acid digestibility and bioavailability (Mansilla et al. 2019).

Nuts and seeds are protein-rich plant ingredients that are typically lower in fibre than legumes, though they present some complications as well. The biological role of a nut or a seed is roughly equivalent to the avian or reptilian egg, as it contains a plant embryo and a concentrated source of energy to supply the growing embryo. As the most energy-dense nutrient, fat is quite prominent in the nutrient profile of many nuts and seeds (Jackson and Hu, 2014). This can reduce their application as sources of dietary protein, as the fat content may exceed the animal's tolerance or imbalance the diet.

In comparison to legumes, nuts and seeds, grains contain less protein, though they can be used to balance the amino acid profile and contribute to the overall protein content of the diet. The concentration of protein in grains can be manipulated with different processing methods, ranging from whole, unprocessed grains, to highly-processed protein concentrate and isolates

(Wang et al. 2019). The protein fractions of legumes, nuts and seeds can also be concentrated by processing.

Few fruits or vegetables contain protein in concentrations relevant to pet food production, with the exception of some fungi, micro- and macro-algae (Dawczynski et al., 2007, Garcia-Vaquero and Hayes, 2016). Limitations exist with these novel protein sources, however, based on limited knowledge of their performance in companion animals and a lack of approval for their use as protein sources in companion animal diets (AAFCO 2020).

Complementary proteins from incomplete plant-based proteins sources, with or without addition of individual amino acids, can be combined to form a complete amino acid profile (Dodd et al. 2018, Young and Pellett, 1994). In this way, it can be possible to formulate plant-based diets meeting the amino acid and protein requirements of dogs and cats. However, issues of digestibility and anti-nutritive factors may remain and require further consideration from an ingredient and/or processing perspective in order to mitigate their impact on the performance of the diet.

1.2.1.1 Arginine

Arginine is a common amino acid found predominantly in meats, as well as plants, such as soy, nuts, cereals and grains (Mirmiran et al., 2016, Górska-Warsewicz et al., 2018).

Nevertheless, insufficiency has been demonstrated in a plant-based cat food product (Zafalon et al., 2020). Arginine is a metabolite in the urea cycle, critical for the detoxification of ammonia

and synthesis of its excretion product, urea (Morris Jr., 2002). Ammonia is generated as a neurotoxic waste product of protein metabolism and requires further metabolism to the less toxic urea for excretion via the kidneys. In cats, synthesis of Arg from citrulline is very poor, and cats fed even a single protein-rich, Arg-deficient meal can show severe, sometimes fatal, clinical signs within hours (Morris and Rogers, 1978).

1.2.1.2 Histidine

Dietary sources rich in His include red meat (and dark muscle fish meat), as well as nuts, seeds and grains (Moro et al., 2020, Górska-Warsewicz et al., 2018). Histidine insufficiency in plant-based pet foods has not been described, and considering the provision of histidine in plant-based protein sources, this amino acid is of little concern in a plant-based diet.

1.2.1.3 Isoleucine, leucine and valine

Together, Ile, Leu and Val make up the branched-chain amino acids (BCAA), known as such due to the physical structure of their unique side chains. Branched chain amino acids are common in many proteins, but are found in the highest quantities in muscle tissues (meat), dairy, and grains, with other plant-based proteins being comparatively poor sources (Merz et al., 2018, Górska-Warsewicz et al., 2018). Insufficiency of Leu has been demonstrated in plant-based cat food products (Kanakubo et al., 2015). The primary role of these amino acids is in protein synthesis. Indeed Leu plays a pivotal role in regulation of protein anabolism and both Leu and Ile are also involved in energy metabolism, particularly within muscle tissues (Shimomura et al., 2015, Duan et al., 2016, Anthony et al., 2001). These functions give the BCAA critical roles in

growth, development, exercise tolerance and performance. Lastly, Leu is an important neurotransmitter precursor, as it crosses the blood-brain barrier more rapidly than other amino acids, generating close to half of brain glutamate and glutamine, critical excitatory neurotransmitters (Yudkoff et al., 2005). Similar to His, the signs of Ile, Leu or Val deficiency are non-specific, related to reduction in food intake, weight loss and negative energy balance (Milner, 1979b, Milner, 1979a, Rogers and Morris, 1979).

1.2.1.4 Lysine

This amino acid is found in high concentration in meats and soy, but comparatively low concentrations in most plant-based protein sources, such as nuts and seeds, grains and root vegetables (Millward, 1999, Young and Pellett, 1994). Indeed, insufficiency of Lys has been demonstrated in plant-based cat food products (Gray et al., 2004, Kanakubo et al., 2015). The role of Lys is specifically related to its structure, with Lys being a key amino acid important for the secondary structure of proteins, the basic shape that the chain of sequential amino acids takes. Specifically, a Lys metabolite, hydroxylysine, is important for the cross-linkages responsible for the structure and function of collagen, the most ubiquitous protein in connective tissues (Yamauchi and Sricholpech, 2012). Similar to His and the BCAA, there are no specific signs of Lys deficiency in dogs and cats other than generalized malnutrition, including reduction in food intake, poor growth, weight loss and negative energy balance (Milner, 1981, Milner, 1979a, Rogers and Morris, 1979). Excess Lys also leads to detrimental effects, these being related to antagonism of Arg and alteration of ammonia detoxification, though the mechanism

behind this antagonism is still unknown (Czarnecki et al., 1985). Often, the amino acid balance of a protein is determined in reference to Lys, as Lys is recognized as a limiting amino acid in the diet of many species, including pigs (van Milgen and Dourmad, 2015).

1.2.1.5 Methionine, cysteine and taurine

Plant-based proteins tend to be relatively poor sources of SAA, which often results in methionine being the first limiting amino acid when using plant-based proteins to meet canine amino acid requirements (Dodd et al., 2018, Mansilla et al., 2019). Previous measurements of SAA in plant-based pet food products have demonstrated inadequacies, failing to meet industry recommended SAA levels (Gray et al., 2004, Kanakubo et al., 2015, Zafalon et al., 2020).

Nevertheless, there have been no published cases of dogs or cats fed PBD having any adverse signs attributable to Met deficiency, potentially due to the ability to use supplemental synthetic Met in products with insufficient SAA from dietary ingredients (Han and Lee, 2000, Zangeronimo et al., 2006). Subclinical deficiency could potentially go undetected and unreported. In cases where SAA intake is impaired, impacts can be detected on one-carbon metabolism, with reduction in the methyl donor *s*-adenosylmethionine, and decreased antioxidant activity from reduced synthesis of glutathione. At the same time, betaine and tetrahydrofolate utilisation is increased to recycle homocysteine (Figure 1.1). Thus, integrated metabolomics may potentially identify sub-clinical SAA deficiency via alterations in these pathways prior to clinical signs being detected.

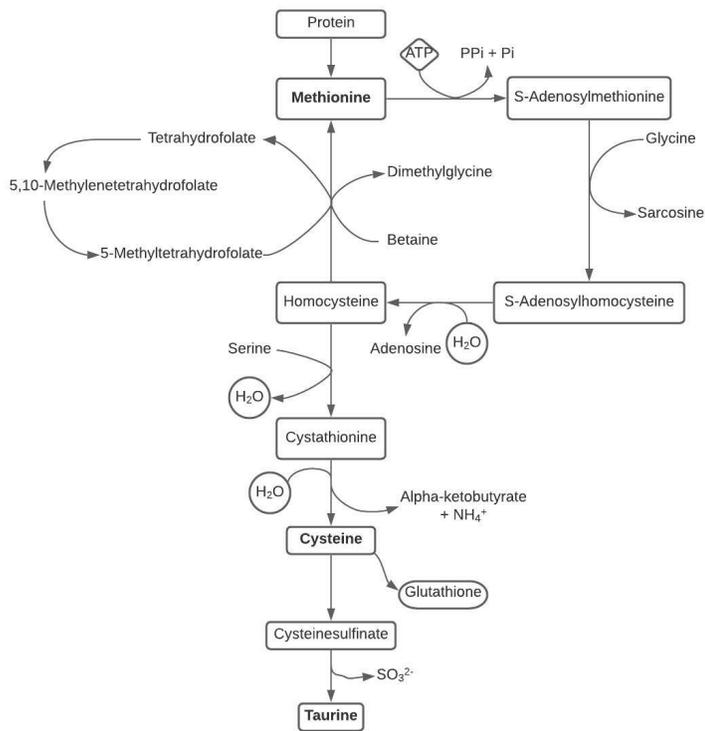


Figure 1.1: Sulfur amino acid metabolism

Methionine, cysteine (Cys) and its oxidized form, cystine, are sulfur-containing amino acids (SAA), with Met and total SAA recognized as indispensable, but not Cys which can be synthesized from Met within the liver (Stipanuk, 1986, Finkelstein, 1990, NRC, 2006). As with Lys, it is the physical structure of Met that gives unique properties to the proteins in which it is incorporated. While Lys forms cross-linkages causing kinks, pleats and twists in polypeptide chains, Met's highly hydrophobic methyl group leads to the majority of Met residues being folded into the interior of proteins (Brosnan et al., 2007). It is this methyl group that also gives Met and its metabolites their main role as the most prevalent methyl donors, responsible for

methylation reactions throughout the body (Brosnan et al., 2007). Methionine also plays a critical role in protein synthesis, in a similar, though less potent way, as Leu (Khayati et al., 2017). Cysteine also has a unique function in determining the secondary structure of proteins into which it is incorporated through the formation of bridges between sulfur molecules (Jahandideh et al., 2009). The sulfur component of the SAA also determines another of their key functions, generation of antioxidant metabolites (Bin et al., 2017), and can be utilised to manipulate urinary pH (Hickey et al., 2015).

Deficiency of Met shares the common characteristics of amino acid deficiency: reduction in food intake and weight loss; as well as demonstrating some unique signs of dermatitis, particularly of high-impact skin (Milner, 1979b, Hirakawa and Baker, 1985, Rogers and Morris, 1979). Data also suggest that insufficient dietary SAA and/or antagonism of SAA and/or the metabolite taurine, may also predispose dogs to dilated cardiomyopathy (Backus et al., 2006). Excess Met also causes adverse signs, most commonly vomiting and ataxia (Hickey et al., 2015).

Taurine is a beta-sulphonic amino acid derived from SAA precursors (Tôrres et al., 2003). As a free amino acid, Tau is not incorporated into proteins, instead it directly plays a role in cardiac and retinal functions (Pion et al., 1987, Fascetti et al., 2003). Taurine content in terrestrial plants is negligible. It is found only sparingly in marine plants, with most Tau in pet foods coming from animal tissues and through addition of supplemental synthetic taurine to compensate for processing-related losses (Pion et al., 1987, Garcia-Vaquero and Hayes, 2016).

In dogs, Tau is considered to be a dispensable amino acid as they are capable of synthesizing sufficient Tau to meet their requirements when provided adequate SAA precursors (Figure 1.1), though for some dogs it may be conditionally indispensable (Backus et al., 2006, Fascetti et al., 2003, Kaplan et al., 2018, Ko and Fascetti, 2016, Tôrres et al., 2003). In cats, decarboxylation of cysteinsulfinate to taurine appears to be impaired, leaving cats unable to synthesize adequate amounts of Tau even when provided sufficient dietary SAA (Morris and Rogers, 1982). Both dogs and cats also have ongoing taurine losses as, unlike most other mammals, they are unable to alternate between glycine and Tau conjugation of bile acids and thus suffer losses of taurocholic acid in excrement (Morris et al., 1994, Ko and Fascetti, 2016). Deficiency of Tau can thus manifest if cats are fed a diet without sufficient Tau, or if dogs are fed a diet without adequate SAA to support sufficient Tau production to exceed the amount lost.

Recently, there has been interest in the possibility of chronic SAA deficiency resulting in Tau deficiency and dilated cardiomyopathy in dogs. In particular, diets with high inclusion of some plant-based proteins have been suggested to increase the risk of Tau deficiency in both dogs and cats due to a number of possible contributing factors: a lack of Tau in protein-rich plants (with the exception of some marine plants (Dawczynski et al., 2007, Garcia-Vaquero and Hayes, 2016)), relative insufficiency of SAA in plant proteins (Reilly et al., 2020), and relative abundance of soluble fibre, trapping taurocholic acids within the intestinal lumen and promoting microbial taurocholic degradation (Kim et al., 1995, Ko and Fascetti, 2016). Taurine insufficiency has been demonstrated in PBD commercially available for cats (Gray et al., 2004,

Kanakubo et al., 2015). Similarly to SAA, however, there are no published reports of Tau deficiency in cats or dogs fed PBD, which, also similar to SAA, may be due to the industry-wide practice of adding synthetic taurine to pet food products, regardless of ingredients, or perhaps due to the predatory nature of free-roaming cats and likelihood of self-supplementing their diet with prey (FEEDAP, 2012, McDonald et al., 2015). Measurement of plasma and whole blood Tau can be an indication of Tau status, and many dogs and cats with taurine-responsive dilated cardiomyopathy have demonstrated reduced Tau and/or Met+Cys levels (Backus et al., 2006, Fascetti et al., 2003, Pion et al., 1987).

1.2.1.6 Phenylalanine + tyrosine, tryptophan

Similar to the SAA, the dietary requirements of the aromatic amino acids (AAA), Phe and Tyr, are considered together, as Tyr is synthesized from Phe (NRC, 2006, Milner et al., 1984). The AAA are synthesized exclusively in plants and microorganisms, making plant-based proteins, especially grains, a common dietary source of AAA (Parthasarathy et al., 2018, Górska-Warsewicz et al., 2018). Regardless, insufficiency of Trp has been demonstrated in plant-based pet food products (Kanakubo et al., 2015). Phenylalanine, via Tyr, is required for synthesis of critical thyroid hormones and catecholamines, as well as for normal hair pigmentation (NRC, 2006, Lehnert and Wurtman, 1993, Watson et al., 2018). The quantity of Phe required to prevent discolouration in black-coated dogs is greater than that required to prevent other signs of deficiency, such as decreased food intake, weight loss or reduced growth rate (Milner et al., 1984, Milner, 1979a, Watson et al., 2018). Aside from its role as a protein constituent, Trp also

serves essential purposes as a precursor for niacin (vitamin B2), and the neurotransmitters kynurenine, serotonin, tryptamine, melatonin, 5-hydroxytryptophan (Richard et al., 2009). When Trp is provided in excess to that required for growth or maintenance, particularly in a high proportion to competing amino acids, it may influence behaviour through increased production of serotonin in particular (DeNapoli et al., 2000). Deficiency of Trp presents similar to deficiency of most other amino acids, characterised by decreased food intake and weight loss (Milner, 1979a, Milner, 1979b, Rogers and Morris, 1979).

1.2.1.7 Threonine

Threonine is found commonly in grains (Górska-Warsewicz et al., 2018), and deficiencies in plant-based pet foods have not been reported. Due to its prevalence in non-animal proteins, there is little concern regarding threonine provision in plant-based pet foods.

1.2.2 Fat and fatty acids

Fat is the most energy dense nutrient, yielding close to double the calories per gram than either protein or carbohydrates (NRC, 2006). In foods, this nutrient is often referred to as fat, though within the body the word lipid is typically used to denote the same compounds. Dogs and cats have a dietary requirement for total fat as a means to meet energy requirements and as a vehicle for fat-soluble vitamins and essential fatty acids. Just as protein is comprised of smaller units, amino acids, fats are comprised of individual fatty acids. Similarly, the physical and chemical characteristics of each fatty acid are determined by their structure, notably the presence or absence of double bonds between carbons in the fatty acid chain, termed unsaturation or

saturation, respectively. Saturated fatty acids thus have two hydrogen molecules bound to each carbon in their chain, while monounsaturated fatty acids have a single pair of carbons with only one hydrogen (i.e., one double bond), and polyunsaturated fatty acids (PUFA) have multiple pairs of carbons in the chain with only one hydrogen bound to them (i.e., more than one double bond) (Velíšek and Cejpek, 2006). These double bonded carbons cause a structural change in the chain, causing a ‘kink’ and changing the shape of the molecule, and giving the fatty acid unique characteristics. Lipids are critical cellular components, forming membranes essential for structure and function of intracellular components and the external cellular membrane. The characteristics of these membranes are influenced by their fluidity, determined by the chemical structure of the fatty acid chains incorporated within them.

Mammals, including dogs and cats, are able to synthesize most of the fatty acids required by their body. Particularly, they can synthesize all of the saturated fatty acids they require if provided with carbon precursors from glucose or amino acids (Hillgartner et al., 1995). However, there are great differences in specific ability to synthesize unsaturated fatty acids, particularly those with a double bond at the 3rd or 6th carbon in the fatty acid chain (Bauer, 2006). These compounds have physiologic roles related to incorporation into cellular membranes, influencing membrane fluidity and fragility as well as the release of eicosanoids during cell membrane insult. As a result, dogs and cats rely on dietary provision of fat to meet their fatty acid needs.

Plant ingredients and oils from plants can be a rich source of fat and easily meet canine and feline total fat requirements. Fats from plants are typically rich in unsaturated fatty acids, particularly PUFA, and low in saturated fatty acids (Kolláthová et al. 2019), making them ideal sources of essential linoleic and alpha-linolenic fatty acids in canine and feline diets. However, terrestrial plants are poor sources of the longer chain fatty acids EPA and DHA, making meeting the requirements of growing puppies and kittens a challenge.

1.2.2.1 Linoleic acid

Linoleic acid is an 18-carbon fatty acid with two double bonds, starting at the 6th carbon from the methyl end of the chain, making LA an n-6 fatty acid (NRC, 2006). It is a common fatty acid available in many plant-based ingredients, such as seed and vegetable oils (Innes and Calder, 2018). Provided dietary LA, dogs are able to synthesize, via progressive steps of desaturation and elongation, longer chain n-6 PUFA including gamma-linoleic acid (GLA) and arachidonic acid (ARA), thus LA is the only indispensable n-6 fatty acid for dogs (Dunbar and Bauer, 2002). As a result of low delta-6 desaturase activity, feline ARA biosynthesis is less efficient than dogs, and cats have potentially conditionally essential requirements for ARA as well (Bauer, 2006). Although LA plays essential roles in the body and must be included in the diet, the eicosanoids formed from its derivatives, the n-6 PUFA, are considered to be pro-inflammatory (Innes and Calder, 2018).

1.2.2.2 Arachidonic acid

Arachidonic acid is not produced by terrestrial plants, thus seed and vegetable oils are inadequate sources, though it may be found in some marine plants and fungi (Garcia-Vaquero and Hayes, 2016, Yu et al., 2003, Dawczynski et al., 2007). Many mammals, including dogs, are capable of synthesizing adequate ARA from precursor LA (Trevizan and Kessler, 2009). Cats, however, lack the ability to synthesize sufficient ARA to meet specific requirements such as gestation, lactation and growth. In adult cats, endogenous synthesis appears to be sufficient to meet requirements for maintenance, especially if provided GLA as an alternative precursor (Bauer, 2006, Trevizan et al., 2012). These pathways are depicted in Figure 1.2.

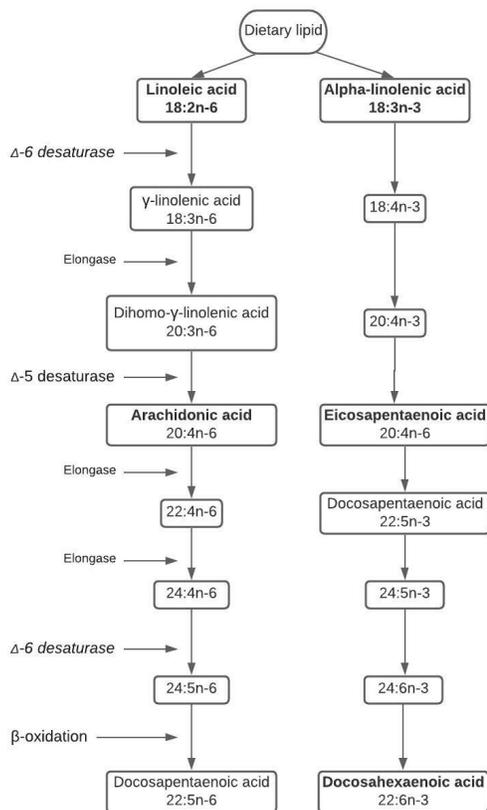


Figure 1.2: Essential fatty acid metabolism. *Italicized* enzymes have low activity in cats.

Although ARA is included in the AAFCO nutrient profiles for both growth and adult maintenance for cats, it appears that this fatty acid may be conditionally, not explicitly, essential. Despite diminished delta-6 desaturase activity, which most mammals use to convert LA to the ARA precursor GLA, adult, non-reproductive cats appear to be able to synthesize adequate ARA to maintain their health status, provided adequate dietary LA or GLA (Figure 1.2) (Trevizan et al., 2012, Bauer, 2006). For feline reproduction, LA may be sufficient to maintain spermatogenesis in males, but females require dietary ARA to support healthy gestation

(MacDonald et al., 1984). To meet industry recommendations, all PBD formulated for cats would nevertheless be required to contain an ARA supplement (AAFCO, 2020). Despite this, ARA insufficiencies have been reported in commercial plant-based cat foods (Zafalon et al., 2020, Gray et al., 2004).

1.2.2.3 Alpha-linolenic acid

1.2.2.4 Alpha-linolenic acid is an 18-carbon fatty acid with two double bonds, starting at the 3rd carbon from the methyl end of the chain, making ALA the n-3 counterpart to LA (NRC, 2006). This fatty acid is found predominantly from plant-based fats, including seed- and vegetable oils (Stark et al., 2008), and insufficiency of ALA has not been reported in plant-based pet foods. Dogs consuming diets rich in ALA are capable of desaturating and elongating ALA to longer chain PUFA including EPA and, minimally, DHA (Purushothaman et al., 2011, Dunbar et al., 2010). ALA is considered essential for growth and development predominantly due to its role as precursor to the longer-chain n-3 PUFA, though dietary sources of EPA and DHA are also considered essential (Bauer, 2016, Bauer, 2006). EPA+DHA

Although typically discussed together, the long-chain PUFA DHA (22:6n-3) and EPA (20:5n-3) have separate functions in the body. Docosahexaenoic acid plays an essential role in neurological development, as a component of neural membranes, and within the retina (Bauer, 2016). Eicosapentaenoic acid is not recognized to have such a critical function, though it can be beneficial for canine health via conversion to anti-inflammatory eicosanoids, resolvins and protectins (Mas et al., 2012). These long-chain PUFA are synthesized only minimally within the tissues of dogs and cats, however, provided adequate ALA, adult dogs and cats may be able to generate sufficient EPA and DHA to meet their requirements (Dunbar and Bauer, 2002, Dunbar et al., 2010, Pawlosky et al., 1994, Pawlosky et al., 1997). Interestingly, suckling puppies appear

to be able to effectively convert ALA to DHA, though puppies suckling from dams fed DHA-enriched diets had improved visual and neural performance (Bauer et al., 2004, Heinemann et al., 2005). At weaning, conversion of ALA to DHA in puppies is reduced, comparable to conversion in adult dogs. Although puppies and kittens from dams fed ALA-rich diets are capable of synthesizing DHA, the improved performance of offspring fed DHA-rich milk and the rapid reduction in ability to continue to produce DHA from ALA suggests a conditionally essential role for the nutrient during lactation and growth (NRC, 2006, Pawlosky et al., 1997). Eicosapentaenoic acid and DHA are not synthesized in significant amounts in terrestrial plants or animals and are provided almost exclusively from marine sources, either directly from the primary source, algae, or through fish who bioaccumulate algae-derived EPA and DHA (Bauer, 2011, Garcia-Vaquero and Hayes, 2016).

1.2.3 Minerals

Dietary minerals are separated into two categories based on their dietary requirements: macrominerals, which must be consumed on the scale of g/100kcal, and microminerals or trace minerals, which are required in lower amount, typically in mg but sometimes ug/100kcal. Ca, P, K, Na, chloride (Cl) and magnesium (Mg) are macrominerals; while iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), iodine (I) and selenium (Se) are microminerals. Macrominerals contribute to maintenance of electrolyte and acid-bases status as well as playing structural and metabolic roles, while microminerals typically function in protein prosthetic groups and enzymatic reactions (NRC, 2006). Supplementary minerals, either as inorganic salts or organic

chelates, are often added to the canine and feline diets regardless of ingredient composition in order to ensure their sufficient provision (Dodd et al. 2018). As such, plant-based diets would not be considered to have any greater challenges than conventional diets in meeting mineral requirements.

1.2.3.1 Macrominerals

1.2.3.1.1 Calcium and phosphorus

Calcium and P can be found in many plants, but not in the concentrations that dogs or cats require. To meet the requirements of these more carnivorous species, Ca-rich supplements must be added to the diet. Calcium is a cation, meaning it has a positive charge as it is ionized within the body, and is the most abundant cation in the mammalian body. It is the most well-known essential mineral as it plays critical roles within the body, with Ca deficiency precipitating rapid and dramatic adverse effects. Calcium homeostasis is regulated via the thyroid gland based on circulating levels of ionized Ca, as well as P and vitamin D (Schenck, 2007, Rosol and Capen, 1997). Because Ca is stored in the bone, compounded with P in hydroxyapatite, dysregulation of Ca homeostasis can have very dramatic results, particularly in young, skeletally immature animals (Dodd et al., 2019a, Verbrugghe et al., 2011, Tal et al., 2018). In addition to its role in skeletal mineralization, Ca is also essential for normal functioning of intracellular signalling pathways, neural transmissions and muscular contraction (Asby and Tepikin, 2001, Rosol and Capen, 1997). Dietary sources of Ca can be organic, coming

from animal tissues such as bone meal, or added as inorganic salts, such as Ca phosphate, Ca carbonate or Ca citrate (AAFCO, 2020).

Along with Ca, P is incorporated into bone as hydroxyapatite. Outside of the skeleton, P is typically found as the anion phosphate (Rosol and Capen, 1997). Other structural functions of P include its incorporation in phospholipids and phosphoproteins, while P also plays a critical metabolic role as a component of enzyme activity regulation (NRC, 2006). In dogs, deficiency of P can also manifest as mild skeletal abnormalities and more generic signs of malnutrition such as reduced growth and poor skin and coat health (Kiefer-Hecker et al., 2018). Deficiency in cats is rare. Muscles have a high concentration of P, thus meat, as well as bone meal, is a rich source of the mineral. Phosphorus is also present in relatively high amounts in many plant-based ingredients as well. Inorganic P-containing salts may also be added to pet foods, though differences in solubility and bioavailability require consideration (Alexander et al., 2019).

Adult dogs and cats have robust homeostatic mechanisms with which to maintain serum total and ionized Ca levels within a tight range (Bronner and Stein, 1995, Schenck, 2007). Given the roles Ca and P play in skeletal mineralization, provided diets with inadequate Ca and/or P, puppies and kittens can rapidly develop dramatic signs of deficiency, and even adults may eventually develop obvious clinical signs (Dodd et al., 2019a, Tal et al., 2018, Verbrugge et al., 2011, Tomsa et al., 1999). There is one case of a cat fed exclusively potatoes, rice and carrots that developed signs of nutritional secondary hyperparathyroidism due to the lack of Ca, vitamin D and P intake provided in the diet (Tomsa et al., 1999). To meet dietary Ca and P requirements,

mineral salts, such as Ca phosphates and Ca carbonate, or amino acid chelates are readily available and used throughout the pet food industry. Nevertheless, insufficiencies in Ca and P have been reported in commercial plant-based pet foods (Gray et al., 2004, Zafalon et al., 2020).

1.2.3.1.2 Potassium

1.2.3.2 Potassium is common in plant-based ingredients (Bolton et al., 2019, Margerison et al., 2013), though, dietary insufficiency has been demonstrated in plant-based pet foods (Zafalon et al., 2020). Potassium is the second most abundant cation and exists predominantly within cells, where it functions in acid-based regulation, nerve impulses and enzymatic reactions (Seifter, 2019). In particular, balance of K in the serum/plasma is essential for normal cardiac function (Cobb and Michell, 1992). Signs of K deficiency are common between dogs and cats, including ventroflexion of the head and neck, weakness of postural muscles and ill thrift, and subclinical deficiency impairs blood pressure, cardiac output and renal blood flow (Abbrecht, 1972, Ruegamer et al., 1946, Hills et al., 1982). Sodium and chloride

Sodium is another main cation, though unlike the mostly intracellular potassium, the greatest concentration of Na is in extracellular fluid. This element is relatively uncommon in most plant-based ingredients, but can be found in animal tissues – although post-mortem losses of extracellular fluid also remove much of the Na that was present within the living animal (Margerison et al., 2013). Sodium is commonly also be added to pet food as a salt, such as NaCl and Na carbonates. Despite the easy provision of inorganic sodium from non-animals sources, insufficiency has been demonstrated in a plant-based dog food product (Zafalon et al., 2020). Sodium is the main driver of osmotic pressure and is thus critical in regulating acid-base balance and extracellular volume (Stanhewicz and Kenney, 2015, Seifter, 2019). Provided adequate access to water, dogs and cats can metabolically adapt to wide variations in dietary Na intake, with deficiency or toxicity rarely occurring (NRC, 2006).

Chloride is the anionic counterpart to Na and is the most prevalent anion in extracellular fluid. It functions alongside sodium to regulate extracellular fluid osmolality and acid-base balance (Seifter, 2019). Chloride has an additional role in digestion, as Cl is excreted as hydrochloric acid, HCl, into the lumen of the stomach. Likely attributable to tight homeostatic regulation, adverse outcomes from insufficient or excess Cl intake are uncommon. Most clinical signs associated with Cl deficiency are actually attributable to resultant hypokalaemia (Miyahara et al., 2009). In comparison to most of the other macrominerals, Cl is not found in great abundance in most food ingredients and is commonly added to pet food as a salt (NRC, 2006).

1.2.3.2.1 Magnesium

Magnesium is naturally found in many plant sources, including greens, nuts, seeds, beans and grains, though dietary supplementation with inorganic Mg salts is also common. Magnesium is a cation, abundant in intracellular fluid and contributes to the structure of bones and teeth. Its main metabolic roles include enzyme function, cell membrane stability, DNA and RNA metabolism and protein synthesis (Rosol and Capen, 1997).

1.2.3.3 Microminerals

1.2.3.3.1 Iron

Iron is naturally present within grains and soy, and inorganic iron can also be added to the diet (Kalpalathika et al., 1991). Iron is a transition metal and is the most prevalent micromineral in the body, with the majority circulating in the blood within hemoglobin in red blood cells (Andreani et al., 2010). As such, Fe plays a critical role in the maturation and

function of red blood cells (Matur et al., 2019). The most prominent sign of Fe deficiency is thus microcytic hypochromic anaemia.

1.2.3.3.2 Copper

Grain ingredients can provide dietary copper, though Cu salts and chelates are typically added to meet canine and feline requirements (Aoyagi and Baker, 1993). Like Fe, Cu is also a transition metal, and is a key factor in enzyme activities, including the metabolism of iron and red blood cell production (Andreani et al., 2010). Copper is also a component of tyrosinase and required for normal melanin pigment production (Watson et al., 2018). Signs of Cu deficiency can thus include discolouration of dark hairs, and reduced reproductive performance has also been reported (Fascetti et al., 2000, Zentek and Meyer, 1991).

1.2.3.3.3 Manganese

1.2.3.3.4 Manganese may naturally be found in whole grains, legumes, nuts and leafy vegetables, though dietary supplementation with inorganic Mn salts is also common. Neither deficiency nor toxicity of Mn have been described in dogs or cats, and the recommended adequate intake was extrapolated from the concentration of Mn in milk (NRC, 2006). Manganese is present in only small amounts within the body, and its role is primarily as a component in metalloenzymes. Zinc

Nuts and legumes can be relatively rich sources of Zn, though Zn salts and chelates are also added to balance pet foods and meet requirements (Pereira et al., 2020). Zinc is found in low concentrations in the body, predominantly in intracellular fluid. Zinc's role is also similar to Cu, in that it acts as a cofactor or catalyst in many enzymatic reactions (Andreani et al., 2010).

Unlike Cu, however, Zn plays an anti-inflammatory role, whereas the Cu is more reactive and

can be pro-inflammatory under certain conditions (Spee et al., 2006, Cummings and Kovacic, 2009). Natural Zn deficiency occurs in some breeds of dog, manifesting primarily as dermatological dysfunction (White et al., 2001).

1.2.3.3.5 Iodine

Iodine content in pet food ingredients can vary greatly, with marine ingredients being rich sources and terrestrial ingredients comparatively poor (NRC, 2006). Iodine's most significant biological role is as a major component of thyroid hormones.

1.2.3.3.6 Selenium

Few conventional pet food ingredients contain high quantities of Se, thus dietary supplementation with Se yeasts, salts or chelates is common (NRC, 2006). Selenium deficiency is uncommon in dogs or cats, though in other species Se deficiency with or without concurrent vitamin E deficiency manifests as a muscular disorder (Löfstedt, 1997). This has been experimentally replicated in the dog (Van Vleet, 1975). Like Zn, Se is a micromineral with antioxidant properties, mainly within the enzyme glutathione peroxidase (Waters et al., 2003, Waters et al., 2005).

1.2.4 Vitamins

There are two classes of vitamins, fat- and water-soluble, as well as the vitamin-like compound, choline, which are recognized as essential in the diets of dogs and cats. Many vitamins can be provided naturally with plant-based ingredients in the diet, though processing

methods may reduce the availability or damage vitamins, often necessitating supplementation in addition to the natural provision from dietary ingredients.

1.2.4.1 Fat-soluble vitamins

1.2.4.1.1 Vitamin A

Natural vitamin A all originates from carotenoids synthesized by plants, converted by herbivores and omnivores into vitamin A analogues such as retinol. Carnivores, including cats, have impaired ability to convert dietary beta-carotene to vitamin A, thus in cats, synthesis from beta-carotene is unlikely to meet dietary requirements and pre-formed vitamin A from animal tissues forms their natural vitamin A intake (Schweigert et al., 2002, Green et al., 2011). Dogs are capable of synthesizing vitamin A from dietary carotenoids, thus their dietary requirements may be considered in terms of retinol equivalents, which may be contributed from both carotenoids and preformed vitamin A (NRC, 2006, Green and Fascetti, 2016). Therefore, in dogs, dietary vitamin A may come from carotenoid-rich plant-based ingredients (Becker and Kienzle, 2013). Nonetheless, synthetic retinyl esters are commonly added to pet foods and could balance PBD for dogs and cats (Thiex et al., 1996). Despite this, insufficiency of vitamin A has been reported in a plant-based cat food (Gray et al., 2004).

Vitamin A, as retinol, plays a critical role in vision, reproduction, cellular division and immune responses (Morris et al., 2012). Transportation of vitamin A is different in dogs, cats and other carnivorous animals, in comparison to non-carnivorous ones. In dogs and cats, vitamin A is transported in the plasma predominantly as retinyl esters, whereas in non-carnivorous

animals retinyl esters only detected following consumption or in cases of excess ingestion (Morris et al., 2012). Signs of inadequate vitamin A intake include vision impairment, skin lesions, ill thrift and impaired immune function (Kemp et al., 1989).

1.2.4.1.2 Vitamin D

Unlike many other mammals, dogs and cats are both reliant on dietary vitamin D intake to maintain vitamin D status, due to inadequate cutaneous synthesis (How et al., 1994). Dietary vitamin D may be provided in two forms: cholecalciferol, or vitamin D₃, and ergocalciferol, vitamin D₂. Cholecalciferol (D₃) is the form of vitamin D produced in the skin of most mammals when exposed to adequate UV light, though in dogs and cats this biosynthesis has been markedly impaired, likely as an evolutionary adaptation to a diet rich in vitamin D, making cutaneous synthesis wasteful. Although the tissues of other animals, particularly oily fish and livers, can contain D₃, commercial pet food products typically have supplementary D₃ added, synthesized predominantly from cholesterol collected from sheep lanolin (Chen et al., 2007, NRC, 2006, Craig, 2010). Cholecalciferol may also be isolated from a handful of terrestrial plants, microalgae and lichen, though D₂ is the more common non-animal form of vitamin D (Wang et al., 2001, Dodd et al., 2018). It is unknown if dogs can utilize D₂ with the same efficacy as D₃. In many animals, D₃ is marginally to markedly more potent than D₂. In cats, for example, there is evidence that D₂ is significantly less effective than cholecalciferol to maintain vitamin D status (Morris, 2002a). At present, there are no differential recommendations for inclusion of vitamin D₂ or D₃ in canine diets.

Vitamin D's most prominent function is in Ca homeostasis and skeletal metabolism, though extra-skeletal roles have been recognized as well (Weidner and Verbrugghe, 2016). Vitamin D is the precursor of calcitriol, the main hormone that controls calcium absorption and bone metabolism (Tam et al., 1986, Hazewinkel and Tryfonidou, 2002). Dietary vitamin D is absorbed and mostly converted to calcidiol, also known as 25-hydroxyvitamin D (25(OH)D), a metabolite of low activity but high stability. When blood calcium levels are low, the parathyroid gland releases parathyroid hormone (PTH), which upregulates renal hydroxylation of 25(OH)D to the active hormone calcitriol, or 1,25-dihydroxyvitamin D (1,25(OH)2D). Calcitriol acts to increase intestinal calcium absorption, decrease renal excretion of calcium, promote mineralization of osteoid and cartilage as well as attenuation of osteoblast activity and accentuation of osteoclast activity to allow release of stored calcium from bone (Hazewinkel and Tryfonidou, 2002). As a result, vitamin D is critical for normal bone mineralization, as skeletal abnormalities may occur with vitamin D deficiency even in the face of appropriate dietary calcium and phosphorus (Hazewinkel and Tryfonidou, 2002).

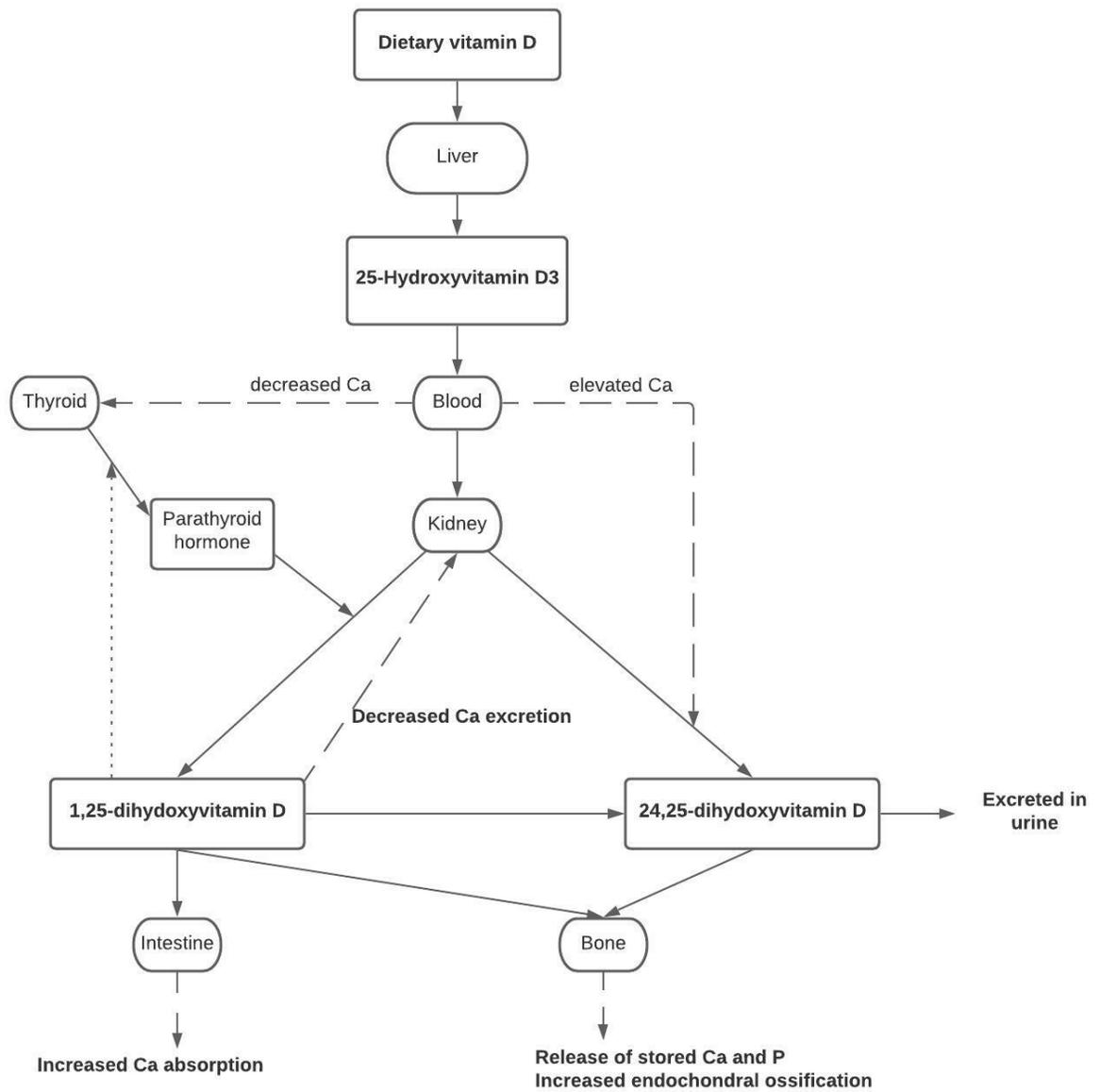


Figure 1.3: Metabolism and activity of vitamin D. Dashed lines represent promotion, dotted lines inhibition.

1.2.4.1.3 Vitamin E

Some plant-based oils, such as wheat germ, sunflower and olive oil, are a rich source of vitamin E, while fruits and vegetables can also provide small amounts (García-Closas et al., 2004). Vitamin E in the form of tocopherol is also often added to pet foods not only to meet dietary requirements of cats and dogs, but also to protect sensitive nutrients, such as fats, from oxidation (Hilton, 1989). Vitamin E is found ubiquitously in the body within lipid membranes where it acts as a fat-soluble anti-oxidant (Piercy et al., 2001) and works in close conjunction with vitamin C, the water-soluble anti-oxidant equivalent (Chan, 1992). Together, vitamins E and C scavenge free radicals and protect highly-metabolic tissues from oxidative damage. In dogs, vitamin E deficiency can cause muscular, neurological and retinal damage (Piercy et al., 2001, Mayhew et al., 1987). In cats, a lack of dietary vitamin E can lead to inflammation and necrosis of fat (Niza et al., 2003).

1.2.4.2 Water-soluble vitamins

In dogs and cats, the only essential water-soluble vitamins are the B vitamins. The B vitamins all play roles in nutrient metabolism and have a major function in the transportation of electrons for the generation of cellular energy.

1.2.4.2.1 Thiamin

Thiamin is present in many plant-based ingredients, including yeast, wheat germ, and legumes, although the form of thiamin in animal or plant tissues may differ. Non-phosphorylated thiamine is the main form found in plants, while in animal tissues it is mostly present as thiamin

pyrophosphate, triphosphate and monophosphate (NRC, 2006). Intestinal uptake of dietary thiamine does not appear to be affected by phosphorylation status and post-absorption (Rindi and Ventura, 1972). Thiamin insufficiency has been reported in a plant-based cat food (Fantinati et al. 2021). The main role of thiamin, also known as vitamin B1, is in energy metabolism, particularly with carbohydrates, and in the synthesis of nucleotides (Kritikos et al., 2017). Signs of deficiency are non-specific and include ill thrift and eventually neurological signs (Kritikos et al., 2017).

1.2.4.2.2 Riboflavin

Riboflavin can be naturally present as different flavins in food and is common in many plant-based ingredients. Insufficiency in a plant-based cat food has been reported (Fantinati et al. 2021). Riboflavin is a component in flavin coenzymes, such as flavin mononucleotide and flavin adenine dinucleotide, which are critical for energy production and the metabolism of other vitamins (Pinto and Zemleni, 2016). Thus, a deficiency of riboflavin is often complicated by deficiencies of other vitamins including folic acid, pyridoxine, niacin, vitamin K and D. Ill thrift is the most common sign of deficiency (Noel et al., 1972).

1.2.4.2.3 Niacin

Niacin may be present in different forms in the diet, with the majority of niacin in plant tissue being nicotinic acid, while in animal tissues it is mostly nicotinamide-adenine-dinucleotide or nicotinamide-adenine dinucleotide phosphate. Furthermore, much of the niacin in plant tissues may be in a bound form and be poorly available to animals consuming it (Carter and Carpenter,

1982). Insufficiency of niacin has been demonstrated in a plant-based cat food product (Gray et al., 2004). Niacin is also referred to as vitamin B3. Similar to riboflavin, which is incorporated in the flavin nucleotides, niacin's main role is within nicotinamide-adenine dinucleotide (Kirkland and Rawling, 2001). Dogs are capable of synthesizing nicotinamide from tryptophan, whereas cats are not (Morris, 2002b). Thus, cats have a greater dietary requirement for niacin than dogs do.

1.2.4.2.4 Pantothenic acid

Vitamin B5, or pantothenic acid, exists ubiquitously in all lifeforms and can be found in both plant and animal ingredients (Miller et al., 2006). In many foods, pantothenic acid can be found incorporated within CoA, acyl-CoA synthase, and within acyl carrier proteins. Natural deficiencies are rare, due to the presence of pantothenic acid in most food ingredients, though experimental deficiencies have been induced and cause signs including ill thrift, gastroenteritis and cardiovascular failure (Shaefer et al., 1942). Pantothenic acid, is a critical component of Coenzyme A (CoA) (Leonardi and Jackowski, 2007). Coenzyme A is required for oxidative metabolism of carbohydrates, acylation and acetylation of proteins and synthesis of lipid metabolites (Miller et al., 2006).

1.2.4.2.5 Pyridoxine

Known as vitamin B6, pyridoxine is found predominantly as the phosphorylated forms of pyridoxamine and pyridoxal in animal tissues, or pyridoxine and pyridoxal in plants. However, plants can also contain high levels of glycosylated pyridoxine, possibly impairing bioavailability

(Sierra and Vidal-Valverde, 1997). Furthermore, insufficiency of pyridoxine has been reported a plant-based cat food products (Gray et al., 2004). Pyridoxine is a key coenzyme in a number of enzymatic reactions, particularly in amino acid transamination reactions, and plays an essential role in gluconeogenesis, niacin synthesis, and neural and erythrocyte function (NRC, 2006). Deficiency of pyridoxine has been demonstrated to cause renal damage, neurological signs and anaemia (Blanchard et al., 1991, Schaefer et al., 1942).

1.2.4.2.6 Folic acid

Folic acid, also known as folate or vitamin B9, is present in many plant-based foods including grains, vegetables and legumes (Suitor and Bailey, 2000). Nevertheless, deficiency of folic acid has been demonstrated in cats fed a plant-based diet (Fantinati et al. 2021). Folic acid is intrinsically related to cobalamin, and plays a large role in one-carbon metabolism, and is also required for normal neural tube maturation during embryological development. Physical deformities as a result of folic acid deficiency in utero are a relatively specific sign of folic deficiency, while in adult animals the signs are similar to those of cobalamin deficiency – namely anemia (Stanley et al., 2018).

1.2.4.2.7 Cobalamin

Cobalamin, or vitamin B12, is not synthesized within animal or plant tissues, but only by microorganisms. Thus, dietary sources are microbial, either directly, as in the case of yeasts, or indirectly, such as the tissues of herbivores which are provided with cobalamin from the metabolism of microbes in their gut. The microbiota of the GI tract of most herbivores

incorporate cobalt into cobalamin, which is then taken up by the host animal and distributed into their tissues. When omnivores or carnivores then consume the tissues of herbivores, they acquire the pre-formed cobalamin. Terrestrial plants, unless colonized by bacteria or fungi, contain no vitamin B12, although marine plants may (Watanabe et al., 2014). Dietary supplementation with purified cobalamin synthesized by microbes is commonplace. Non-animal sources of dietary cobalamin include fermented legumes, fungi and algae, none of which are commonly used in commercial pet food production (Watanabe et al., 2014, AAFCO, 2020). However, supplementation with purified cobalamin from microbial synthesis is commonplace. Nevertheless, cobalamin insufficiency has been documented in a plant-based cat food (Gray et al., 2004). No reports of cobalamin deficiency in dogs or cats fed PBD have been published, though a recent report described two cats with folate deficiency secondary to eating a PBD providing insufficient folate, thiamin and riboflavin, and, when their food intake was considered, daily cobalamin intake was also below recommendations, though the cats were not symptomatic for cobalamin deficiency (Fantinati et al., 2021, FEDIAF, 2020).

Cobalamin functions as adenosylcobalamin and methylcobalamin, with roles in methyl transfer and carbon skeleton rearrangement. Most notably, cobalamin, along with folate, is a key factor in the synthesis of red blood cells, thus anaemia is one of the key clinical signs of cobalamin deficiency (Oh and Brown, 2003).

1.2.4.2.8 Choline

Dietary choline is primarily in the form of phospholipids or lecithin (Zeisel and da Costa, 2009). Soy lecithin is the richest plant-based source, with grains and vegetables contributing little, and choline may be added directly or as lecithin to plant-based pet foods. Choline is sometimes referred to as a vitamin-like compound, as opposed to a vitamin, as many animals can synthesize choline in their liver (NRC, 2006). It is a key component of phosphatidylcholine, also referred to as lecithin, though its main roles are in cholinergic neurotransmission and as a methyl donor, in association with folic acid and cobalamin (Al-Humadi et al., 2012).

1.3 Surveying of pet health and diet

Surveying pet owners regarding their pets have been used extensively throughout veterinary research. Indeed, the American Veterinary Medical Association regularly conducts surveys of pet owners and releases information regarding their demographics, attitudes and characteristics (AVMA, 2018). With particular respect to nutrition, surveys have played a role in collecting epidemiological evidence of dietary practices in both humans and companion animals for decades. Examples of nutrition survey topics that have been reported in the literature include supplement use in dogs with cancer, lifestyle and dietary practices of humans, and dog feeding practices (Davey et al., 2002, Fraser et al., 2000, Bianco et al., 2020, Robertson, 1996). There are limitations associated with cross-sectional and self-reported data, namely: no ability to contrast current and past practices, follow-up, or predict future practices; observer bias; recall bias; and response bias (Embree and Whitehead, 1993). In attempt to reduce these biases, validation of

surveys prior to data collection can be undertaken, or previously validated questions may be used (Freeman et al., 2016, Lavan, 2013).

With respect to PBD and pets, one research group has published findings from surveying 515 vegetarians and vegans regarding their motivations for abstinence from animal consumption, pet ownership, pet diet, guilt experienced from feeding their pet a diet containing animal products, and perceived appropriateness of animal-based pet foods (Rothgerber, 2013). They found that pet ownership among vegetarians, specifically of cats and/or dogs, was higher than the national average, supporting an affinity of vegetarians for animals. Vegans reported the most guilt associated with feeding animal products to their pet and were the most likely to feed a PBD to their pet; around a quarter of vegans fed their pet a diet containing less than 25% products from animals. These findings prompted the research team supporting this PhD thesis to perform a survey-based investigation of plant-based pet feeding practices to determine its prevalence and explore the perceptions of pet owners regarding PBD (Dodd et al., 2019b). Supporting the finding of higher than average pet ownership amongst vegetarians and vegans (Rothgerber, 2013), it was found that disproportionately more vegetarians and vegans were identified amongst the 3,673 surveyed pet owners than reported for the general population. Vegans, more than vegetarians or any other group, reported a concern for the rights of farmed animals, likely contributing to the reported guilt amongst vegans feeding animal products to their pets (Rothgerber, 2013). In support of the previous findings, around one quarter of vegans fed a

strictly PBD to their pet, while three quarters of those who did not expressed an interest in doing so.

One earlier publication included a survey of 86 predominantly vegetarian cat owners as a component of their overall study on cats fed vegetarian diets (Wakefield et al., 2006). All cat owners feeding a vegetarian diet (n = 34) to their cat were themselves a vegetarian (further classification of vegetarian or vegan was not discussed). Similar to the findings of Rothgerber (2013) and our research group's previous findings (Dodd et al. 2019), Wakefield and colleagues (2006) reported over three quarters of the cat owners feeding a vegetarian diet chose the diet due to ethical concerns, whereas vegetarians feeding a conventional (meat-based) diet to their cat did so out of concern for health, convenience, a lack of information regarding vegetarian diets or their cat's preference. They also reported differences in opinions between vegetarian feeders and conventional feeders regarding commercial cat foods and health risks and benefits of vegetarian diets for cats.

Previous surveys have focused on owner beliefs and values (Rothgerber, 2013, Dodd et al., 2019b), or else were limited in scope to a single species and small sample size (Wakefield et al., 2006). To date, no larger scale surveys of owner perception of cat or dog health with respect to PBD have been published.

1.4 Nutrient analysis of plant-based diets

Measurement of nutrients in pet foods of specific interest, such as therapeutic diets, homemade diets, or unconventional diets, have long been used to disseminate information regarding their risks and benefits (Davies, 2014, Stockman et al., 2013, Kanakubo et al., 2015, Funaba et al., 2001, Dillitzer et al., 2011). To account for variabilities between labs, the AOAC International publish official methods of analysis, and AAFCO publish analytical variations as well as quality assurance and quality control guidelines for analytical laboratories working with animal feeds (AAFCO, 2020, AOAC, 2019). Third party analyses of commercial pet food products should thus present the analytical methods utilized in order to defend their findings, particularly if they differ from those reported by the manufacturer of the product.

Considering that domestic dogs and cats evolved from carnivorous predecessors, their anatomy and physiology evolved to acquire, masticate, digest and utilize nutrients from prey animals (Morris, 2002b, Bosch et al., 2015). As such, their dietary requirements include nutrients abundant in animal tissues that may not always be found in adequate concentrations in many plants. Studies investigating the nutrient content of plant-based pet foods have been published, though they have been limited with respect to the number of diets analyzed or the nutrients evaluated. An early study looking at nutritional sufficiency of two plant-based products for cats found numerous insufficiencies (Gray et al., 2004). One product was a commercial canned food, one was a supplement to be added to a home-cooked diet - the supplement was added to a recipe provided by the manufacturer and prepared into a home-cooked food prior to analysis. In

comparison to AAFCO recommendations for adult maintenance, both products demonstrated multiple amino acid insufficiencies, and inadequate ARA and pyridoxine. The canned product also contained insufficient calcium (Ca), phosphorus (P), niacin, cobalamin and vitamin A. Furthermore, the canned product bore a statement of nutritional adequacy for feline growth, yet failed meet AAFCO minimum growth recommendations for lysine (Lys), methionine (Met), methionine+cysteine (Met+Cys) and taurine (Tau), in addition to the aforementioned vitamins and minerals. A more recent study analyzed the nutrient content of four products (three labelled for dogs, one for cats) in Brazil and compared to FEDIAF and AAFCO recommendations (Zafalon et al., 2020). They reported finding inadequate Met in one of the dog products, inadequate arginine (Arg) and arachidonic acid (ARA) in the cat product, and mineral insufficiencies in each (Ca), potassium (K) or sodium (Na) for the dog products, K for the cat product; inadequate Ca:P in all four.

To date, the largest-scale nutrient analysis on vegetarian pet foods was performed in the United States, measuring amino acids in 24 products (Kanakubo et al., 2015). Thirteen were dry extruded diets (9 labelled for dogs, 3 for cats and 1 for both) and 11 were canned (8 labelled for dogs, 2 for cats, 1 for both). One of the canned diets listed egg as an ingredient, the rest listed only plant-based protein sources. All seventeen products compared to canine adult maintenance met all AAFCO minimum amino acid recommendations. Insufficiencies in Met+Cys and tryptophan (Trp) were found when compared to recommendations for canine growth – although it was not discussed whether the diets were labelled for adult dogs or for puppies. In the seven

diets compared to feline adult maintenance, insufficiencies in leucine (Leu), Met, Met+Cys were detected, as well as Tau in all three canned products. When compared to feline growth, inadequate Leu, Lys, Met, Met+Cys and Trp were reported, as well as Tau in all three canned products, though it was not discussed whether the diets were labelled for adult cats or for kittens.

1.5 Diet trials

Controlling dietary intake to perform diet trials in dogs and cats has been used extensively in veterinary and nutrition research. However, trials utilising PBD for pets are limited both in number and in scope. One study compared the effects of varying concentrations of animal (chicken and chicken meal) and vegetable (corn gluten meal) protein in diets with inadequate (12% dry matter) and high (28% dry matter) protein content (Wakshlag et al., 2003). Muscle wasting was reported in the dogs fed the inadequate protein diets, which was worse when the low-protein diet was predominantly vegetable protein, as opposed to animal protein, attributed to worse insufficiencies and imbalances in essential amino acids with an inadequate provision of vegetable protein. Another study compared haematologic parameters in racing sled dogs fed a meat-free or meat-containing diet for four months (Brown et al., 2009). No differences were detected between groups, only increases in red blood cell and haemoglobin values over time, consistent with the intensive training and exercise the dogs were enduring. More recently, a study compared digestibility of vegetarian diets to a diet supplemented with feather meal fed to dogs for 10 days and found the digestibility comparable between all diets (El-Wahab et al.,

2021). The same team also investigated the effect of the same diets on the fecal microbiome, citing beneficial effects on the ratio of Firmicutes/Bacteroidetes from the PBD, particularly when rye was included (Hankel et al., 2020). Other trials have been performed demonstrating comparable performance of plant-based protein sources to conventional animal-based proteins in pet foods in protein digestibility, availability, and quality (Carciofi et al., 2009, Yamka et al., 2003, Yamka et al., 2005). No diet trials have been published that examined the health, nutritional status or body composition of dogs fed nutritionally complete and balanced PBD.

1.6 Metabolomics

Metabolomics is the comprehensive analysis of metabolite concentrations in order to study the effect of different dietary components (Zhang et al., 2015). By examining individual metabolites, the end effects of genetic expression of the animal, their microbiome, and dietary composition are all considered. The study of metabolomics includes analytical chemistry techniques, such as gas chromatography or liquid chromatography (LC) coupled with mass spectrometry (MS), to generate metabolic profiles, upon which bioinformatics tools are used to evaluate relationships, such as pathway analysis, heat mapping and network analysis (Pang et al., 2021). Advantages of MS, coupled with LC, includes rapid, high sensitivity quantitative profiling of separated molecular entities (Zhang et al., 2015).

The popularity of this investigative methodology in veterinary research has grown, improving our understanding of the influences of disease states and dietary parameters on the health and wellbeing of companion animals (Ottka et al., 2021, O’Kell et al., 2020, Muñoz-Prieto

et al., 2021, Moore et al., 2020, Li et al., 2020, Li et al., 2021). Because metabolomic features can be altered by diet, this modality lends itself well to the investigation of effects of different dietary regimes (Rezzi et al., 2007). In dogs, metabolomics have been applied to diet trials to compare effects of different diets on canine fecal and serum metabolomes. (Schmidt et al., 2018, Allaway et al., 2013). In humans PBD have been demonstrated to alter the metabolome, with lower creatine, creatinine, carnitine, acetylcarnitine, taurine, trimethylamine-N-oxide and glutamine excretion in the urine, and lower cholesterol and LDL in plasma (Wu et al., 2014b, Hovinen et al., 2021). These alterations reflect the metabolism of plant-derived nutrients in vegans versus metabolites from higher lipid (cholesterol, LDL) and amino acid (creatine, creatinine, carnitine, acetylcarnitine, taurine, trimethylamine-N-oxide, glutamine) intake from animal products in omnivores. Cholesterol and LDL in humans have been associated with risk of atherosclerosis and cardiac disease, which are common pathologies amongst humans, but not dogs or cats, and PBD have been employed to resolve these lipid abnormalities (Klag et al., 1993, Stone, 1993, McDougall, 1999). In children eating strictly plant-based diets, a shift in bile acid synthesis pathways to conserve taurine has been detected, as well as differences in essential fatty acid, vitamin A and vitamin D metabolism, again reflecting the influence of nutrients from plant versus animal sources (Hovinen et al., 2021).

Considering that diets for dogs and cats are formulated to meet specific nutrient profiles, the influence of ingredients on metabolic pathways may not be as significant as in humans for whom changes in dietary ingredients may affect the absolute intake of particular nutrients.

However, the balance of essential nutrients and non-essential nutrient concentrations may differ between PBD and non-PBD for dogs and cats, and may influence metabolomics in ways previously undemonstrated. Presently, no studies have been published that investigated serum metabolomics in dogs fed PBD, thus the impact of plant-derived nutrients in a diet formulated to meet a canine maintenance nutrient profile has yet to be described.

1.7 Conclusion

Despite their evolutionary history as carnivorous omnivores, in theory, the nutrient requirements of domesticated dogs can be met with diets devoid of animal-derived ingredients. Though studies demonstrating nutritional insufficiencies have been published, the size (number of diets analyzed) and scope (number of nutrients measured) of these investigations have in the past been limited (Kanakubo et al., 2015, Gray et al., 2004, Zafalon et al., 2020). Thus, there are some nutrients that have been identified as being particularly likely to be insufficient in diets lacking animal-derived ingredients, the nutritional profile of commercial plant-based diets is still relatively unclear. Despite the possibility of nutritional imbalance, previous studies have eluded to a perception of health benefits, or a lack of health risks, among pet owners feeding plant-based diets to dogs and cats (Wakefield et al., 2006, Dodd et al., 2019b). However, comparison of owner perception of health and wellbeing between dogs and cats fed plant-based or conventional diets are lacking. Furthermore, data regarding pet health and nutritional status collected from longitudinal feeding trials is lacking. This PhD study therefore aims to: perform comprehensive nutrient analyses in plant-based diets commercially available in Ontario, Canada; describe the

perception of pet owners regarding their pet's health and wellbeing for pets fed plant-based and meat-based diets; and investigate the effects of a nutritionally complete and balanced plant-based diet on dog wellness, nutritional status, body composition, and serum metabolomics

1.8 References

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2 CHAPTER TWO: Research aims and structure

2.1 Statement of the problem

The term plant-based diet (PBD), for the purposes of this thesis, will refer to products devoid entirely of any animal-derived ingredients or supplements. Meat-based diets (MBD) will refer to diets including some degree of animal-derived products, and unless processing is described (ie: extruded, homecooked, raw, etc) these may be raw or processed, homemade or commercial. Conventional diets refers to commercially heat-processed MBDs.

Most pet owners feeding their pets a PBD have been reported to do so out of ethical motivation, although some have reported concerns about perceived negative effects of animal products in pet food, presumably extrapolating from the effects of some animal products on human health, and/or extending perceived benefits of plant-based foods (Dodd et al., 2019b). There is a dearth of information on how exclusively PBD may affect carnivorous companion animals.

Specifically, we sought to question pet owners' perceptions of health and wellbeing in their pets, with a goal of contrasting these perceptions between owners feeding PBD and owners feeding MBD. Additionally, we sought to identify health conditions or body systems affected by PBD by comparing to a control population – the non-plant-based feeders responding to the same survey.

When applied to carnivorous animals, including the facultatively carnivorous dog and the obligatory carnivorous cat, challenges may arise in meeting their nutritional requirements using only plant-based ingredients. Based on existing investigations performed in other countries, it was suspected that plant-based pet food products available commercially in Ontario, Canada may have insufficiencies and imbalances in provision of dietarily essential nutrients for dogs and cats (Zafalon et al., 2020, Kanakubo et al., 2015, Gray et al., 2004). These may predispose dogs and cats to nutrient deficiencies, which may contribute to adverse health outcomes in cats and dogs fed PBD.

While cross-sectional data collected from surveys of pet owners and snapshot nutrient analyses of plant-based pet foods would provide a background understanding of the scope of the problem, longitudinal data are required for any inference of causality. The requirements for dietary essential nutrients, rather than ingredients, is well accepted and thus, there should be little concern, in theory, regarding complete and balanced PBD for dogs and cats. However, this assumes that PBD provide all the essential nutrients in appropriate quantities and that those nutrients are as digestible and bioavailable for carnivorous species as nutrients derived from animal tissues. In practice, those assumptions may not be appropriate, and the metabolic impacts of exclusively PBD on canine and feline body systems require additional research.

2.2 Research aims

This thesis comprised three projects:

- 1) a survey of pet guardians comparing the perceived health and wellbeing of dogs and cats fed

PBD or MBD;

- 2) analyses of essential nutrients in PBD commercially available in Ontario, Canada; and
- 3) a diet trial comparing general health status, body composition, serum amino acid profile, vitamin D status, bone mineralization, and serum metabolomics, in dogs fed a PBD or MBD.

The specific aims of this research were as follows:

1. Understand the perception of pet health held by dog and cat owners, using measures such as reported health conditions, body condition score and previously validated questions regarding general health and wellbeing, and contrast these between owners feeding plant-based and MBD (Chapters Three and Four)
2. Identify health conditions or body systems commonly affected as reported by owners feeding PBD to dogs and cats (Chapters Three and Four)
3. Analyze all nutrients of concern in PBD commercially available in Ontario, Canada (Chapter Five)
4. Evaluate general parameters of health (veterinary wellness examination, CBC, biochemistry, urinalysis) in dogs fed an experimental extruded PBD for three months (Chapter Six)
5. Analyze body composition and measure nutritional status of proteins and amino acids in serum of dogs fed an experimental extruded PBD for three months (Chapter Six)
6. Analyze bone mineral content and density and measure vitamin D status and vitamin D metabolites in dogs fed an experimental extruded PBD for three months (Chapter Six)

7. Compare serum metabolomics between dogs maintained on an experimental extruded PBD or a conventional extruded animal-based diet (Chapter Seven)

2.3 Hypotheses

1. Guardians of cats and dogs fed a plant-based will report a higher prevalence of health disorders (Chapters Three and Four).
2. Conditions previously postulated to be attributable to PBD, such urinary tract disorders, will be more prevalent in pets fed a PBD than those fed a MBD (Chapters Three and Four).
3. Plant-based diets commercially available in Ontario, Canada will have insufficiencies and imbalances in one or more essential nutrients (Chapter Five). It is expected that nutrients previously identified as at risk, including sulfur amino acids, taurine, arachidonic acid, EPA+DHA and vitamin A will not be provided in the recommended amounts.
4. After a three-month diet trial, dogs fed a complete and balanced PBD will have comparable haemogram and serum biochemistry to dogs fed a MBD. Urine pH may increase and struvite crystalluria may be more prevalent in dogs fed the PBD compared to those fed the MBD (Chapter Six).
5. Body composition and serum amino acid profile of dogs will not be affected by eating a PBD for three months (Chapter Six).
6. Vitamin D status and bone mineralization will not be affected by eating a PBD for three months (Chapter Six). It is expected that some vitamin D metabolites may be affected by

the provision of ergocalciferol in the PBD, though total vitamin D status is anticipated to remain stable

7. After a three-month diet trial, dogs fed a PBD will demonstrate differences in the serum metabolome, specifically with respect to metabolism of limiting amino acids (Chapter Seven)

2.4 Overall thesis structure

Figures 2.1 and 2.2 depict the research aims and overview. The concepts of plant-based nutrition, the nutritional requirements of dogs and cats, and the challenges in meeting those requirements without inclusion of animal products in the diet were introduced in Chapter One. The aims and overall structure of the research accumulated in this thesis is presented here in Chapter Two. Chapters Three and Four include published papers from studies of pet owner perception of health and wellbeing of their pets, with a comparison between pets fed plant-based or MBD (Dodd et al., 2021a, Dodd et al., Submitted). The findings from Chapter Three were presented virtually at the 24th congress of the European Society of Veterinary and Comparative Nutrition (ESVCN) (Dodd et al., 2020c), while those of Chapter Four were presented at the 23rd ESVCN congress in Turin, Italy (Dodd et al., 2019c). Chapter Five consists of a published paper from a study of nutrient analyses in commercially available plant-based pet food in Ontario, Canada (Dodd et al., 2021b). The findings from Chapter Five were presented virtually at the 20th symposium of the American Academy of Veterinary Nutrition and the American College of Veterinary Internal Medicine forum (Dodd et al., 2020b, Dodd et al., 2020d). Chapters Six and

Seven describe results from a diet trial in which healthy adult dogs were maintained on either a commercially available animal-based extruded diet or on an experimental plant-based extruded diet, for three months. The results of these two chapters were not yet presented at the time of thesis submission. Chapter Eight discusses the main findings, implications, limitations, and future directions presented from the research accumulated herein.

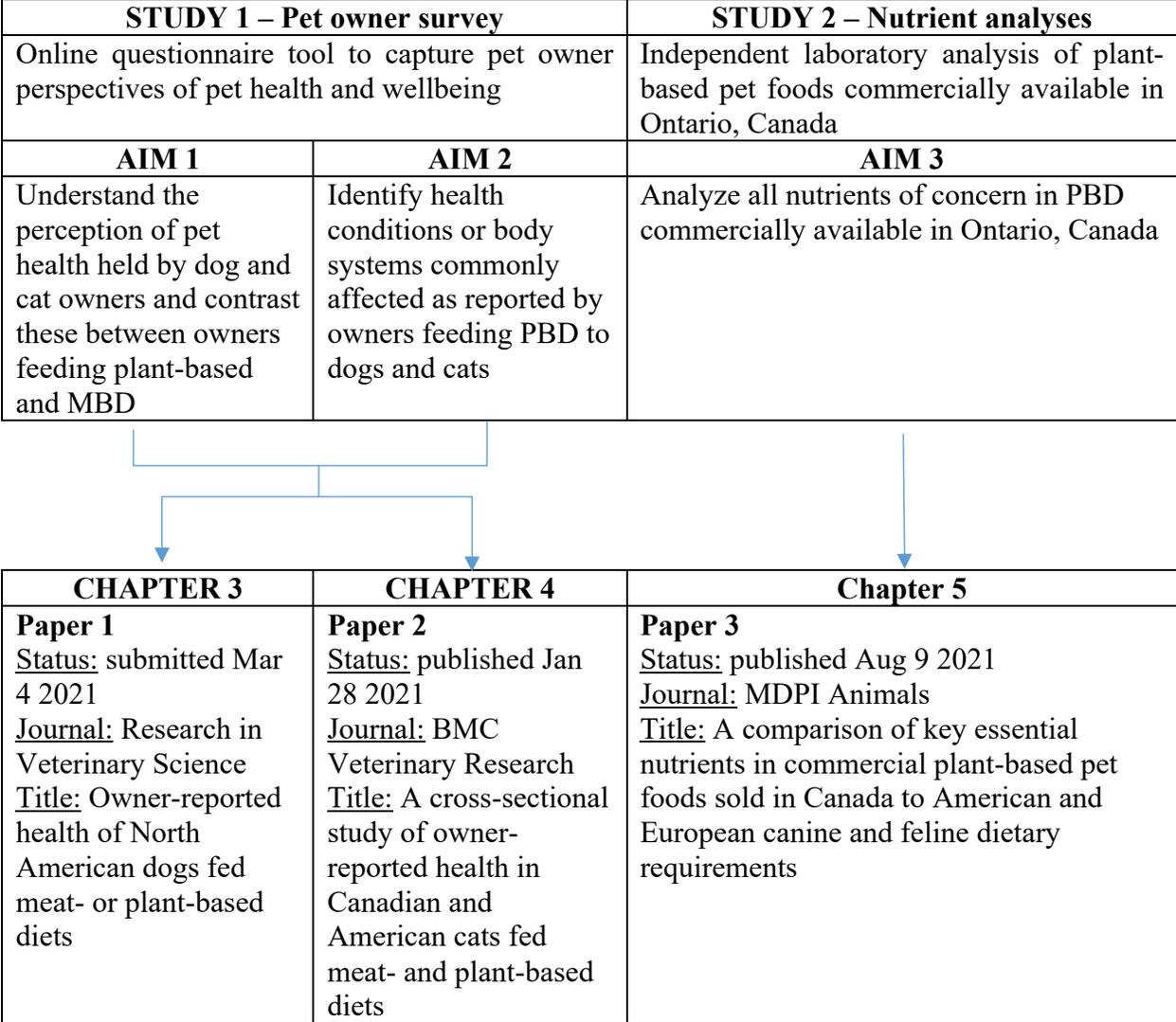


Figure 2.1: Flow diagram representing research aims and overview, part 1.

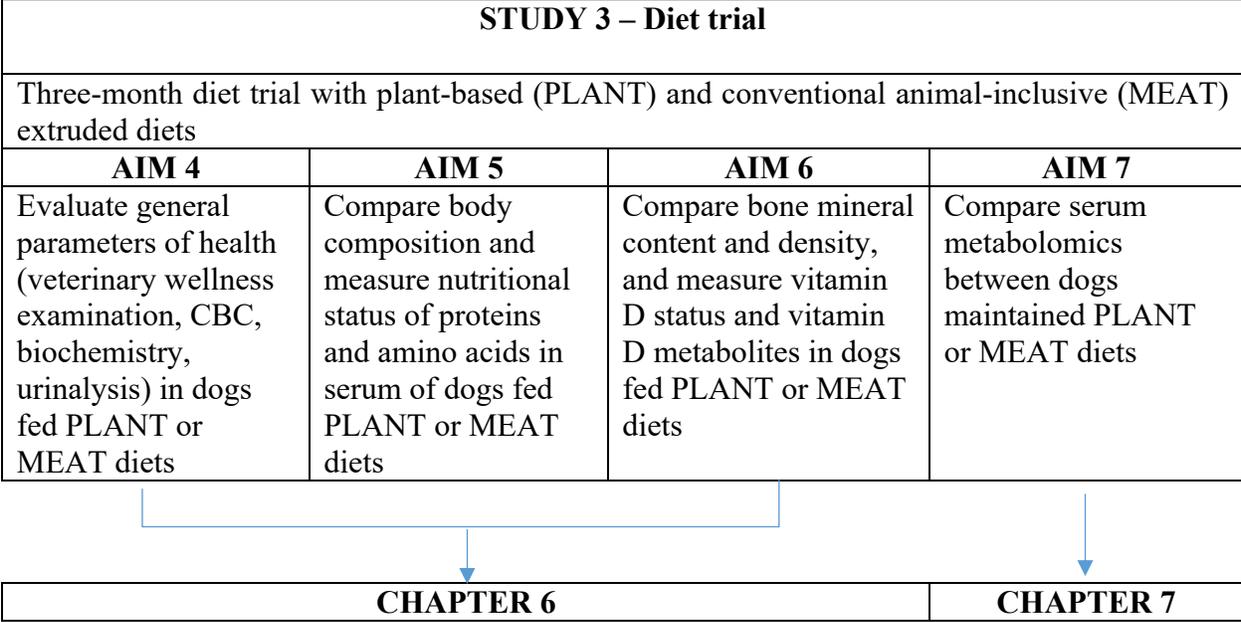


Figure 2.2: Flow diagram representing research aims and overview, part 2.

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3 CHAPTER THREE: Owner perception of health of North American dogs fed meat- or plant-based diets

Adapted from:

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Owner perception of health of North American dogs fed meat- or plant-based diets

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3.1 Abstract:

Background: Some dog owners elect to feed their dog a plant-based food either as part of or for their entire dietary intake. Being omnivores or facultative carnivores, a strictly plant-based diet is not the natural type of food dogs evolved to consume, leaving some question as to whether this feeding management strategy is safe and healthy for dogs.

Objectives: This study surveyed owner perceptions of health and wellbeing of dogs and compared between those fed meat-based and plant-based diets.

Methods: A web-based questionnaire was distributed to pet owners to collect data on dog characteristics, husbandry, health and wellbeing. Univariate comparisons between diet groups was made by chi square analyses or Kaplan-Meier tests as appropriate, with a significance cut-off value of 0.05. Multivariate models were negative binomial and logistic regression for count and categorical data, respectively.

Results: Owners feeding plant-based diets to their dogs reported fewer health disorders, specifically with respect to ocular or gastrointestinal and hepatic disorders. Dog longevity, determined from lifespan of previously-owned dogs, was greater for dogs fed plant-based diets. Owners feeding plant-based diets to their dogs relied less on veterinary associates for nutrition information, versus dog owners feeding meat-based diets.

Conclusions: Dog owners feeding a plant-based diet did not perceive adverse health effects in their dogs. The results might suggest an association between feeding a plant-based diet and improved perceived health and longevity, however inherent bias and limitations associated with

surveys of owner perception must be considered, and objective research is required to determine if plant-based diets truly affect canine health.

3.2 Introduction

The domestic dog (*Canis lupus familiaris*) belongs to the order Carnivora, though this taxonomic nomenclature may come across as misleading. As one may expect from the name, order Carnivora contains the obligate carnivores, Felidae, but it also includes the herbivorous Ailuropodidae (pandas) and the omnivorous canids (Wozencraft, 2005). Though it is commonly thought that the diets of dogs, and their ancestors the wolves, must be comprised of the tissues of other animals, their natural diets can vary greatly, with some being composed predominantly from plant matter (Zlatanova et al., 2014). Most conventional dog foods (commercial kibble and wet products) are typically composed of animal-derived ingredients from the agriculture system producing products for human consumption and thus contain large quantities of commonly farmed animals, namely chicken, cattle, swine, sheep, duck, turkey, deer and a number of fish species. At this time, diets containing ‘novel’ proteins are also popular, including ingredients from more exotic animals such as bison, rabbit, kangaroo, and alligator. Comparatively, few dog foods are devoid of animal ingredients, likely resulting from a combination of the traditional cooperation of the human and pet food production systems, as well as the general acceptance of the dog as being a facultative carnivore who thrives on a meat-based (MB) diet.

Though there is some disagreement among both pet keepers and pet health professionals, it is accepted that dogs can thrive when fed nutritionally complete and balanced diets devoid of animal ingredients (FEDIAF, 2017). Meat-free therapeutic diets have been designed to treat and

manage certain health conditions, with indications for dietary hypersensitivity, gastroenteritis, inflammatory bowel disease, lymphangiectasia, protein-losing enteropathy, pancreatitis, exocrine pancreatic insufficiency, malabsorption, hepatic encephalopathy, urate urolithiasis, cystine urolithiasis, hyperlipidemia and liver disease reported by the manufacturers (Purina® Pro Plan Veterinary Diets® HA Hydrolyzed Canine Formula; Rayne Nutrition™ Plant-Based™ Royal Canin® Veterinary Exclusive Vegetarian Canine). However, it is recognized that formulation and production of complete and balanced diets for dogs is more challenging when limited exclusively to non-animal ingredients (FEDIAF, 2017, Dodd et al., 2018, Kanakubo et al., 2015). In particular, plants are limited in sulfur amino acids and the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. Additionally, plants do not contain all essential vitamins, though they may contain precursors (Dodd et al., 2018). For example, plants do not contain retinol (vitamin A), though they do contain carotenoids, meaning synthetic vitamin A must be added and/or the diet must contain sufficient precursor carotenoids for dogs to convert to active vitamin A (Deming and Erdman Jr, 1999). Similarly, though the dietary requirement of vitamin D is defined in terms of cholecalciferol (vitamin D₃), yet the form of vitamin D typically found in non-animal ingredients is ergocalciferol (vitamin D₂) for which there is no defined dietary requirement for dogs (NRC, 2006, Hazewinkel and Tryfonidou, 2002, How et al., 1994). Vitamin B₁₂, also known as cobalamin, is also lacking in plants, though this nutrient is produced by microbes, including yeasts and microalgae, as well as the microbes within animals' digestive tracts (NRC, 2006). Plant provision of minerals, particularly calcium, can also be low in comparison to the dietary requirements of dogs, though non-animal inorganic sources are common and widely used throughout the petfood industry.

Few previous publications have examined the impact of feeding a plant-based (PB) diet on the health of domestic dogs. While nutrient deficiencies have been documented in PB dog foods (Semp, 2014, Zafalon et al., 2020), no adverse health outcomes attributable to diet have been reported. Indeed, even in dogs with exceptional exercise demands, a vegetarian diet was demonstrated to maintain the limited parameters measured within normal limits (Brown et al., 2009). No case studies have been published either demonstrating efficacy of plant-based diets in maintenance of canine health, nor identifying negative health outcomes associated with feeding a plant-based diet to dogs. Nevertheless, being such a novel feeding trend practiced by approximately 2% (Dodd et al., 2019b) of dog owners (around 1 million dog owners in the USA alone) (AVMA, 2018) the incidence of reporting such dietary-associated conditions may be under-represented. To date, owner perspectives on health of dogs fed plant-based, a topic which may reveal areas in which further research regarding health effects of plant-based diets is indicated, have not been investigated. The objective of this study was thus to survey a sample of dog owners and compare owner perception of health and wellbeing of dogs fed PB and MB diets.

3.3 Methods

This study was approved by the University of Guelph Research Ethics Board (REB # 18-07-039).

3.3.1 Survey Design

An online virtual questionnaire was designed and distributed using the Qualtrics (Qualtrics XM, Provo, Utah, USA) online platform. Questions were based on previously

validated survey items (Schneider et al., 2010, Lavan, 2013) and were piloted by the authors and pet owners before the survey was made available to potential participants. Inclusion criteria were owners of cats or dogs living in the United States of America or Canada. (Data relating to cats are presented elsewhere (Dodd et al., 2021a). Exclusion criteria included age younger than 18 years, non-ownership of a dog or cat, and living outside of the USA or Canada. Incentive to participate was provided as a random draw to obtain a gift certificate for a pet store of the respondent's choosing. Eight prizes of \$25CAD gift cards were available, and participants could choose to enter the prize draw by entering their email address at the end of the questionnaire. Identifying data were removed from the main data and stored separately until the end of the data collection period, such that participant responses were not identifiable. After the prize draw was conducted and the winning participants were contacted, all participant emails were deleted.

The questionnaire included 36 multiple-choice and Likert items, 8 short answer, 7 sliding scale (0-100) and 1 ranking questions. The dataset included responses for cats as well as dogs, cat data is presented elsewhere (Dodd et al., 2021a). The questionnaire used flow logic to reveal species-specific questions, depending on the type of pet the participant was answering for. Owners of both cats and dogs were presented both sets of questions. Participants could choose not to answer or skip questions, as per the requirements of the Research Ethics Board. Questions were designed to collect demographic information about pet owners, pet species, breed, sex, age, acquisition, lifestyle (indoor/outdoor), as well as wellness indicators based on previously validated survey items (Schneider et al., 2010, Lavan, 2013). Body condition score (BCS) was selected by the respondents based on images from the World Small Animal Veterinary Association body condition scoring chart (WSAVA, 2013b), randomly ordered to avoid bias. For

this 9-point method, scores from 1 to 3 are underweight, 4 to 5 are ideal, and 6 through 9 are overweight. Faecal condition score was selected by the respondent based on images corresponding to the Bristol stool chart (Anonymous, 2017), randomly ordered to avoid bias. By the Bristol stool chart, faecal scores of 1 to 2 are abnormally hard to constipated, 3 to 4 is normal, and 5 through 7 range from soft to diarrhoea. To determine prevalence of health disorders, body systems and common disorders were listed, along with an “other” category, and respondents were asked to select the appropriate system and describe their dog’s specific disorder. These included: behavioural, cancer, cardiovascular (heart), dental, dermatologic (skin), diabetes, ear, endocrine, eye, gastrointestinal, hyperthyroid, kidney, liver, musculoskeletal, neurologic, obesity, parasites, reproductive, seizures, trauma/injury, urinary, other. The survey instrument can be found in Appendix I.

3.3.2 Survey Distribution

A link to the survey was distributed via email and postcards to customers of Canadian and American pet food retailers, including deliberate distribution to clients of the largest PB pet food retailer in Ontario, Canada. This convenience sampling was utilised to increase the sample size of dogs fed PB, as it was expected only around 2% of dogs are fed plant-based exclusively (Dodd et al., 2019b) and a statistically significant sample size would be difficult to obtain using random sampling. As well, the survey was promoted in online dog-related groups on social media (Facebook, Inc., Menlo Park, California, USA). The questionnaire, which was available in English only, was made accessible for nine months, from June 2018 to March 2019. Respondents represented a convenience sample of pet owners voluntarily participating in the study.

3.3.3 Survey Analyses

Survey responses were included for analysis for each question that was completed, incomplete or skipped questions were not included for analysis. Dog breeds were categorized into breed types based on phenotypic and genetic similarities (Turcsán et al., 2011, Voith et al., 2009). Description of dog diet was collected in an open-text question and, where sufficient information was obtained, categorized based on ingredients (MB, PB) and processing (commercial heat-processed, homemade, raw) or a combination of the aforementioned (ie: some dogs were fed more than one type of food on a regular basis). The term PB referred to a diet that contained no animal ingredients, while the term MB referred to any diet including animal-derived ingredients. Thus, while a MB diet could also include plant-based ingredients, a PB diet included no animal-derived ingredients. In addition to the main diet, information was also collected regarding the feeding of treats (commercial heat-processed, raw, homemade), table foods and supplements, and categorized based on ingredients (MB or PB). Dogs fed a PB diet but also given MB treats or snacks and/or supplements containing animal-derived products, were categorized as PB+MB. Dogs reported to have free access outdoors and/or ability to hunt were not considered to have a strictly PB diet even if fed PB. These dogs were re-categorized as PB+MB/H, a group that included dogs fed a PB diet but either supplemented with a MB treat, snack or supplement and dogs fed only PB but with hunting ability. Dog wellness measured by Likert item was reported directly (e.g.: poor, fair, adequate, good, very good), while sliding scale data were translated by the survey software into a value ranging from 0 to 100. Dog health disorders were offered in a multiple-choice question, with the option of ‘other’ for input by the respondent. Owner-reported ‘other’ health disorders were categorized by a veterinarian (SD) and

included for analyses. Based on the responses, aural, behavioural, musculoskeletal, parasites, reproductive and trauma/injury were removed as categories and not included in statistical analysis. The majority of aural disorders were actually external ear infections, a dermatopathy, and thus considered in that category. True behavioural disorders were difficult to differentiate from issues of training or lifestyle (such as “he hates when anyone touches his paws”, “unsociable”, and “she doesn’t listen very well”). Reported musculoskeletal and trauma/injury issues were difficult to interpret, with ambiguous descriptions and anecdotes of incidents that were believed to have occurred prior to current ownership, such as “she has scars of dog bites from when she lived on the streets before being rescued”, “she had two broken legs when I rescued her, she limps a little. Not sure how her legs were broken”, and “had shell casings from a type of pellet in her ear and shoulder”. The parasite category was dropped as responses were typically regarding puppy worming or simply indicated that the dog was regularly treated with endo- or ectoparasite medications; reproductive disorders were similarly dropped as the most common reported issue was either having puppies or desexing surgery. The remaining variables were recategorized to: cardiac, dental, dermatologic, endocrine, gastrointestinal and hepatic, lower urinary tract, neoplasia, obesity, ophthalmic, and renal.

3.3.4 Statistical Analyses

All statistical analyses were performed using Stata/IC 15.1 (StataCorp, College Station, Texas, USA) statistical software package. Descriptive statistics included frequency (n) and percentage (%) presented for most data (type of pets, location and time of dog acquisition, breed, indoor/outdoor management, diet and supplementation, motivators for selection of dog food, resources for information about canine nutrition). Frequency and percentage were also used for

data collected using Likert items for ranked data. Mean and standard deviation were presented for normally distributed data (dog age, duration of dog ownership, duration of feeding and lifespan of previous dogs [all measured in years]). Median and range was presented for ordinal data (BCS, FS) and count data (number of dogs kept, number of health disorders per dog).

Univariate comparisons between diet categories and responses related to pet food purchasing behaviours and pet nutrition information resources were conducted using χ^2 testing (see Tables 4 and 5). These analyses were performed when comparing between diet categories only (pet food purchasing, nutrition resources), without consideration of potential confounders. Age of previously owned dogs at death was compared among diet groups using Kaplan-Meier statistic.

Statistical models were selected based on the nature of the variable of interest – either count, binary or continuous data. The relationship between number of health disorders per dog, measured as count data, and diet type was modelled using multivariate negative binomial regression. Within the model, the dependent variable was the number of health disorders per dog. Independent variables considered included dog diet (3-level categorical: PB, PB+MB/H compared to MB), dog age (years), dog age squared (years squared), sex (2-level categorical: male compared to female), sex status (2 level categorical: intact compared to desexed), breed type (14-level categorical: collies and small herding dogs, large shepherds and livestock dogs, mastiffs and bulldogs, Nordic breeds, retrievers, scent hounds, sighthounds, spitz and primitive dog breeds, terriers, toys, working and hunting dogs, small mixed breeds, large mixed breeds, compared to mixed breed dogs of unspecified size), and BCS (ordinal: 3, 5, 7, 9 compared to 1).

Variables were assessed for correlations and collinearity prior to inclusion in the final model, no collinearity correction was required. Using backward stepwise elimination to remove non-significant variables, the final model included dog age, age squared, sex status, breed type and diet. Model fit was visually evaluated by assessment of distribution of the residuals.

Logistic regression models were designed to assess the relationship between individual health disorders and diet. One model was developed for each individual health disorder as the dependent variable (cardiac, dental, dermatological, endocrinological, GI and hepatic, neoplastic, neurological, obesity, ocular, renal and urinary), measured as a binary present/absent outcome. Independent variables considered included dog diet (MB, PB, PB+MB/H), dog age, sex, sex status, breed type, and BCS. Variables were assessed for correlations and collinearity prior to inclusion in the model, no corrections for collinearity were required. Backward stepwise regression was used to eliminate non-significant variables from the final multivariate model for each health disorder. Significant independent variables kept in each multivariate model differed based on health disorder (Table 3.7). Though non-significant for some disorders, age was forced into the model due to the known associations between increasing age and risk of health disorders as was diet, as it was the variable of interest. Model fit was assessed by Hosmer-Lemeshow goodness-of-fit test.

Ordered logistic regression modelling was used to evaluate the relationship between owner perception of dog health, ranked in four levels: “poor”, “fair”, “good”, and “very good”. The dependent variable was health ranking, with odds ratios reported in comparison to the referent outcome “very good”. Independent variables considered included dog diet (MB, PB,

PB+MB/H), dog age, sex, sex status, breed type and BCS. Variables were assessed for correlations and collinearity prior to inclusion in the model, no collinearity corrections were required. Backward stepwise regression was used to eliminate non-significant variables from the final multivariate model. Significant independent variables kept in the final multivariate model were dog age, sex and diet.

For all analyses, statistical significance was set at $P < 0.05$. A-priori sample size estimations were made using data regarding prevalence of canine health disorders (O'Neill et al., 2014b, Lund et al., 1999). Considering the suggested increased risk of urinary tract diseases in dogs fed PB diets (Knight and Leitsberger, 2016), this health disorder was chosen for sample size estimation. Assuming the proportion of dogs fed MB with urinary tract disorders was 3%, calculation of the sample size comparing two different proportions yielded a required sample size of 171 dogs per group. Post-hoc power calculations were performed comparing the mean values or proportions of each variable of interest between diet groups, considering an α value of 0.95 and a β of 0.8 as the cut-off points. Power less than 80% was considered too low and represented an insufficient sample size to detect significant ($P < 0.05$) differences.

3.4 Results

3.4.1 Response rate and demographic information

A total of 1,413 questionnaires were undertaken and included for analysis. Partially completed surveys were included, thus the number of responses for each question varied.

Demographic data of respondents are shown in Table 3.1. Post-hoc power calculations confirmed

the sample size was adequate to attain statistical significance for comparison between dogs fed different diets.

Nearly twice as many respondents kept dog(s) only (916/1383, 65%) when compared to those who kept dog(s) and cat(s) (497/1413, 35%). The median number of dogs kept per respondent was one (range 1 – 13). Most dogs were acquired from shelters, rescues or veterinarians (601/1344, 45%), followed by purchase from registered breeders (347/1344, 26%), inherited or gifted from friends and family (132/1344, 10%) or purchased online (136/1344, 10%). Less commonly, dogs were purchased from backyard breeders, farms, or homebred (98/1344, 7%) or from pet stores (30/1344, 2%). Most dogs were acquired by respondents at puppyhood (786/1307, 60%), with less than half acquired later in their life (523/1307, 40%), and had been kept by the respondents for 2 weeks to 18 years (mean 5.1 years, std. dev.3.9).

3.4.2 Dog characteristics

A summary of dog characteristics is shown in Table 3.2, grouped by diet type. One hundred and eighteen specific breeds were reported, available in Table S1. An additional 162 named crossbreeds were also reported, the rest were mixes or unknown. There was no association between dog diet and breed type.

Median dog age was six years, with a range from four months to 18 years. There were no associations between dog diet or breed type and dog age. Most dogs lived indoors with controlled access to the outdoors (1156/1351, 86%), some had uncontrolled access to the outdoors (184/1351, 14%), and few lived outdoors exclusively (11/1351, 0.8%). The majority of respondents indicated that their dogs did not hunt prey (1184/1350, 88%). Some dogs were

reportedly capable of hunting, though their owners did not believe they did so (106/1350, 7.9%), and few were known to hunt prey (60/1350, 4.4%).

3.4.3 Dog diet

Diet information was collected for 1,189 dogs, with half (665/1189, 56%) being fed MB, a third (357/1189, 30%) being fed strictly PB, and few being fed a combination of PB with MB (63/1189, 5%) or indeterminable (104/1189, 9%). Dogs fed a PB diet but known to hunt prey were re-categorized as PB+MB/H for health outcome comparisons, reducing the number of plant-based dogs to 339, and increased the number of PB+MB/H to 81. Table 3.2 shows a summary of dog feeding practices, by dog demographic. Just over half of dogs were reported to receive treats (710/1196, 59%), while half also received table scraps (595/1195, 50%) in addition to their main diet. Significantly ($P < 0.001$) fewer dogs fed PB received treats (175/357, 49%) as compared to dogs fed MB (440/665, 66%). There were no differences in feeding of table scraps, though the types of table scraps differed. Regardless of overall diet, the most common scraps given to dogs were fruit and vegetables (199/665, 30% MB vs 156/357, 44% PB). Aside from fruits and vegetables, among the dogs fed MB, meat scraps were most commonly offered (118/665, 18%), followed by eggs and dairy (92/665, 14%). Among the dogs fed PB, nuts, seeds and legumes were common scraps (43/357, 12%), followed by grain products like pasta, bread, rice and ancient grains (38/357, 11%). Supplements were given to a quarter of dogs (315/1189, 26%). Among the 315 dogs given supplements, the median number of supplements per dog was 1, with a range from 1 – 7. Supplement use varied significantly between diet groups, with 33% of PB (119/357) compared to 25% of MB (165/665) dog receiving supplements ($P = 0.002$). The most common supplements given to dogs fed MB (165/665, 25%) were sources of omega-3 fatty

acids, glucosamine, chondroitin and ingredients indicated for joint health (107/665, 16%) and vitamins, minerals or coenzymes (44/665, 6.6%). Supplements offered more commonly to dogs fed PB (119/357, 33%) were vitamins, minerals or coenzymes (47/357, 13%), yeast (36/357, 10%), or nuts and seeds (26/357, 7.3%). Supplement use was also variable dependent on food processing, used by 24% of owners feeding commercial heat-processed diets, 41% feeding commercial or homemade raw MB diets, and 54% feeding cooked or raw homemade diets. Supplement use differed significantly ($P = 0.006$) with presence of health disorders. Provision of dietary supplements increased with reporting of health disorders, ranging from 23% (112/482) in dogs with no health disorders to 41% (14/34) in dogs with 4+ disorders. The odds of using one or more supplements were 1.2 times higher per disorder present. Specifically, supplement use was significantly higher for dogs with dermatological (78/247, 32%, $P = 0.04$) and musculoskeletal (48/100, 48%, $P < 0.001$) disorders.

Diet selection was primarily based on whether a diet was complete and balanced (852/1184, 72%), all selection criteria are shown in Table 3.3 and compared between dog diets. Significant differences were found between feeders of MB and PB diets regarding specific ingredients they wished to avoid or they particularly wanted included in their dog's food. Among the MB feeders, avoiding grains (64/661, 9.7%) or specific animal ingredients (52/661, 7.9%) were most common. Among the PB feeders, avoiding all animal ingredients (350/357, 98%) or grains (12/357, 3.4%) were most common. Respondents not only indicated what their selection criteria were, but they also ranked the importance of their chosen selection criteria (Figure 3.1). Respondents indicated the resources they used for information about canine nutrition. Veterinary professionals (802/1106, 68%), and the internet (765/1106, 65%) were equally the most common

reported sources of canine nutrition information, though proportion varied by diet (Table 3.4). Owners feeding PB relied more on the internet (285/357, 80%) while owners feeding MB relied more on veterinarians (498/664, 75%).

Dogs had reportedly been fed their current diet for their whole life (650/1205, 54%) or had changed diet at least once prior to being fed their current diet (555/1205, 46%). On average, dogs had been fed their current diet for 3 years (range 0.05 – 15 years). There was no difference in duration of feeding between dogs fed MB (median 3 years, range 0.05 – 18) or PB (median 3 years, range 0.05 – 16). Of the dogs fed a PB diet, 20% (72/357) had unsupervised access to the outdoors and 12% (42) were reported to be capable of hunting prey, while 5.3% were known to hunt (19/357) and could thus supplement their otherwise plant-based diet with prey.

3.4.4 Dog health and wellbeing

Owners reported dog BCS ranging from 1 to 9, with most owners reporting their dogs to be in ideal condition (679/1187, 57%). More owners reported their dogs to be overweight (312/1187, 26%) than underweight (196/1187, 17%). There were no significant differences in owner-reported BCS based on diet type. Based on Kruskal Wallis test, there were significant differences in BCS based on breed type, with the lowest BCS (mean BCS 4, Std. Dev. 1.6) for sighthounds and the highest (mean BCS 5.6, Std. Dev. 1.3) for retrievers ($P = 0.0001$). Mean reported faecal score was 2.6 (Std. Dev. 1.05) on a scale from 1 to 7. There were significant ($P = 0.02$) differences in faecal score depending on diet, with fewer owners of dogs fed PB reporting constipation (0/336, 0% vs 8/652, 1.2%) or hard faeces (178/318, 56% vs 431/652, 66%), and

more reporting slightly soft faeces (44/318, 14% vs 51/652, 7.8%) in comparison to dogs fed MB.

Health disorders were reported by the dog owners and categorized by body system or as systemic disorders, as appropriate. The number of owner-reported disorders per dog ranged from 0 to 7 (mean 0.97, Std. Dev. 1.17), with nearly half of all dogs (624/1413, 44%) having no reported health disorders. Age was positively associated with the number of health disorders reported, with every year of age increasing the number of health disorders by 0.08 ($P < 0.001$). Difference in number of owner-reported health disorders were also detected by breed type, with toy breeds, retrievers, scent hounds, terriers, mastiffs and bulldogs, working and hunting dogs, sighthounds, small crossbreeds and large crossbreeds having more health disorders than mixed breed dogs. A negative binomial regression was designed to investigate the association between diet and number of health disorders, including potential confounders age, age squared, sex status and breed type in the model. In comparison to owners of dogs fed a MB diet, owners of dogs fed strictly PB reported 0.13 (95% CI 0.03 – 0.22) fewer health conditions ($P = 0.009$). Prevalence of the most common health disorders reported are shown in Table 3.5. Logistic regression was used to investigate diet and specific health disorders, with potential confounders age, sex, sex status, and dog type also included in the models (see Table 3.6). Owners of dogs fed plant-based diets had reduced odds of reporting GI and hepatic or ocular disorders in their dogs.

Owners also ranked their dog's health from poor to very good. The majority considered their dog's health to be very good (849/1187, 72%), regardless of diet (PB 268/338, 80%; MB 459/665, 69%). Indices of wellness were also reported (Table 3.8).

3.4.5 Dog lifespan

Respondents were asked about previous dogs, with indicated lifespans ranging from 8 months to 23 years (median 13 years). The diets fed to previous dogs were MB (907/1201, 76%), PB (103/1201, 8.6%), a combination of PB with MB (42/1201, 3.5%) or indeterminable (149/1201, 8.8%). Previous dogs reportedly fed a PB diet had significantly ($P < 0.01$) longer lifespan than dogs fed MB (mean 14.1 years, 95% CI 13.5 - 14.7 vs mean 12.6 years 95% CI 12.3 - 12.8, respectively) (Figure 3.2).

3.5 Discussion

The convenience sampling strategy employed was successful in targeting owners feeding PB to their dogs. The proportion of owners feeding PB diets in this study, 30%, was greater than what has been estimated for the general pet-keeping population, which as previously documented by this research group was around 2% (Dodd et al., 2019b). This was expected, as the study was not intended to document the prevalence of particular pet feeding practices, but to compare the owner-reported perceived health and wellbeing of dogs fed PB and MB diets. The convenience sample strategy targeting PB feeders was utilized to obtain a comparable sample size between PB and MB diet feeders. When compared to previous reports, participants in this survey were typical of the demographic of pet food and pet health survey respondents (Dodd et al., 2019b, Morgan et al., 2017, Rajagopaul et al., 2016). Furthermore, dog keeping characteristics, including number of dogs and indoor/outdoor lifestyle, as well as dog characteristics such as median age, sex and sex status, were similar to previous publications from Canada, the United States of America and worldwide (Pugh et al., 2015, Heuberger and Wakshlag, 2011, Wan et al., 2009).

Aside from the high previously mentioned high prevalence of PB feeding, the feeding practices documented here are comparable to other recent reports (Stull et al., 2013, Connolly et al., 2014, Dinallo et al., 2017, Dodd et al., 2020a). The feeding of treats and table scraps was expected, though daily intake from treats and scraps could not be quantified. The risk this posed to unbalancing a complete diet could thus not be estimated, though a lack of correlation between treat/scrap feeding and BCS or health disorders would suggest this practice did not pose a significant risk. The proportion of fed supplements was lower in this study population as compared to previous estimates, though the range of supplements fed per dog and the proportion of supplement types were similar (Bianco et al., 2020). Supplementation of vitamins and minerals is indicated when homemade diets are being fed, but were reported by only half of the respondents feeding a homemade diet. Commercial diets labelled for growth or maintenance sold in the USA and Canada are typically formulated or undergo feeding trials to ensure they meet the nutritional requirements of dogs and are considered to provide complete and balanced nutrition for the lifestage and species to which they are intended to be fed (FDA, 2020). Feeding of additional vitamins and minerals in combination with commercial diets, as practiced by nearly 10% of study respondents, may actually be counterproductive and could induce toxicities or interactions between excess vitamins and other nutrients. For example although supplementation up to five times the recommended allowance has been demonstrated to be safe in dogs with vitamin D deficiency (Young and Backus, 2016), clinical hypervitaminosis D has been reported in dogs when their diet contained large excess (Mellanby et al., 2005). The higher prevalence of supplementation of vitamins and minerals in dogs fed PB diets suggests that those owners either have concerns about the vitamin and mineral content of PB diets for dogs, or that there is a

perceived benefit of additional vitamin and/or mineral supplementation. Previous work has demonstrated that 60% of vegan pet owners did have concerns that a PB diet would provide incomplete nutrition (Dodd et al., 2019b), so it is possible the higher prevalence of vitamin and mineral supplementation, as compared to feeders of MB diets, reflects an attempt to balance the diet. Alternatively, it may reflect vegans' behaviours regarding their own diet, as the majority of vegans supplement their daily intake with a vitamin supplement, over five times more than the general population (Vollmer et al., 2018).

In addition to vitamins and minerals, dogs were also given fatty acids, amino acids and their derivatives and herbal supplements. Over supplementation of fatty acids for anti-inflammatory effects and joint or coat health may also have adverse effects, such as altered platelet function, vomiting, diarrhoea, pancreatitis, altered wound healing, lipid peroxidation, and altered immune function (Lenox and Bauer, 2013). Excessive doses of amino acids and amino acid derivatives can also have adverse effects, ranging from mild gastrointestinal upset to death (Gwaltney-Brant et al., 2000). Even herbal remedies, which pet owners often believe are safe and natural products, are not without risk and severe to life-threatening toxicities are possible (Ooms et al., 2001, Means, 2002, Poppenga, 2001). Furthermore, some supplements that may be safe when fed to healthy pets, could be counter-productive and provide excess nutrients when given with the intention of treating particular health (Shmalberg et al., 2013). Considering that dogs with health disorders were more likely to receive dietary supplements than healthy dogs, this could pose a very real risk to dog health.

The most frequently reported criterion for diet selection was the labelling of the diet as being complete and balanced, and it was also valued as the second most important criterion (Figure 3.1). The most important criterion was veterinary recommendation. Of all the criteria reported by dog owners, complete and balanced and veterinary recommendation are the two criteria which relate most to the suitability of the diet for the animal, while most of the other criteria relate to the suitability of the diet for the owner. This suggests dietary choices are being made with the best interest of the animal as a top priority, which is not surprising, considering the role of dogs as family members (Case, 2008) and previous research regarding the prioritization of dog nutrition (Kamleh et al., 2020). The resources dog owners are using to educate themselves about canine nutrition are shown in Table 3.4 and it can be seen that veterinary professionals and the internet are similarly indicated. Among dog owners feeding MB, veterinarians outweigh the internet considerably, though among those feeding PB, the inverse relationship was seen. It is recognized that veterinary clinics may provide some nutrition-related information on their website, and veterinary professionals also run social media websites and blogs regarding nutrition. These resources were not differentiated from other internet resources in this study, thus, it is possible for the internet and veterinary categories to overlap. Nevertheless, the inverse relationship between internet and veterinary professional reported by PB vs MB feeders is interesting. It has been demonstrated that feeders of diets considered unconventional or alternative may have less trust in veterinary professionals as a source of nutritional information (Morgan et al., 2017). This phenomenon likely includes feeders of PB diets, as only 20% of vegans regarded veterinary approval as being an important factor in determining whether or not they would consider feeding their pet a PB diet (Dodd et al., 2019b).

Of note, neither diet group reported high usage of pet food manufacturers or distributors as sources of information regarding canine nutrition. Whether this indicates a lack of trust in the pet food industry, as has been previously suggested (Sprinkle, 2018), or whether pet owners make some decisions about pet food purchases based on information acquired prior to going to a store or online retail site could not be determined from this data.

With respect to the impact of diet on health and wellbeing, it must be noted that very few nutritionally-associated outcomes arise rapidly, particularly in adult animals, and thus long-term feeding of a diet is typically required in order to see a response or change in a given parameter. In young, growing puppies, nutritional imbalances and inadequacies can rapidly manifest with severe clinical signs (Dodd et al., 2019a, Hutchinson et al., 2012, McMillan et al., 2006, Tal et al., 2018). However, in adult dogs, these can take months to years to develop prior to diagnosis (Verbrugge et al., 2011, de Fornel-Thibaud et al., 2007). Thus, the duration of feeding was an important factor to extrapolate any association between diet and health. Half of dogs had been fed their current diet for as long as they had been owned, with the median duration of ownership being 4 years. The median duration of feeding the current diet was also 3 years. Association between health status and diet of the participants' dogs was therefore considered possible, as much as could be attributed to diet.

The proportion of dogs reported to be ideal body condition (57%) is likely an overestimation, as the proportion of dogs reported to be overweight (26%) is likely an underestimation. In 1995, the prevalence of overweight and obesity among US dogs was close to the findings of the current study (Lund et al., 2006), but has been trending upwards since then

(Chandler et al., 2017). A survey of American veterinary clinics in 2018 found that, based on veterinary assessment of BCS, only 43.1% of dogs were ideal, 55.8% were overweight (36.9%) or obese (18.9%), only 1.1% were underweight (Ward, 2019). This discrepancy was expected, to a degree, as it has been previously described that pet owners fail to appropriately identify overweight conditions in their pets (White et al., 2011) and that untrained evaluators tend to underestimate BCS (Shoveller et al., 2014). So, while the overall reporting may be an underestimation of the true BCS, the factor of most interest in this study was a comparison of the owner-reported BCS between dogs fed MB and PB diets. In humans, a difference in body composition has been reported between vegans and non-vegans, with vegans being leaner with a lower body mass index (Rosell et al., 2006, Le and Sabaté, 2014, Berkow and Barnard, 2006, Melina et al., 2013), and a PB diet being recommended for treatment and prevention of obesity (Turner-McGrievy et al., 2017, Kahleova et al., 2018, Wright et al., 2017). The same was not found for dogs, with no difference in BCS attributable to diet, suggesting that PB diets do not protect against overweight in dogs.

Overall the reported faecal consistency was firmer than is considered normal, with the majority of respondents indicating a faecal score of 2, which is considered slightly harder and drier than ideal. Whether these dogs were truly exhibiting abnormal faeces, or whether this represents a challenge using pictographic faecal scoring systems with untrained pet owners could not be determined. However, it is interesting to note that dogs fed PB were reported to have softer stool than dogs fed MB, with more faecal scores consistent with normal stool. This may be attributable to the greater presence of dietary fibre in plant ingredients compared to animal

ingredients, as fibre has noted effects on faecal consistency (Jackson and Jewell, 2019, Propst et al., 2003, Fritsch et al., 2019).

In this study, results relied on owner reporting of perception of health, no review of medical records or confirmatory examination could be performed. The proportion of dogs with one or more health disorder reported by the owner was greater in this study than has previously been documented by other surveys of pet owners (Heuberger and Wakshlag, 2011, Freeman et al., 2006). The data captured by this study includes conditions that dogs may have had in the past, but have overcome, as opposed to including only disorders affecting them at the present time. Conversely, as expected, the proportion of dogs with health disorders was lower than what is reported for dogs attending veterinary clinics, as the majority of visits to veterinary clinics are as result of a health disorder (Lund et al., 1999). The prevalence of specific conditions, however, was comparable to data collected from primary healthcare facilities, with dental disease and dermatopathies most commonly reported (Lund et al., 1999, O'Neill et al., 2014b). As noted above, the prevalence of obesity reported in this study, 3.7%, is likely an underestimation. Nevertheless, the number of dogs reported to be obese did agree somewhat with the number of dogs reported to have a BCS of 9, suggesting that owners did recognize such an extreme degree of overweight as being a pathological condition, though owners of overweight dogs with a BCS of 7-8/9 did not appear to recognize those BCS as obesity. Prevalence of health conditions varied among dog breed types. It has been documented that mixed breed dogs in the UK had significantly lower prevalence of obesity and dermatological and ophthalmological disorders than purebred dogs (O'Neill et al., 2014b). In the present study as well, owners of mixed breed dogs reported a lower number of health disorders.

Dog owners reported fewer disorders per dog reported for dogs fed PB than MB diets. Specifically, an association between dogs fed PB and reduced odds of ocular or GI and hepatic disorders was detected, though the reduced odds of ocular disorders was suspected to be a spurious finding. Further, more owners feeding their dog PB considered their dog to be in very good health as compared to owners feeding their dog MB. Whether this reveals dogs fed PB truly have better health, or that owners are biased in their perceptions and feeding PB have a more positive perception of their dogs' health could not be determined. Previously, it has been reported that vegetarians (of which 17% were vegan) had more positive attitudes towards pet animals as compared to non-vegetarians (Preylo and Arikawa, 2008). Based on the previous finding that pets being fed PB were almost all owned by vegans, and the remainder by vegetarians (Dodd et al., 2019b), it was assumed that a majority of dogs fed PB in the present study were owned by vegans or vegetarians, and it is possible this increased positive perspective on dog health may be attributable in part to a more positive attitude towards the dog in general. Furthermore, meat-avoidance has been associated with a more 'health conscious' identity and improved perception of health, which vegetarian or vegan pet owners may be applying to their pet as well (Bedford and Barr, 2005, Corrin and Papadopoulos, 2017). These dog owner attitudes and beliefs should be considered by veterinary practitioners when discussing diet and health with owners of dogs fed PB diets.

An alternative possibility is that upon diagnosis with a health condition, the diet of dogs may have been changed on recommendation of a veterinary practitioner. Thus, it is possible that dogs previously fed a PB diet may have been switched to a meat-containing diet, thus giving the erroneous appearance that fewer dogs fed PB diets suffer from GI-related disorders, although a

change from a meat-based to a plant-based therapeutic diet may also be recommended by veterinary practitioners. Some veterinary therapeutic diets are indicated for adverse food reactions, chronic gastrointestinal disease, exocrine pancreatic insufficiency, food allergic dermatitis, food allergic gastroenteritis, food intolerance, hepatic encephalopathy, hyperlipidemia, inflammatory bowel disease, liver disease, lymphangiectasia, malabsorption, pancreatitis, protein-losing enteropathy and urate and cystine urolith prevention are plant-based, though most therapeutic diets indicated for canine health conditions are meat-based.

Based on owner reporting of the lifespan of their previously-owned dogs, longevity reported in this study is in close agreement with other studies (Galis et al., 2007, Yordy et al., 2020, Adams et al., 2016, O'Neill et al., 2014b). Interestingly, despite the lack of differences in perceived health detectable amongst the respondents' current dogs, diet had a significant association with lifespan, with dogs fed PB reportedly living 1.5 years longer. It must be recognized that a retrospective survey collecting owner-reported data based on their recollection of information does not provide the same level of evidence as a prospective paired cohort lifetime study. However, it does present an interesting insight, if accurate. In humans, aside from prevention and treatment of certain diseases, including obesity, cardiovascular disease and diabetes, a PB diet has also been proposed to slow aging and increase longevity (McCarty et al., 2009). However, the main mechanism by which this is proposed is via a reduced intake of methionine, which may not be translatable to dogs and dog diets, as dietary methionine content must meet a minimum recommended level in order for the product be labelled as complete and balanced for either puppy growth or adult maintenance. In a study investigating the amino acid content of commercial PB dog foods in Canada, the methionine content ranged from 84 to 504%

(median 181%) of minimum requirements according to the National Research Council (Dodd et al., 2020b). This doesn't support the idea of PB diets increasing longevity as a consequence of low provision of methionine. Furthermore, in dogs, methionine restriction may result in insufficient endogenous synthesis of taurine and adverse health outcomes associated with sulfur amino acid and taurine deficiencies (Backus et al., 2006, Fascetti et al., 2003). Alternative theories include the ability of a PB diet to down-regulate insulin-like growth factor I and thus 'slow' the aging process (McCarty, 2003), or to boost production of 'pro-longevity' fibroblast growth factor 21 (McCarty, 2015). Research investigating the effects of PB diets on lifespan in dogs is necessary.

An alternative reason for the owner-reported longer lifespan in dogs fed PB diets could be a delay of euthanasia in aged and end-of-life dogs. The unique perspectives of individual dog owners, with respect to their thoughts, feelings and experiences, may impact decision-making regarding euthanasia of aged and ill dogs (Spitznagel et al. 2020). It was assumed, based on previous research, that the majority of respondents feeding a plant-based diet to their pet followed a vegetarian or vegan lifestyle themselves (Dodd et al. 2019). It is thus possible that, given the strong affinity of meat-avoiders for companion animals (Rothegeber 2013, Herzog et al. 1991), that making end of life decisions may be more drawn out, resulting in a longer lifespan. In contrast, vegetarians and vegans may have greater concern for animal suffering and greater empathy for animals (Filippi et al. 2010) when compared to non-vegetarians, which would suggest compassionate consideration of euthanasia when dogs enter end-of-life criteria. More research investigating owner beliefs and feelings towards animals and their decision making surrounding end-of-life and euthanasia are needed.

Being a retrospective, convenience sampled, cross-sectional study, the findings presented in this study must be interpreted with recognition of the biases and limitations inherent due to study design. The sampling strategy employed allowed for self-selection into the study which likely introduces bias with respect to the nature of the participants. Notably, pet owners with exceptional interest in pet health and wellness would be most likely to voluntarily participate in the study, which could affect the results. Pet owners with specific interest in their pet's health and wellness may be highly perceptive and aware of conditions affecting their pet, resulting in their pet being presented to their veterinarian more often. This could either prevent health disorders by implementing appropriate prevention strategies or result in earlier diagnoses. These two potential outcomes could have opposing effects on the number of health disorders reported as prevention would decrease the number of health disorders occurring while timely diagnosis could increase the number of health disorders reported. By collecting health data reported by dog owners and not health professionals, objective evaluation of dog health could not be performed. Collection of data from veterinary practitioners has limitations as well since many veterinary visits are as a result of a health disorder (Bartlett et al., 2010), which could result in overestimation of the prevalence of health disorders. Utilising a survey of pet owners, if representative of the general pet-owning population, may better represent owners of healthy pets as well as pets with health disorders. This was the goal of the study presented here. With respect to the methodology employed, the sampling strategy likely also captured pet owners with particular interest in unconventional diets as it has been demonstrated that pet owners feeding unconventional diets have a strong interest in pet health and wellness (Morgan et al., 2017). This would potentially bias the results to include a higher proportion of pets fed unconventional diets,

which, as unconventional diets have been suggested to increase risk of health disorders, may have resulted in reporting of more health disorders than would be expected in a general population of pets. In order to compensate for this, the questionnaire was not only advertised online, but also to customers of pet retail stores, with the expectation that many of the customers would purchase commercial pet foods from these stores and thus be representative of pet owners feeding conventional foods. However, other commercial establishments where pet foods are sold, such as grocery stores or “big box” stores, were not targeted for survey advertising, which could impact the types of respondents represented in the study. As the questionnaire was available online, the number of potential pet owners who saw the link but chose not to participate (non-respondents) was indeterminable. As such, there was no ability to evaluate the response rate, nor quantify the proportion of responses obtained through advertisement a pet stores as compared to online. This impairs interpretation of how representative the sample is of the general pet-owning population. Another limitation of survey-based studies in general is the reliance on accurate reporting and representation by the pet owner, a form of recall bias. In this case, despite careful review of the reported diet, potential for misclassification exists if the diet reported by the dog owner did not accurately reflect what the dog was actually being fed. This is challenging to control or compensate for. Lastly, the findings presented here represent the opinions and beliefs of dog owners, not the definitive health status of the dogs, and must be interpreted as such. No causation can be inferred and interpretation of the findings must thus be made with this consideration in mind.

3.6 Conclusion

This study surveyed a sample of dog owners from Canada and the USA to describe the owner perception of health and wellbeing of dogs fed PB and MB diets. No adverse health outcome was associated with feeding PB diets to dogs, indeed feeding of PB diets was perceived to be potentially protective against health disorders, and the lifespan of dogs fed PB was reportedly longer than for dogs fed MB. Owners who fed PB reported a more positive perception of their dogs' health than owners who fed a MB diet. This perception must be considered by veterinary practitioners when diet-related health risks or benefits are discussed with dog owners, particularly as dog owners feeding PB may seek information regarding dog diet from alternative resources. Further prospective research is warranted to determine if PB alter canine health.

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3.9 Tables:

Table 3.1: Respondent demographics as reported by participants in the “Pet Health and Wellbeing” survey.

		n = 1413	%
Country	Canada	1000	71
	United States of America	413	29
Gender	Female	1,208	86
	Male	170	12
	Prefer not to disclose	29	2
Age	Less than 18 years	19	1
	18 – 24	163	12
	25 – 34	327	23
	35 – 44	279	20
	45 – 54	299	21
	55 – 64	212	15
	65 – 74	86	6
	75 – 84	9	1
	Greater than 85 years	0	0

Table 3.2: Characteristics of dogs as reported by participants in the “Pet Health and Wellbeing” survey.

Dog characteristic		MB		PB		PB+MB	
		n = 665	%	n = 357	%	n = 63	%
Sex	Male (n = 679)	317	48	174	49	38	60
	Female (n = 688)	347	52	181	51	25	40
Sex status	Intact (n = 150)	86	13	29	8.2	5	7.9
	Desexed (n = 1214)	577	87	326	92	58	92
Age (years)	Less than 1 (n = 59)	36	5.6	6	1.7	5	8.3
	1-2 (n = 224)	129	20	41	12	9	15
	3-4 (n = 206)	114	18	41	12	11	18
	5-6 (n = 221)	92	14	72	21	10	17
	7-8 (n = 206)	88	14	65	19	4	6.7
	9-10 (n = 178)	72	11	51	15	5	8.3
	11-12 (n = 118)	57	9.0	33	9.5	9	15
	13-14 (n = 74)	35	5.5	25	7.2	4	6.7
	15 and greater (n = 35)	14	2.2	14	4.0	3	5.0
Breed type	Toy (n = 168)	75	11	51	14	5	7.9
	Retrievers (n = 105)	70	11	17	4.8	1	1.6
	Collies and herding dogs (n = 66)	40	6.0	13	3.7	3	4.8
	Terriers (n = 60)	35	5.3	12	3.4	3	4.8
	Shepherds and livestock dogs (n = 60)	35	5.3	9	2.5	1	1.6
	Working and hunting dogs (n = 65)	35	5.3	14	3.9	2	3.2
	Scent hounds (n = 37)	19	2.9	13	3.7	1	1.6
	Mastiffs and bulldogs (n = 61)	25	3.8	14	4.8	5	7.9
	Nordic breeds (n = 30)	19	2.9	13	3.7	1	1.6
	Sighthounds (n = 23)	11	1.7	4	1.1	1	1.6
	Spitz and primitive dogs (n = 16)	9	1.4	6	1.7	0	0
	Small cross (n = 186)	76	11	57	16	13	21
	Medium cross (n = 166)	81	12	46	13	7	11
	Large cross (n = 244)	107	16	68	19	13	21
	Unknown mix (n = 69)	32	4.8	24	6.7	5	7.9

Table 3.3: Factors influencing pet food purchasing as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between owners of dogs fed different diets.

Criteria	Total		MB		PB		PB+MB	
	n =	%	n =	%	n =	%	n = 63	%
	1184		661		357			
Complete and balanced	852	72	488	74	251	70	47	75
Convenience	337	28	196	30	98	27	19	30
Homemade	174	15	70	11 ^a	84	24 ^b	10	16 ^{a,b}
Human grade	433	36	226	34	142	40	33	52
Natural/organic/holistic	375	32	153	23 ^a	156	44 ^b	32	51 ^b
Palatability	282	24	177	27	68	19	15	24
Presence/lack specific ingredient	181	15	135	20 ^a	20	5.6 ^b	9	14 ^{a,b}
Price	315	27	190	29	71	20	20	32
Raw	128	11	124	19 ^a	0	0 ^b	2	3.2 ^{a,b}
Skin/coat health	318	27	202	31 ^a	68	19 ^b	21	33 ^a
Stool quality/odour	204	17	133	20	53	15	10	16
Therapeutic/vet recommended	100	8.5	86	13	3	0.8	3	4.8

Superscript characters denote significant ($P < 0.05$) differences between the diet categories.

Note: values add up to >100% since respondents could indicate that they sought information from more than one source and numbers of dogs per category may not add up to total due to non-responders and indeterminable diet type.

Table 3.4: Resources used to acquire canine nutrition information as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between owners of dogs fed different diets.

Source of information	Total		MB		PB		PB+MB	
	n = 1186	%	n = 664	%	n = 357	%	n = 63	%
Book, pamphlet or printed resource	247	21	16	25	82	23	16	25
Breeder, trainer or groomer	86	7	80	12	2	0.6	1	1.6
Discussion group	345	29	185	28	116	32	16	25
Friends and/or family	233	20	130	20	63	18	11	17
Internet and social media	765	65	368	55 ^a	285	80 ^b	47	75 ^{a,b}
Pet food vendor or manufacturer	239	20	161	24	46	13	10	16
Education or experience	59	5.0	43	6.5	12	3.4	3	4.8
Veterinary technician, practitioner, specialist, student	802	68	498	75 ^a	194	54 ^b	41	65 ^{a,b}

Superscript characters denote significant ($P < 0.05$) differences between the diet categories.

Note: values add up to >100% since respondents could indicate that they sought information from more than one source and numbers of dogs per category may not add up to total due to non-responders and indeterminable diet type.

Table 3.5: Prevalence of canine health disorders as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between dogs fed different diets.

Health disorder	Total		MB		PB		PB+MB/H	
	n = 1171	%	n = 656	%	n = 339	%	n = 81	%
Cardiac disease	29	2.4	14	2.1	11	3.2	3	3.7
Dental disease	232	20	128	19	65	19	15	19
Dermatopathy	245	21	132	20	63	19	21	26
Endocrinopathy	21	1.8	16	2.4	5	1.5	0	0
Gastrointestinal and hepatic diseases	145	12	96	15	26	7.8	9	12
Lower urinary tract disease	61	5.2	32	4.9	19	5.7	5	6.3
Neoplasia	36	3.0	20	3.0	13	3.8	2	2.5
Neurological	48	4.1	28	4.3	10	3.0	5	6.2
Obesity	43	3.7	22	3.4	14	4.2	5	6.3
Ophthalmic disorders	100	8.5	67	10	23	6.8	5	6.2
Renal disease	14	1.2	12	1.8	0	0	1	1.3

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

The numbers of dogs per category may not add up to total due to non-responders and indeterminable diet type.

Table 3.6: Results from multivariable logistic regression models (one model per disorder) of associations between reporting of health disorders and dog diet, after controlling for confounders (age, breed type, sex, sex status).

Health Disorder	Variable	Odds Ratio	95% CI	P-value
Cardiac	Age	1.17	1.068 - 1.285	0.001
	Diet, PB	-	-	0.431
	Diet, PB+MB/H	-	-	0.363
Dental	Age	1.16	1.116 - 1.212	< 0.001
	Breed type, toy	6.77	1.968 - 23.287	0.002
	Breed type, scent hound	7.04	1.764 - 28.122	0.006
	Breed type, terrier	4.11	1.065 - 15.886	0.040
	Breed type, spitz	11.16	2.323 - 53.609	0.003
	Breed type, small mix	5.32	1.546 - 18.323	0.008
	Diet, PB	-	-	0.147
	Diet, PB+MB/H	-	-	0.634
Dermatological	Age	1.07	1.027 - 1.109	0.001
	Sex status, intact	0.34	0.162 - 0.719	0.005
	Diet, PB	-	-	0.374
	Diet, PB+MB/H	-	-	0.277
Endocrine	Age	1.23	1.104 - 1.381	< 0.001
	Diet, PB	-	-	
	Diet, PB+MB/H	-	-	0.247
GI and hepatic	Diet, PB	0.57	0.358 - 0.900	0.016
	Diet, PB+MB/H	-	-	0.727
Lower urinary tract	Age	1.10	1.029 - 1.174	0.005
	Sex, male	0.28	0.147 - 0.550	< 0.001
	Diet, PB	-	-	0.747
	Diet, PB+MB/H	-	-	0.397
Neoplasia	Age	1.26	1.151 - 1.381	< 0.001
	Diet, PB	-	-	0.946
	Diet, PB+MB/H	-	-	0.383
Neurological	Age	1.18	1.088 - 1.272	< 0.001
	Breed type, sight hound	7.74	1.587 - 37.736	0.011
	Diet, PB	-	-	0.287
	Diet, PB+MB/H	-	-	0.287
Obesity	Age	1.13	1.028 - 1.205	0.008
	Diet, PB	-	-	0.652
	Diet, PB+MB/H	-	-	0.569
Ocular	Age	1.10	1.039 - 1.158	0.001
	Breed type, toy	3.06	1.374 - 6.822	0.006
	Diet, PB	0.53	0.315 - 0.898	0.018
	Diet, PB+MB/H	-	-	0.464
Renal	Age	1.16	1.019 - 1.316	0.024
	Diet, PB	-	-	
	Diet, PB+MB/H	-	-	0.666

Referent categories: Breed type = mixed breed, Diet = meat-based, sex = female, sex status = desexed. For non-significant variables ‘-’ replaces the OR and CI.

Table 3.7: Responses to seven Likert item questions asking respondents to rank indices of dog wellness, with comparison between dogs fed different diets.

Wellness Indicator		Total		MB		PB		PB+MB/H	
		n = 1105	%	n = 605	%	n = 324	%	n = 79	%
Frequency of vomiting	Not at all	852	77	456	75	256	79	66	84
	A little	249	23	147	24	67	21	12	15
	Quite a bit	4	0.4	2	0.3	1	0.3	1	1.3
Frequency of inactivity	Not at all	866	78	462	76	268	83	63	81
	A little	207	19	127	21	45	14	13	17
	Quite a bit	32	2.9	17	2.8	10	3.1	2	2.6
Happy appearance	Not at all	1	0.1	0	0	0	0	1	1.3
	A little	12	1.1	8	1.3	3	0.9	1	1.3
	A moderate amount	243	22	129	21	72	22	16	21
	A great deal	841	77	464	77	246	77	59	77
Distress vocalization	Not at all	757	69	413	69	216	67	55	71
	A little	281	26	151	25	90	28	18	23
	A moderate amount	47	4.3	4	5.2	13	4.0	4	5.2
	A great deal	13	1.2	8	1.3	3	0.9	0	0
Demonstration of affection	Not at all	0	0	0	0	0	0	0	0
	A little	12	1.1	7	1.2	3	0.9	1	1.3
	A moderate amount	200	18	109	18	61	19	12	16
	A great deal	885	81	485	81	257	81	64	83
Contact avoidance	Not at all	932	85	508	85	276	86	64	83
	A little	154	14	86	14	42	13	12	16
	A moderate amount	11	1.0	6	1.0	4	1.2	1	1.3
	A great deal	1	0.1	1	0.2	0	0	0	0
Curious behaviour	Not at all	14	1.3	8	1.3	1	0.3	2	2.6
	A little	68	6.2	40	6.7	19	5.9	5	6.5
	A moderate amount	369	34	200	33	110	34	22	29
	A great deal	646	59	352	59	192	60	48	62

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

Table 3.8: Supplementary table: Dog breeds and breed type categorization

Breed type	Breed
Collies and small herding dogs	Australian cattle dog, Australian shepherd, Border collie, Corgi, Miniature American shepherd
Large shepherds and livestock guardians	Anatolian shepherd, Belgian malinois, Belgian tervuren, Bohemian shepherd, Bouvier, Dutch shepherd, German shepherd, Great Pyrenees, Maremma, Polish tatra, Tibetan shepherd, White shepherd
Molossers and bulldogs	American Bulldog, Boxer, Bullmastiff, Cane corso, Dogo Argentino, Dogue de Bordeaux, English bulldog, English bull terrier, English mastiff, French bulldog, Great Dane, Guatemalan dogo, Pitbull, Presa Canario, Rottweiler, Staffordshire Bull Terrier
Nordic	American Eskimo, Canadian Eskimo, Husky, Keeshond, Malamute, Norwegian Elkhound, Samoyed
Retrievers	Chesapeake Bay Retriever, Flatcoat Retriever, Golden retriever, Labrador retriever, Newfoundland dog, Nova Scotia Duck Tolling Retriever
Scent hounds	Basset hound, Beagle, Bluetick coonhound, Coonhound, Dachshund, Hamilton Stovare, Redtick coonhound, Treeing walker coonhound
Sight hounds	Greyhound, Italian greyhound, Whippet
Spitz and primitive dogs	Chow chow, German spitz, Pomeranian, Schipperke, Shiba inu, Shar pei
Terriers	Airedale terrier, Border terrier, Boston terrier, Cairn Terrier, Dandie Dinmont terrier, Fox terrier, Jack Russell terrier, Norfolk terrier, Parson Russell terrier, Rat terrier, Scottish terrier, Silky terrier, Tibetan terrier, West Highland White Terrier, Wheaten Terrier
Toy	Bichon Frisé, Cavalier King Charles spaniel, Chihuahua, Chinese crested, Coton du Tulear, Griffon, Havanese, Japanese chin, Lhasa apso, Lowchen, Maltese, Miniature Dachshund, Miniature Pinscher, Miniature Schnauzer, Papillon, Peckinese, Pomeranian, Pug, Shih tzu, Tibetan spaniel, Toy poodle, Yorkshire Terrier
Working and hunting dogs	Barbet, Brittany spaniel, Cocker spaniel, Doberman, English pointer, English setter, Formosan mountain dog, German shorthaired pointer, Hungarian vizsla, Irish setter, Karelian bear dog, Mountain cur, Portuguese pondengo, Portuguese water dog, Rhodesian ridgeback, Saint Bernard, Shetland sheepdog, Springer spaniel, Standard poodle, Standard schnauzer, Swiss mountain dog, Texas blue lacy, Weimeraner

3.10 Figures:

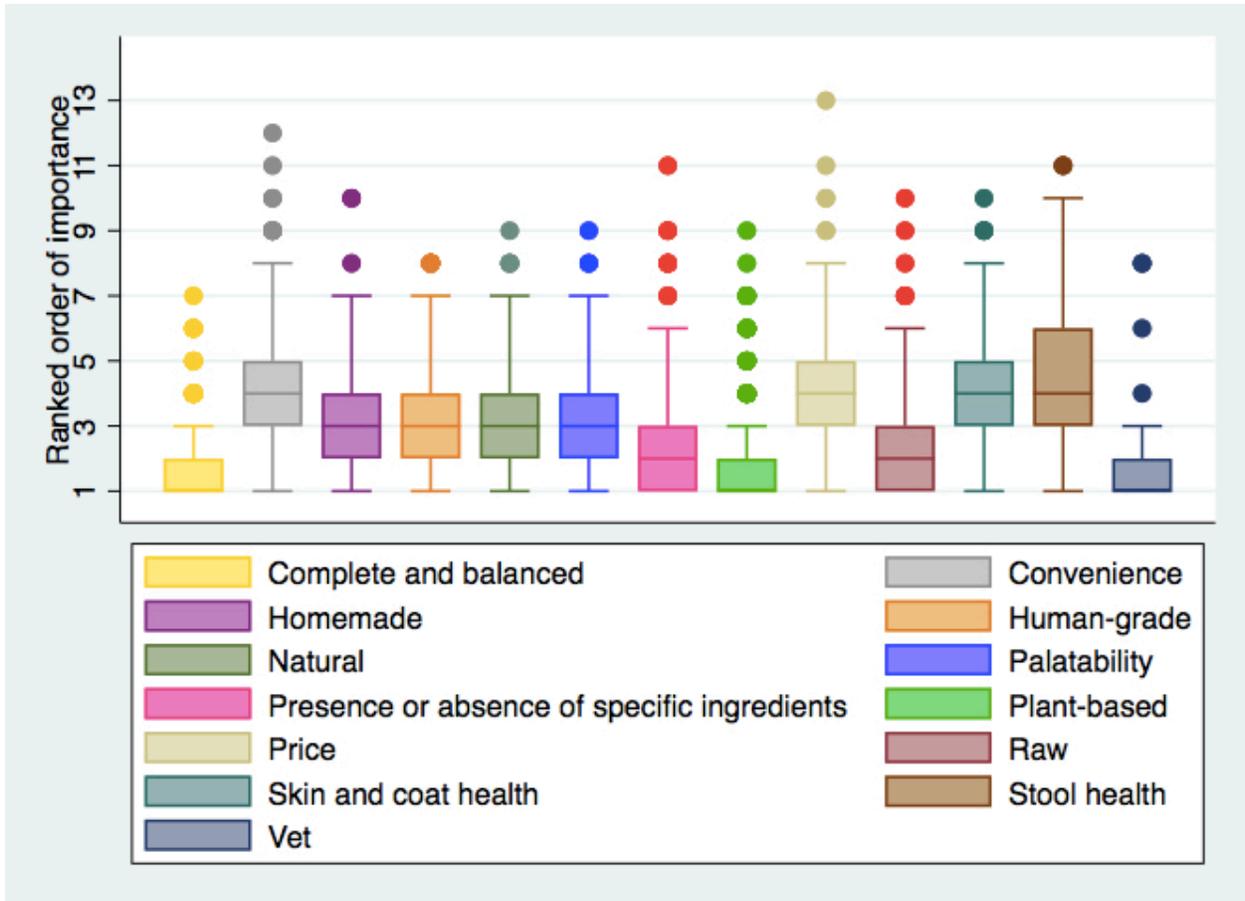


Figure 3.1: Box and whisker plot demonstrating importance of each reported criterion for diet selection, ranked from 1 (most important) to 13 (least important). Responses closest to 1 were most important, further from 1 were less important.

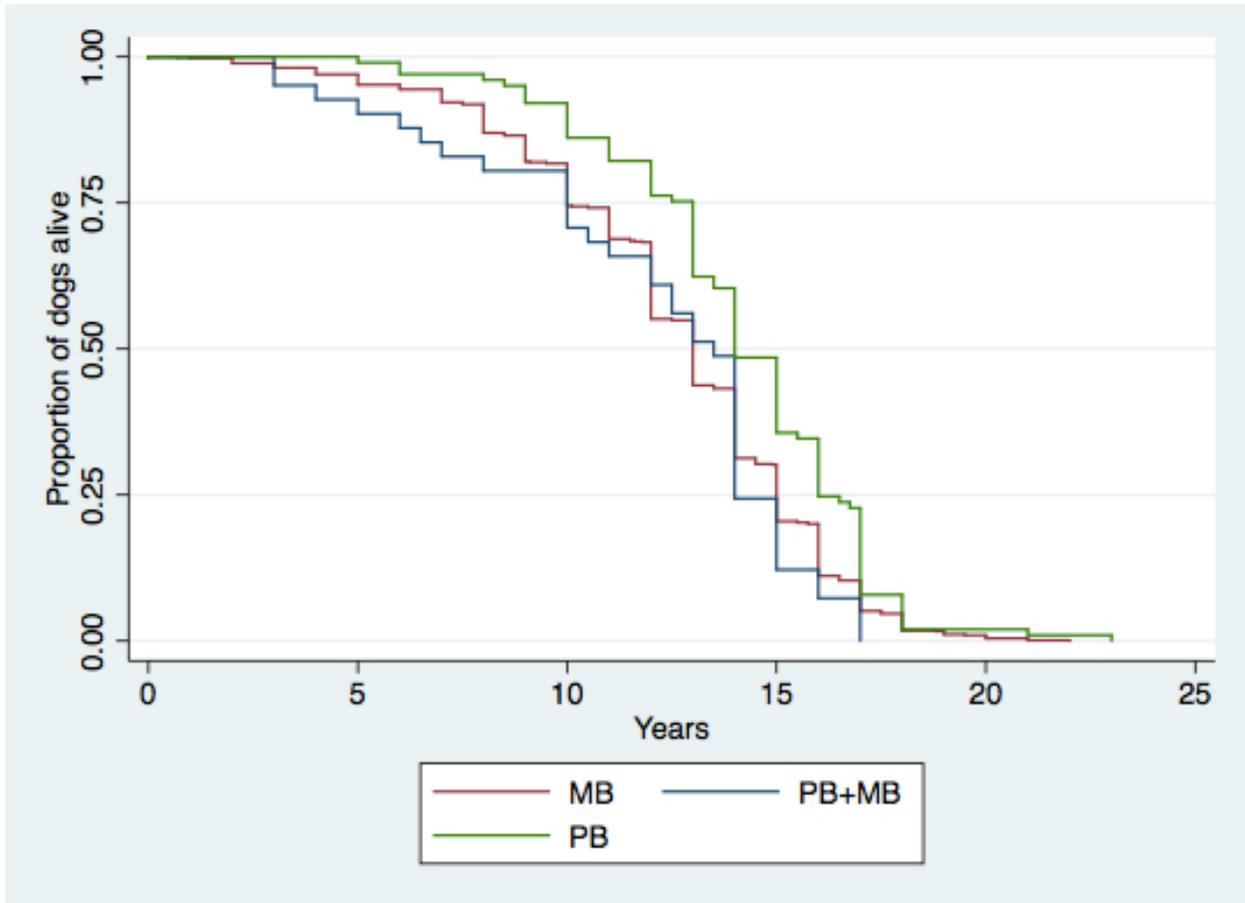


Figure 3.2: Kaplan-Meier survival plot depicting lifespan of previously owned dogs as reported by owners, comparing dogs fed plant-based (PB, n = 103), meat-based (MB, n = 907) or a combination (MB+PB, n = 42) diets.

4 CHAPTER FOUR: A cross-sectional study of owner-reported health in Canadian and American cats fed meat- and plant-based diets.

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RESEARCH ARTICLE

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A cross-sectional study of owner-reported health in Canadian and American cats fed meat- and plant-based diets



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A cross-sectional study of owner-reported health in Canadian and American cats fed meat- and plant-based diets.

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4.1 Abstract

Background: Cats, being obligate carnivores, have unique dietary requirements for nutrients most commonly found in dietary ingredients of animal origin. As such, feeding a diet devoid of animal-derived ingredients has been postulated as a possible cause of nutrient imbalances and adverse health outcomes. A small proportion of cat owners feed strictly plant-based diets to the cats in their care, yet the health and wellness of cats fed these diets has not been well documented.

Objectives: This study surveyed owner-reported health and wellbeing of cats and compared between those fed meat-based and plant-based diets.

Materials: A web-based questionnaire was distributed to pet owners to collect data on cat characteristics, husbandry, health and wellbeing. Univariate comparisons between diet groups was made by chi square and Kaplan-Meier tests as appropriate. Multivariate models were negative binomial and logistic regressions for count and categorical data, respectively.

Results: A total of 1,325 questionnaires were complete enough for inclusion. The only exclusion criterion was failure to answer any questions. Most cats, 65% (667/1026), represented in the survey were fed a meat-based diet and 18.2% (187/1026) were fed a plant-based diet, with the rest fed either a combination of plant-based with meat-based (69/1026, 6.7%) or indeterminable (103/1026, 10%). Cat age ranged from 4 months to 23 years, with a median of 7 years, and was not associated with diet type. No differences in reported lifespan were detected between diet types. Fewer cats fed plant-based diets reported to have gastrointestinal (GI) and hepatic disorders. Cats fed plant-based diets were reported to have more ideal body condition scores than

cats fed a meat-based diet. More owners of cats fed plant-based diets reported their cat to be in very good health.

Conclusions: Cat owner perception of the health and wellness of cats does not appear to be adversely affected by being fed a plant-based diet. Contrary to expectations, owners perceived no body system or disorder to be at particular risk when feeding a plant-based diet to cats. This study collected information from cat owners and is subject to bias, as well as methodological limitations. Further research is warranted to determine if these results are replicable in a prospective investigation.

4.2 Introduction

The domestic cat, *Felis catus*, is a small mammal of the order Carnivora considered to be an obligate carnivore, based on their evolutionary anatomical, physiological and metabolic adaptations to a diet exclusively comprised of prey (Verbrugghe and Bakovic, 2013, Plantinga et al., 2011). As a consequence, cats, have unique nutritional adaptations resulting in particular dietary requirements (Hecker et al., 2019, Morris, 2002b). Briefly, in comparison to their omnivorous counterpart, the domestic dog, the cat's requirement for total protein is higher, and they require dietary provision of taurine, long-chain polyunsaturated fatty acids and vitamin A (Bauer, 2006, Eisert, 2011, Verbrugghe and Bakovic, 2013, Green et al., 2011, Trevizan et al., 2012). Protein content and quality, in terms of amino acid digestibility, bioavailability and balance, is typically higher in animal-derived as opposed to plant-derived ingredients (Funaba et al., 2002). Moreover, taurine, arachidonic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and vitamin A are predominantly or exclusively found in animal tissues (Dawczynski et al., 2007, Spitze et al., 2003). Nevertheless, plant-based [PB] diets (diets entirely devoid of animal-derived ingredients, aka vegan diets) exist commercially and are marketed for feeding to domestic cats.

Associations between nutrition and health are known regarding domestic cats. Not only do cats require a diet providing balanced nutrition in order to avoid adverse health outcomes, certain disease states are also known to be associated with imbalances or inappropriate provision of particular nutrients (Freeman et al., 2011, Villaverde and Fascetti, 2014, Dijcker et al., 2011, Polizopoulou et al., 2005, Niza et al., 2003). Considering this, the type of diet a cat is fed may influence their day-to-day health, disease status, and even their longevity. As obligate carnivores,

it has long been considered that cats require a diet which contains animal-derived ingredients to provide the essential nutrients they require, and the implications of feeding PB diets to cats are yet to be well described. However, nearly 1% of all cat owners, and 10% of vegan cat owners, choose to feed an entirely PB diet to their cat (Dodd et al., 2019b).

Previous publications regarding feeding of PB diets to carnivorous cats have focused either on the content of some essential nutrients in PB cat food or on measurement of some indicators of nutrient status in the blood of cats fed PB diets for at least one year (Kanakubo et al., 2015, Gray et al., 2004, Wakefield et al., 2006, Semp, 2014). Results from these studies have varied, with dietary nutrient insufficiencies documented (Kanakubo et al., 2015, Gray et al., 2004, Semp, 2014), but no adverse health or nutritional outcomes detected (Semp, 2014, Wakefield et al., 2006). Nevertheless, it has been suggested that feeding PB diets to cats may predispose them to health disorders, including lower urinary tract diseases (Knight and Leitsberger, 2016, Semp, 2014). Other studies have investigated the motivations and attitudes of cat owners towards feeding their cat, finding that meat-abstainers were the only ones who fed their cats PB diets, and that their impetus to do so was based largely on ethics or morality (Dodd et al., 2019b, Wakefield et al., 2006, Rothgerber, 2013). Currently, only one study investigating owner perception of health in cats fed PB has been published (Wakefield et al., 2006). The objective of this study was to survey a wide sample of cat owners in order to describe beliefs and practices regarding cat health and nutrition and compare between owners of cats fed PB or meat-based [MB] diets. It was hypothesized that resources used for feline nutrition and factors influencing dietary decisions would differ between owners feeding cats PB or MB diets and that there would be a higher prevalence of health disorders among cats fed PB diets. In particular, it

was hypothesized that more cats fed PB diets would be reported to have lower urinary tract disorders as compared to cats fed MB diets.

4.3 Methods

The study was approved by the University of Guelph Research Ethics Board (REB # 18-07-039).

4.3.1 Survey Design

A questionnaire was designed by the authors using the Qualtrics (Qualtrics XM, Provo, Utah, USA) online platform. The questionnaire included questions based on previously validated survey items and was piloted by the authors before being made available to potential participants. Respondents were incentivised to participate by a random draw to obtain a gift certificate for a pet store of their choosing. Eight prizes of \$25CAD gift cards were available, and participants could choose to enter the prize draw by including their email address at the end of the main questionnaire. These sensitive data were removed from the main data and stored separately until the end of the data collection period. After the winning participants were contacted, all sensitive data were deleted. The questionnaire included 36 multiple-choice, 8 short answer, 7 Likert items (0-100), and 1 ranking questions. The dataset included responses for dogs as well as cats, dog data is presented elsewhere (Dodd et al., 2020c). The questionnaire used flow logic to show questions related to cats only to participants indicating that they owned cats. Questions were designed to collect demographic information about pet owners, pet species, breed, sex, age, acquisition, lifestyle (indoor/outdoor), as well as information on pet health, clinical signs and wellness. Cat BCS was selected by the respondents based on images from the

World Small Animal Veterinary Association BCS chart, randomly ordered to avoid bias (WSAVA, 2013a). For this 9-point score system, a score between 1 and 3 is considered under ideal, 5 ideal, and 7 to 9 over ideal. Faecal score (FS) was selected by the respondent based on images corresponding to the Bristol stool chart (Anonymous, 2017), randomly ordered to avoid bias. A score of 1 to 2 being abnormally hard to constipated, 3 to 4 being normal, and 5 through 7 ranging from soft to diarrhoea. To determine prevalence of health disorders, body systems and common disorders were listed, along with an “other” category, and respondents were asked to select the appropriate system and describe their cat’s specific disorder. These included: behavioural, cancer, cardiovascular (heart), dental, dermatologic (skin), diabetes, ear, endocrine, eye, GI, hyperthyroid, kidney, liver, musculoskeletal, neurologic, obesity, parasites, reproductive, seizures, trauma/injury, urinary, other. Fifteen indicators of cat wellness were adapted from previously validated survey items (Noble et al., 2018, Tatlock et al., 2017, Freeman et al., 2016), and answered as seven Likert item questions and eight on a visual sliding scale. The survey instrument can be found in Appendix I.

4.3.2 Survey Distribution

A link to the survey was distributed via email and postcards to customers of Canadian and American pet food retailers, including deliberate distribution to clients of the largest PB pet food retailer in Ontario, Canada. As well, the survey was promoted in online groups of pet owners on social media (Facebook, Inc., Menlo Park, California, USA). The survey, which was available in English only, was made accessible for nine months, from June 2018 to March 2019. Respondents represented a convenience sample of cat owners voluntarily participating in the study.

4.3.3 Survey Analyses

Surveys were included for analysis for each question that was completed. Breeds were categorized into breed types based on phylogenetic similarities and historical origin (Lipinski et al., 2008). Description of cat diet was collected in an open-text question and, where sufficient information was obtained, categorized based on ingredients (MB, PB) and processing (commercial heat-processed, homemade, raw) or a combination of the aforementioned (i.e.: some cats were fed more than one type of food on a regular basis). The term PB referred to a diet that contained no animal ingredients, while the term MB referred to a diet that included animal-derived ingredients. Thus, while a MB diet could include plant-derived ingredients (e.g. a kibble made from chicken and soy), a PB diet could include no animal-derived ingredients. In addition to the main diet, information was also collected regarding the feeding of treats (commercial heat-processed, raw, homemade), table foods and supplements, and categorized based on ingredients (MB or PB). Cats fed a PB diet but also given treats, table foods, snacks and/or supplements containing animal-derived products, were categorized as PB+MB. . All cats fed a MB diet were included in the MB category, even if their diet included PB treats, snacks and/or supplements. Due to the likelihood of eating prey even if not reportedly known to hunt (Loss et al., 2013, McDonald et al., 2015, Blancher, 2013), cats fed PB diets with unrestricted outdoor access or ability to hunt, were added to the PB+MB category, which was then re-classified as PB+MB/H for comparative analyses. For comparisons of pet food purchasing motivations (Table 4.2) and use of nutrition resources (Table 4.3) comparison was made between cats fed PB, PB+MB, and MB. For comparison between health outcomes (Table 4.4, Table 4.5, Figures 4.1 and 4.2), consideration of ability to hunted prey (PB+MB/H) was included as this may have an effect on

cat health as compared to a strictly PB diet. Cat wellness indices measured by Likert items were reported directly, while sliding scale data was translated by the survey software into a value reported ranging from 0 to 100. Cat health disorders were offered in multiple-choice questions, with the option of ‘other’ for input by the respondent. Owner-reported ‘other’ health disorders were categorized by a veterinarian (SD) and included for analyses. Based on the responses, behavioural, musculoskeletal, parasites, reproductive and trauma/injury were dropped. True behavioural disorders were difficult to differentiate from issues of training or lifestyle (such as “a little too sassy”, “jealous”, “grumpy” or “claws door frames and harasses our girl cat). Reported musculoskeletal issues were difficult to interpret, with ambiguous descriptions often associated with suspected previous injuries. The parasite category was dropped as responses were typically regarding kitten worming or indicated that the cat was regularly treated with endo- or ectoparasite medications, similarly the most common reproductive disorder reported was either having kittens or desexing surgery. The trauma/injury category contained mostly anecdotes of incidents that were believed to have occurred prior to current ownership, such as “broken jaw before we adopted him”, “picked up by a bird of prey prior to rescue from the street at 6 weeks”, “one rear leg amputated from abuse” and “he sustained an injury before I got him that made his back half a bit wonky”. The remaining variables recategorized to: cardiac, dental, dermatologic (including ear infections and polyps), endocrine, GI and hepatic, lower urinary tract, neoplasia, obesity, ocular and renal.

4.3.4 Statistical Analyses

All analyses were performed using Stata/IC 15.1 (StataCorp, College Station, Texas, USA) statistical software package. Descriptive statistics included frequency (n) and percentage

(%) presented for most data (type of pets, location and time of cat acquisition, breed, indoor/outdoor management, hunting activity, diet and supplementation, motivators for selection of cat food, resources for information about feline nutrition) (Pérez-Vicente and Expósito Ruiz, 2009). Frequency and percentage were also used within category for data collected using Likert items and for ranked data. Mean and standard deviation were presented for normally distributed data (cat age, duration of cat ownership, duration of feeding (measured in years), and lifespan of previous cats)(Yin et al., 2016). Median and range were presented for ordinal data (BCS, FS) and count data (number of cats kept, number of health disorders per cat).

Univariate comparisons between diet categories and responses related to pet food purchasing behaviours and pet nutrition information resources were conducted using χ^2 testing (see Tables 4.2 and 4.3). These analyses were performed when comparing between diet categories only (pet food purchasing, nutrition resources, hunting activity), without consideration of potential confounders. Age of previously owned cats at death was also compared among diet groups using Kaplan-Meier statistic(Che-Castaldo et al., 2019).Age of previously owned cats at death was also compared among diet groups using Kaplan-Meier statistic (Che-Castaldo et al., 2019).

Statistical models were selected based on the nature of the variable of interest – count, binary, or continuous data (Stryhn et al., 2009). The relationship between number of health disorders per cat, measured as count data, and diet type was modelled using multivariate negative binomial regression. Within the model, the dependent variable was the number of health disorders per cat. Independent variables considered included cat diet (3-level categorical: PB,

PB+MB/H compared to MB) and potential confounders: cat age (years), sex (2-level categorical: male compared to female), sex status (2 level categorical: intact compared to desexed), breed type (9-level categorical: domestic medium hair, domestic longhair, mixed breed, Asian, American, European, other, unknown, compared to domestic shorthair), BCS (ordinal: 3, 5, 7, 9 compared to 1) and indoor/outdoor access (3-level categorical: indoor/outdoor access, outdoors only, compared to indoor only). Variables were assessed for correlations and collinearity prior to inclusion in the final model, no collinearity correction was required. Using backward stepwise elimination to remove non-significant variables, the final model included cat age, sex and diet. Model fit was visually evaluated by assessment of distribution of the residuals.

Logistic regression models were used to assess the association between individual health disorders and diet. One model was developed for each individual health disorder as the dependent variable (cardiac, dental, dermatological, endocrinological, GI and hepatic, neoplastic, obesity, ocular, renal and urinary), measured as a binary present/absent outcome. Independent variables considered included cat diet (MB, PB, PB+MB/H) and potential confounders: cat age, sex, sex status, breed type, BCS and indoor/outdoor access. Variables were assessed for correlations and collinearity prior to inclusion in the model, no corrections for collinearity were required. Backward stepwise regression was used to eliminate non-significant variables from the final multivariate model for each health disorder. Significant independent variables kept in each multivariate model differed based on health disorder (Table 4.5). Though non-significant for some disorders, age was forced into the model due to the known associations between increasing age and risk of health disorders as was diet, as it was the variable of interest. Model fit was assessed by Hosmer-Lemeshow goodness-of-fit test.

Ordered logistic regression modelling was used to evaluate the relationship between owner perception of cat health, ranked in four levels: “poor”, “fair”, “good”, and “very good”. The dependent variable was health ranking, with odds ratios reported in comparison to the referent outcome “very good”. Independent variables considered included cat diet (MB, PB, PB+MB/H), cat age, sex, sex status, breed type, BCS and indoor/outdoor access. Variables were assessed for correlations and collinearity prior to inclusion in the model, no collinearity corrections were required. Backward stepwise regression was used to eliminate non-significant variables from the final multivariate model. Significant independent variables kept in the final multivariate model were cat age, sex, BCS and diet.

For all analyses, statistical significance was set at $P < 0.05$. A-priori sample size estimations were made using data regarding prevalence of feline health disorders (O’Neill et al., 2014a). Considering the suggested increased risk of urinary tract diseases in cats fed PB diets (Knight and Leitsberger, 2016), this health disorder was chosen for sample size estimation. Assuming the proportion of cats fed MB with urinary tract disorders was 4%, calculation of the sample size comparing two different proportions with the estimation that three times as many cats fed PB would have urinary tract disorders (12%), yielded a required sample size of 116 cats per diet category. Post-hoc power calculations were performed comparing the mean values or proportions of each variable of interest between diet groups, considering an α value of 0.05 and a β of 0.8 as the cut-off points. Power less than 80% was considered too low and represented an insufficient sample size to detect significant ($P < 0.05$) differences.

4.4 Results

4.4.1 Response rate and demographic information

A total of 1,325 questionnaires were voluntarily undertaken and included for analysis, responses were excluded if they failed to answer all questions in the questionnaire. Partially completed surveys were included, thus the number of responses for each question varied as a result of non-responses to individual questions. Demographic data of respondents are shown in Supplementary Table S1. Post-hoc power calculations confirmed the sample size was adequate to attain statistical significance for comparison of the number of health disorders, specific health disorders (GI and hepatic) and lifespan between cats fed PB and MB diets.

The proportion of respondents keeping cat(s) only (653/1325 49%) did not differ from those keeping dog(s) and cat(s) (672/1325, 51%). The median number of cats kept per respondent was two (range 1 – 18). Most cats were acquired from shelters, rescues or veterinarians (664/1241, 54%), followed by tamed stray, feral or found cats (214/1241, 17%) or inherited or gifted from friends and family (167/1241, 13%). Less commonly, cats were purchased online (64/1241, 5.2%), from backyard breeders, farms, or homebred (59/1241, 4.8%), from registered breeders (44/1241, 3.5%) or from pet stores (29/1241, 2.3%). Cats were acquired by respondents at kittenhood (645/1241, 55%) or later in their life (538/1241, 45%). Cats had been kept by the respondents for less than 1 to 25 years (mean 6.0 years, std. dev. 4.85).

4.4.2 Cat characteristics

Cat characteristics are shown in Table 4.1, grouped by diet type. There was a significant association between diet and breed type, with more MB cats being domestic longhairs (66/656,

10% MB; 8/182, 4.4% PB), while more PB cats were mix breeds (58/656, 8.8% MB; 29/182, 16% PB), or their breed unknown (46/656, 7.0% MB; 25/182, 14% PB) ($P = 0.004$). No significant differences in breed types were detected between PB+MB and PB or MB. Mean cat age was reported to be 7.5 years (std. dev. 4.85). There was no association between cat age and diet. Most cats lived indoors only (749/1246, 60%), many had unlimited outdoor access (373/1246, 30%), some had limited or controlled outdoor access (113/1246, 9.1%), and few lived outdoors exclusively (11/1246, 0.88%). The majority of respondents indicated that their cats did not hunt prey (949/1241, 76%). Some respondents recognized that their cats could hunt but they did not believe that they did so (91/1241, 7.3%) and less than a quarter acknowledged that their cat did hunt prey (201/1241, 16%). Though there was no association between access to the outdoors and diet, significantly ($P = 0.005$) more PB cats were reported to not to hunt (129/186, 69%) or to have the ability to hunt but not do so (23/186, 12%). Of the cats fed a PB diet, 35% (65/187) were reported unlimited access to the outdoors, suggesting their diet could be supplemented with hunted prey to some degree (PB+MB/H).

4.4.3 Cat diet

Diet was described by 1,026/1,325 respondents. Most cats were fed a MB diet (667/1026, 65%), less than a quarter were fed strictly PB (187/1026, 18%), and a small proportion were fed a combination of PB and MB (PB+MB, 69/1026, 6.7%). Diet type was indeterminable for 10% (103/1026) of cats. For cat health and wellness comparative analyses, cats that were fed PB but had access to the outdoors and ability to hunt were re-classified as PB+MB/H (139/1026, 14%).

Cats had been reportedly fed their current diet their whole life (534/1034 52%) or had changed from at least one previous diet to their current diet (500/1034, 48%). Cats had been fed their current diet for a mean of 3.8 years (std. dev. 3.95). There was no difference in duration of feeding the current diet between cats fed MB (mean 3.6 years, std. dev. 3.69) PB (mean 3.6 years std. dev. 4.38) or PB+MB (mean 3.0, std. dev. 3.27) diets. Less than half of cats received treats (386/1026, 38%) or table scraps (220/1026, 21%) in addition to their main diet. More cats fed MB (308/667, 46%) than PB (29/187, 16%) or PB+MB (16/69, 23%) received treats ($P < 0.001$), though more cats fed PB (63/187, 34%) than MB (128/667, 19%) received table foods ($P < 0.001$). No significant differences in feeding of table foods were detected between PB+MB and PB or MB. Significant differences in the feeding of table foods was evident between PB and MB cats ($P < 0.001$). The most common table foods fed to MB cats were meat (66/667, 9.9%), dairy or eggs (64/667, 9.6%) and fruits or vegetables (20/667, 3.0%). The most common table foods fed to PB cats were fruits or vegetables (34/187, 18%), nuts, seeds, legumes or grains (21/187, 11%), and plant-based meat alternatives (16/187, 8.6%). Cats fed PB+MB were most commonly offered dairy or eggs (10/69, 14%), fruits or vegetables (7/69, 10%), or meat (5/69, 7.3%). Supplements were fed to less than a quarter of cats ($n = 193/1026$, 19%), more so to those fed PB (75/187, 40%) than PB+MB (17/69, 24%) or MB (91/667, 14%) ($P < 0.001$). Not only were the number of cats offered supplements different between diet groups, but also the types of supplements given. Overall, 'functional foods' such as cranberry powder, coconut oil and yeast were the most common supplements offered (77/923, 8.3%), followed by fibre, pre- or probiotics (38/923, 4.1%) and vitamins and/or minerals (36/923, 3.9%). Cats fed PB were given more 'functional foods' (55/187, 29%), supplements marketed for specific disorders (14/187, 7.5%),

and digestive enzymes (8/187, 4.3%), than cats fed PB+MB (functional foods 6/69, 8.7%; specific disorders 2/69, 2.9%; enzymes (0/69, 0%) or MB (functional foods 16/667, 2.4%; specific disorders 19/667, 3.9%; enzymes 9/667, 1.4%). There were no differences between diet groups and the feeding of marine derived fatty acids, multivitamin/minerals, fibre or pre/probiotics, amino acids, cannabis products, or herbs.

4.4.4 Cat health and wellness

Cat BCS ranged from 1 to 9. Most cats were reported to be in ideal condition (677/1233, 55%), and more cats were overweight (405/1233, 33%) than underweight (151/1233, 12%); median BCS was 5 with a range of 1 to 9. Cat BCS differed significantly between cats fed PB and MB ($P = 0.13$). More owners of cats fed strictly plant-based reported their cat to have an ideal BCS (83/117, 71%) and fewer reported overweight (23/117, 20%), as compared to cats fed MB (358/666, 54% ideal; 226/666, 34% overweight). No difference was detected for PB+MB/H compared to PB ($P = 0.196$) or MB ($P = 0.635$).

Based on FS, most cats were reported to have normal faeces (920/1047, 88%), few were constipated (33/1047, 3.2%) or had soft to diarrhoeic faeces (94/1047, 9.0%); median FS was 2 (range 1 -7) on a 1-7 scale, with 3-4 being ideal, 1 being constipation and 7 being diarrhoea. There were no significant differences in FS between cats fed MB, PB or PB+MB/H diets.

Health disorders were reported by the respondent and categorized by body system or systemic disorders as appropriate. The number of health disorders reported per cat ranged from 0 – 6 (median 0), and half of all cats (628/1208, 52%) were reported to have no health disorders. Prevalence of the most commonly reported health disorders are shown in Table 4.4. A negative

binomial model was designed to determine if the number of health disorders a cat had was associated with diet. With cat age and sex included in the model, the number of disorders per cat differed significantly based on diet, after controlling for demographic and lifestyle variables. Cat age (Coef. 0.09, 95% CI 0.070 – 0.100, $P < 0.001$) and male sex (Coef. 0.16, 95% CI 0.004 – 0.308, $P = 0.044$) were associated with increased number of disorders, while PB (Coef. -0.40, 95% CI -0.641 – 0.155, $P = 0.001$) and PB+MB/H (Coef. -0.33, 95% CI -0.589 - -0.075, $P = 0.011$), as compared to MB, were associated with fewer disorders. The relationships between diet and individual health disorders were investigated using logistic regression models, results are shown in Table 4.5. Overall, after controlling for age, sex, breed type, and body conditions score, diet was significantly associated only with dental, GI and hepatic, and ocular disorders. Age was associated with most health disorders.

Respondents' perception of their cat's health, from poor to very good, was predominantly very good (813/1206, 67%). Few respondents indicated their cat was in fair (47/1206, 3.9%) or poor (11/1206, 0.91%) health. An ordered logistic regression model was designed to determine if owner perception of cat health was associated with diet. Increasing cat age (Odds Ratio [OR] 0.84, 95% CI 0.808-0.863, $P < 0.001$) and male sex (OR 0.71, 95% CI 0.523 – 0.976, $P = 0.035$) were associated with lower odds of ranking cat health as very good. Conversely, a BCS of 5 (OR 7.72, 95% CI 2.67 – 22.29, $P < 0.01$) or 7 (OR 4.55, 95% CI 1.552 – 13.337, $P = 0.006$) as compared to 1, and feeding a PB (OR 1.99, 95% CI 1.194 – 3.329, $P = 0.008$) or PB+MB/H (OR 2.19, 95% CI 1.360 – 3.511, $P = 0.001$), as compared to MB, were associated with greater odds of ranking cat health to be very good, after controlling for cat demographics. Respondents also

ranked their cat's wellness based on seven Likert items (Table 4.6), and eight visual scales ranging from 0 (lowest) to 100 (highest) (Figures 1 and 2).

Lifespan was indicated when respondents were asked about previous cats. Mean lifespan was reported to be 14 years (std. dev. 4.88). Previous cats were fed MB (841/1161, 72%), PB (77/1161, 6.6%), a combination of MB and PB (41/1161, 3.5%) or indeterminable (258/1161, 22%). There was no significant difference in reported lifespan based on diet detected by log rank test (Figure 4.3).

4.4.5 Diet changes

Owners typically fed their current cat the same type of diet (i.e. PB or MB) as they fed their previous cat(s). Of the owners who fed their previous cat(s) a PB diet, only 4.0% (2/50) added some animal products and 2.0% (1/50) changed to an MB diet for their current cat. Of the owners who fed a PB+MB to their previous cat(s), most continued to feed PB+MB diet (10/25, 40%), while relatively even proportions changed to a completely PB (6/25, 24%) or MB (8/25, 32%) diet for their current cat. Of the owners who previously fed their cat(s) an MB diet, the majority of owners continued to feed this diet type to their current cat (514/637, 81%), though more changed to an entirely PB diet (73/637, 11%) than a PB+MB (31/637, 4.9%).

4.4.6 Diet Selection

Cat food was predominantly chosen based on whether or not it was complete and balanced (775/1106, 70%). Table 4.2 shows the most common criteria reported for cat food selection. Of the 14% of owners (156/1106) who reported concern regarding specific ingredients in their cat's diet, significant differences were found between feeders of MB, PB and PB+MB

diets. Owners feeding their cats MB most commonly reported a desire to avoid ingredients like by-products, grains, ‘fillers’ or additives (4/187, 2.1% PB; 2/69, 2.9% PB+MB; 60/667, 9.0% MB; $P = 0.002$), and for a diet to contain animal ingredients high on the list of ingredients (0/187, 0% PB; 0/69, 0% PB+MB; 30/667, 4.5% MB; $P = 0.008$). More owners feeding their cats a PB diet were wanted to see inclusion of particular nutritional additives (e.g. specific vitamins) (12/187, 6.4%) as compared to PB+MB (0/69, 0%) or MB (8/667, 1.2%) ($P < 0.001$). There were no differences between diet groups and the desire to avoid specific plant-derived ingredients (e.g. corn, soy). Respondents indicated the resource(s) they used for information about feline nutrition. The common sources of information and significant differences between diet groups are shown in Table 4.3. Veterinary professionals were the most common (707/1022, 64%), followed by internet and social media (681/1022, 62%). Of the respondents who indicated using the internet as a resource for feline nutrition, over half (396/681, 58%), also used a veterinarian.

4.5 Discussion

4.5.1 Response rate and demographic information

Participants in this study typically represented the demographics of previously reported pet food and pet health survey respondents (Dodd et al., 2019b, Morgan et al., 2017, Rajagopaul et al., 2016), supporting the likelihood that the sampled population was consistent with other publications in the field. However, the sampling methodology was not random, but invited cat owners to self-select to participate in the study. This may have biased the types of owners and cats represented by the study. The sampling included owners of both dogs and cats, not just cats exclusively, though only responses relating to cats were included in these analyses. In the USA,

almost half (47%) of cat owners also own dogs (AVMA, 2018), making the input of these owners as relevant as the input of cat-exclusive owners.

4.5.2 Cat characteristics

Cat keeping characteristics, including number of cats, cat acquisition, and indoor/outdoor lifestyle, as well as cat characteristics such as median age, breed, sex and sex status, were similar to previous publications in Canada, the USA and worldwide (Johnston et al., 2017, Toribio et al., 2009, AVMA, 2002), supporting the likelihood that the sampled population was representative of the general cat population in the regions of interest.

4.5.3 Cat diet, health and wellness

Despite their carnivorous physiology and metabolism, approximately 1% of cat owners feed a PB diet to their cat (Dodd et al., 2019b). A previous study evaluated the attitudes of cat owners and compare between cats fed vegetarian and conventional MB diets (Wakefield et al., 2006). In that study, over three quarters of owners feeding their cats a vegetarian diet considered the diet to be beneficial for decreased risk of cancer, healthy coat, longevity, weight control and/or reduced risk of allergies, and just over ten percent considered there to be no benefit. When queried regarding the risks of vegetarian diets, over three quarters reported concern for retinal atrophy, taurine deficiency, lower urinary tract disease, protein deficiency, dilated cardiomyopathy, while less than a quarter considered there to be no risk to health. In a recent investigation, though nearly two-thirds of owners feeding their pet a PB diet reported concern for the diet providing incomplete nutrition, though less than half reported a concern regarding risk to health (Dodd et al., 2019b). This apparently positive perception of PB diets previously

documented was supported by the findings of the present study, where more owners of cats fed PB reported their cat to be in very good, as opposed to good, overall health. Additionally, owners of cats fed PB diets reported fewer health disorders in their cats. In previous evaluations of the health of cats fed PB diets, no adverse health outcomes attributable to diet were found, and most parameters measured were within the normal reference range or above minimum thresholds (Wakefield et al., 2006, Semp, 2014).

Among veterinarians, animal nutritionists and veterinary nutritionists, it is generally considered contraindicated to feed a strictly PB diet to cats (Parr and Remillard, 2014, Fox, 2005, Gray et al., 2004). Considering this, it was predicted that feeding a strictly PB diet to cats may result in poorer health and/or wellness. However, this was not supported by the findings of this study. Owner perception of health and wellness, as reported by participants in the study, were largely comparable between cats fed MB and PB diets (Tables 6-8, Figures 1 and 2), and in some cases significantly better for cats fed PB. PB diets have also been effectively used as growth and maintenance diets for other captive carnivores, including American alligators and carnivorous fish (Reigh and Williams, 2018, DiGeronimo et al., 2017, Ytrestøyl et al., 2015, Burel et al., 2000), suggesting that the maintenance of carnivorous cats using PB diets may not be as novel or unconventional as is commonly considered. It must be noted, however, that diets for animals intended for human consumption are typically designed to maximize some component of production and are not necessarily designed for optimal animal health or longevity. Nevertheless, longevity of cats, as reported by the study participants, did not vary between cats fed PB or MB diets, and was comparable to the recognized lifespan of domestic cats (Pittari et al., 2009).

Specific concerns raised regarding PB diets for cats have typically been for the total protein content in comparison to feline requirements, risk of taurine deficiency and related disorders, and the carbohydrate content of the diet and risk of obesity and diabetes. Though total protein in commercial PB cat foods has been demonstrated to be sufficient, the amino acid profiles may be variable. In one study, five of six diets intended for feeding to cats failed to meet the recommended minimum value for one or more amino acids (Kanakubo et al., 2015). In the two canned products, taurine was below the recommended minimum. Thus, concern for taurine deficiency and protein malnutrition appears warranted. Nevertheless, to the authors' knowledge, no case reports of protein malnutrition or taurine deficiency in cats fed PB diets have been published. Interestingly, despite the concern for amino acids, a common supplement in human PB nutrition, few cats were offered protein or amino acid supplementation. Though three times as many cats fed PB than MB received supplements in addition to their food, the supplements fed were most commonly 'functional foods' (30%), and those marketed for treatment or prevention of specific health disorders (7.5%), neither of which are likely to have contributed greatly to the nutritional value of the cats' diet.

In addition to concerns for health disorders related to inappropriate protein and amino acid provision, concerns regarding the carbohydrate content of PB diets have also been raised. Carbohydrates have been suggested by some to contribute to feline obesity (Zoran, 2002, Rand et al., 2004). The proportion of cats reported by the owner to be overweight in this study was in close agreement with the proportion reported to be overweight by the largest USA-based survey of pet owners (AVMA, 2018). Interestingly, BCS were reportedly more ideal and less overweight in cats fed PB than MB diets. The current body of evidence points towards

imbalanced energy intake versus expenditure as being the predominant cause of obesity, as opposed to intake of any single macronutrient, though fat, being the most energy-dense and lipogenic nutrient, is of more concern than carbohydrates as a predisposing factor for feline obesity (Russell et al., 2000, Loftus and Wakshlag, 2015, Hamper, 2016). For a recent review of the role of carbohydrates in feline nutrition, see Verbrugghe and Hesta, 2017 (Verbrugghe and Hesta, 2017). It is possible that PB foods fed to cats may have lower dietary fat than MB diets, and likely that the higher fibre levels in plant ingredients versus meat ingredients may reduce energy density, help to regulate glucose tolerance and insulin sensitivity, and thus be protective against obesity, as has been demonstrated in humans (Musco et al., 2017, Mori et al., 2009, Turner-McGrievy et al., 2017, Wright et al., 2017). Similarly, though diets with high levels of carbohydrates have been postulated to contribute to faecal abnormalities in cats (Verbrugghe and Hesta, 2017, Kienzle, 1993), with high levels of digestible carbohydrates and soluble fibres potentially causing diarrhoea and high levels of insoluble fibres potentially contributing to constipation, no difference in FS was attributable to a PB compared with a MB diet. Body and faecal condition are two health markers pet owners can easily keep track of at home. Similarly, hair condition, behaviour and activity levels are also commonly considered by pet owners and veterinarians alike to be general indicators of health.

Prevalence of health disorders in the overall study population were comparable to the reported prevalence in general populations as determined from veterinary visits (O'Neill et al., 2014a, Bartlett et al., 2010). When total number of disorders was compared, significantly fewer were reported in cats fed PB compared to MB diets. When comparing individual disorders, reporting of GI and hepatic disorders was lower in cats fed PB as opposed to MB diets. No

disorders were reportedly higher in cats fed PB. It is possible that PB diets confer some protection against these particular disorders, namely renal disorder and GI and hepatic disorders; indeed this has been demonstrated in humans (Wiwanitkit, 2007, Kim et al., 2019a, Chiba et al., 2010, Joshi et al., 2018). In humans the health benefits of a PB diet have primarily been with respect to obesity, diabetes, cardiac and neoplastic disorders (Craig, 2009, Appleby and Key, 2016, Dinu et al., 2017, Le and Sabaté, 2014, Trap and Barnard, 2010), and, unlike in humans, no statistically significant differences were reported in the prevalence of these disorders in cats. Of interest, the reported prevalence of disorders expected to be higher in cats fed PB diets, such as urinary tract disease (Knight and Leitsberger, 2016), did not differ between diet groups in this study. At this time, to the authors' knowledge, no cases of any adverse health outcome associated with PB diets in cats have been published, though a lack of evidence should not be interpreted as evidence of lack of risk. Nutrient deficiencies and imbalances may take many years to develop clinical signs, particularly in adult animals, and may go undetected.

4.5.4 Diet changes

It is possible that upon diagnosis of a health disorder a cat's diet is changed, most likely to a therapeutic diet. Considering no current therapeutic diets for cats are PB, it is possible that some cats diagnosed with a health disorder are changed from a PB to a MB diet, thus reducing the number of cats with health disorders in the PB category. Given the study design, this information was not able to be discerned. Interestingly, when comparing the diet of previous cats to current cats, few owners changed diet type, most continued to feed either PB or MB to current cats if that is what they fed their previous cat. Only a single owner who fed PB previously changed entirely to a MB diet, while about 10% of owners who previously fed MB changed

entirely to a PB diet. Comparing diets of previous cats to current cats does not give any indication as to whether the diet of the current cat was changed in response to diagnosis of an adverse health disorder, but the lack of dietary change between cats may support a hypothesis that the incident of diet change from PB to MB may be low. Further research would be required to determine if this is indeed the case. The present data only indicates that cat owners who fed PB to previous cats continue to feed PB to new cats, while a proportion who fed MB to previous cats may adopt a PB diet for their new cat. This supports the suspicion that this trend is likely actively growing at this point in time, as has previously been hypothesized (Dodd et al., 2019b). It is suspected that this may occur in conjunction with increasing proportions of people choosing to follow a vegan lifestyle for themselves, and not because of a perception of PB diets being healthier for cats. Previous research has demonstrated that a lack of concern for risk of negative impact on health was a determining factor for vegans feeding a PB to their pet, though a perception of improved health has not been reported (Dodd et al., 2019b). This warrants further investigation as to what health benefits may be perceived to be associated with PB diets for cats.

4.5.5 Diet Selection

Of interest are the differences in the sources where cat owners sought information about feline nutrition and the criteria used to select a diet for their cat reported in the present study. Overall, veterinary professionals were equally represented with the internet and social media as being the resources used by the most cat owners, which is in good agreement with previous reports (Kamleh, 2019). It is unclear if this referred to using a veterinary professional as well as a separate internet resource, or whether this indicated the use of a virtual veterinary resource, such as a veterinary blog or clinic website. However, when comparing between owners of cats fed PB

or MB, significantly fewer owners feeding PB considered their veterinary team, instead relying much more heavily on the internet and social media, as well as discussion groups and books and printed resources to a lesser extent. This finding is not surprising, as it has been suggested that pet owners feeding PB diets may not feel comfortable discussing their feeding practice with their veterinarian and may not view their veterinarian as an informed resource for discussing PB nutrition (Dodd et al., 2019b). This may be an issue not specific to PB feeding practices, per se, but to practices considered unconventional or alternative to the mainstream, as it has been reported that feeders of raw MB diets also show a decreased trust in veterinarians and seek nutritional information elsewhere (Morgan et al., 2017). A large risk of reliance on resources online or in print is the lack of quality control and critical evaluation these media undergo. While some websites, books, or other media may be useful resources with up-to-date and accurate information, it may not be apparent to the pet owner which are useful and which may be misleading and potentially harmful. For this reason, discussion of pet diet with the pet's veterinarian is recommended. The criteria used to select pet food differed as well between feeders of MB or PB diets. In comparison to the owners feeding MB, those feeding PB had a greater concern for the diet being labelled complete and balanced, being a human grade food product, being marketed as natural, organic or holistic, and had less concern for the palatability of the diet or the presence or lack of specific ingredients. While human grade, natural, organic or holistic products are not recognized by many practitioners to be superior to their conventional counterparts, it is positive to note the increased awareness or concern of owners feeding PB regarding the labelling of a diet being complete and balanced. While a statement of nutritional adequacy in accordance with industry guidelines is not a guarantee that the product is indeed

appropriate for the animal for which it is intended to be fed (Gosper et al., 2016, Burdett et al., 2018), it is a benchmark by which pet foods are compared and is a minimum standard to ensure nutritional sufficiency.

4.5.6 Limitations

The findings presented in this study must be interpreted with recognition of the inherent bias and limitation associated with the methodology. The sampling strategy employed allowed for self-selection into the study which likely introduces bias with respect to the nature of the participants. This sampling strategy was employed as the intention was to collect a large number of responses from broad sample of pet owners, representing the diversity within the pet owning population and avoiding targeting of specific subsections only. It is likely, however, that pet owners with exceptional interest in pet health and wellness would be most likely to voluntarily participate in the study. This could affect the results in different ways. Firstly, pet owners with specific interest in their pet's health and wellness may be highly perceptive and aware of conditions affecting their pet, resulting in their pet being presented to their veterinarian more often. This could either prevent health disorders by implementing appropriate prevention strategies or result in earlier diagnoses. These two potential outcomes would have opposing effects on the number of health disorders reported as prevention would decrease the number of health disorders occurring while timely diagnosis could increase the number of health disorders reported. By collecting health data reported by cat owners and not health professionals, objective evaluation of cat health could not be performed. Collection of data from veterinary practitioners has limitations as well, since less than half of cat owners present their cats to a veterinarian on a regular basis (AVMA, 2018), and many veterinary visits are as a result of a health disorder

(Bartlett et al., 2010). This could result in overestimation of health disorders. Utilising a survey of pet owners, if representative of the general pet-owning population, should better represent owners of healthy pets as well as pets with health disorders. This was the goal of the study presented here. With respect to the methodology employed, the sampling strategy likely targeted pet owners with particular interest in health and wellness and it is likely that pet owners with particular interest in unconventional diets were included as it has been demonstrated that pet owners feeding unconventional diets have a strong interest in pet health and wellness (Morgan et al., 2017). This would potentially bias the results to include a higher proportion of pets fed unconventional diets. As unconventional diets have been suggested to increase risk of health disorders, this may have resulted in reporting of more health disorders than would be expected in a general population of pets. In order to compensate for this, the questionnaire was not only advertised online, but also to customers of pet retail stores, with the expectation that many of the customers would purchase commercial pet foods from these stores and thus be representative of pet owners feeding conventional foods. However, other commercial establishments where pet foods are sold, such as grocery stores or “big box” stores, were not targeted for survey advertising, which could impact the types of respondents represented in the study. As the questionnaire was available online, the number of potential pet owners who saw the link but chose not to participate (non-respondents) was indeterminable. As such, there was no ability to evaluate the response rate, nor quantify the proportion of responses obtained through advertisement at pet stores as compared to online. This impairs interpretation of how representative the sample is of the general pet-owning population. Another limitation of survey-based studies in general is the reliance on accurate reporting and representation by the pet owner,

a form of recall bias. In this case, despite careful review of the diet reported utilised to determine what type of diet the cat was fed, a potential for misclassification exists if the diet reported by the cat owner did not accurately reflect what the cat was actually being fed. This is challenging to control or compensate for and represents a limitation of the study methodology that warrants consideration. Lastly, the findings presented here represent the opinions and beliefs of cat owners, not the definitive health status of the cats, and must be interpreted as such.

4.6 Conclusions

Owners who fed their cats PB diets had a positive perception of their cats' health, and reported a belief of better general health, better body condition, and fewer health disorders as compared to owners who fed their cats MB diets. Furthermore, the reported lifespan of cats did not differ based on diet type. While these data are owner reported and thus warrant follow-up research involving more objective evaluations, the hypothesis that owners of cats fed a PB diet would report higher prevalence of negative health outcomes was not supported by these findings.

4.7 References

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4.8 Tables

Table 4.1: Characteristics of cats as reported by participants in the “Pet Health and Wellbeing” survey.

		MB (n = 667)	PB (n = 187)	PB+MB (n = 69)
Sex	Male (n = 611)	321 (48%)	94 (50%)	34 (49%)
	Female (n = 653)	342 (51%)	93 (50%)	35 (51%)
Sex status	Intact (n = 41)	17 (3%)	10 (5%)	3 (4%)
	Desexed (n = 1223)	646 (97%)	177 (95%)	66 (96%)
Breed type	DSH (n = 660)	362 (54%)	93 (50%)	36 (52%)
	DMH (n = 60)	35 (5%)	6 (3%)	5 (7%)
	DLH (n = 95)	66 (10%)	8 (4%)	1 (1%)
	Asian (n = 50)	32 (5%)	8 (4%)	2 (3%)
	American (n = 35)	21 (3%)	4 (2%)	1 (1%)
	European (n = 58)	34 (5%)	8 (4%)	2 (3%)
	Other (n = 9)	2 (0%)	1 (0%)	0 (0%)
	Mix (n = 134)	58 (9%)	29 (16%)	9 (13%)
	Unknown (n = 131)	46 (7%)	25 (14%)	11 (16%)
Age (years)	Less than 1	29 (4%)	7 (4%)	3 (4%)
	1-2	92 (14%)	28 (15%)	6 (9%)
	3 – 4	105 (16%)	24 (13%)	6 (9%)
	5-6	83 (12%)	34 (18%)	14 (20%)
	7 – 8	90 (13%)	24 (13%)	6 (9%)
	9 – 10	65 (10%)	19 (10%)	12 (17%)
	11 – 12	61 (9%)	21 (11%)	5 (7%)
	13 – 14	41 (6%)	18 (10%)	5 (7%)
	15 – 16	40 (6%)	6 (3%)	6 (9%)
	17 – 18	16 (2%)	6 (3%)	2 (3%)
	19 – 20	9 (1%)	2 (1%)	0 (0%)
	Greater than 20	1 (0%)	0 (0%)	0 (0%)

Numbers of cats per category may not add up to total due to non-responders and indeterminable diet type. Thirty-four specific breeds were reported, including: Abyssinian, American bobtail, Balinese, Bengal, Birman, Bombay, British shorthair, Burmese, Chantilly, Chartreux, Chaussie, domestic shorthair (DSH), domestic medium hair (DMH), domestic longhair (DLH), Havana brown, Himalayan, Korat, Maine coon, Manx, Norwegian forest cat, Oriental, Persian, Ragdoll, Rex, Russian blue, Siamese, Siberian, Snowshoe, Somali, Sphynx, Tonkinese, Toyger, Turkish angora, Turkish van.

Table 4.2: Factors influencing pet food purchasing as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between owners of cats fed different diets.

Criteria	Total		MB		PB		PB+MB	
	n = 1025	%	n = 667	%	n = 187	%	n = 69	%
Complete and balanced	775	70	462	69 ^a	151	81 ^b	51	74 ^{a,b}
Convenience	321	29	198	30	51	27	23	33
Human grade	150	14	84	13 ^a	39	21 ^b	7	10 ^a
Natural/organic/holistic	327	30	152	23 ^a	95	51 ^b	36	52 ^b
Palatability	316	29	218	33 ^a	47	25 ^b	16	23 ^b
Price	314	28	204	31 ^a	42	22 ^b	22	32 ^a
Skin/coat/hairball health	272	25	174	26	44	24	11	16
Specific ingredients	156	14	115	17 ^a	21	11 ^b	4	5.8 ^b
Stool quality	133	12	83	12	25	13	8	12
Therapeutic/vet recommended	186	17	144	22 ^a	4	2 ^b	7	10 ^c

MB = meat-based, PB = plant-based, PB+MB = plant-based with animal-derived treats/snacks/supplements.

Superscript characters denote significant ($P < 0.05$) differences between the diet categories.

Note: values may add up to >100% since respondents could indicate that they selected pet food based on more than one criterion and numbers of cats per category may not add up to total due to non-responders and indeterminable diet type.

Table 4.3: Resources used to acquire feline nutrition information as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between owners of cats fed different diets.

Source of information	Total		MB		PB		PB+MB	
	n = 1022	%	n = 665	%	n = 187	%	n = 69	%
Book, pamphlet or printed resource	174	16	95	14 ^a	43	23 ^b	16	23 ^b
Breeder or shelter	20	2	17	2.6	1	0.5	0	0
Discussion group	272	25	145	22 ^a	67	36 ^b	24	35 ^b
Friends and/or family	186	17	100	15 ^a	30	16 ^a	18	26 ^b
Internet and social media	681	62	361	54 ^a	164	88 ^b	53	76 ^c
Manufacturer	21	2	13	2.0	3	1.6	3	4.4
Pet store or vendor	229	21	143	22 ^a	26	14 ^b	17	25 ^a
School or courses	39	4	31	4.7	2	1.1	0	0
Veterinary technician, clinician, specialist, student	707	64	465	70 ^a	87	47 ^b	38	55 ^b

MB = meat-based, PB = plant-based, PB+MB = plant-based with animal-derived treats/snacks/supplements.

Superscript characters denote significant ($P < 0.05$) differences between the diet categories.

Note: values may add up to >100% since respondents could indicate that they sought information from more than one source and numbers of cats per category may not add up to total due to non-responders and indeterminable diet type.

Table 4.4: Prevalence of feline health disorders as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between cats fed different diets.

Health disorder	Total		MB		PB		PB+MB/H	
	n = 1208	%	n = 667	%	n = 117	%	n = 139	%
Cardiac disease	26	2.2	17	2.6	2	1.7	20	0
Dental disease	208	17	131	20	21	18	16	11.5
Dermatopathy	137	11	82	12	12	10.3	13	9.4
Endocrinopathy	38	3.2	24	3.6	3	2.6	3	2.2
GI and hepatic diseases	126	10	90	13	3	2.6	11	7.9
Lower urinary tract disease	132	11	74	11	13	11	12	13
Neoplasia	21	1.7	10	1.5	2	1.7	4	2.9
Neurological	19	1.6	15	2.3	3	2.6	0	0
Obesity	100	8	64	9.6	7	6.0	9	6.5
Ocular disorders	57	5	39	5.9	4	3.4	1	0.7
Renal disease	42	3	31	4.7	1	0.9	2	1.4

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

Numbers of cats per category may not add up to total due to non-responders and indeterminable diet type. No statistically significant differences were detected.

Table 4.5: Results from multivariable logistic regression models of associations between reporting of health disorders and cat diet type, with confounders (age, breed type, sex, body condition score) controlled for.

Health Disorder	Variable	Odds Ratio	95% CI	P-value
Cardiac	Age	1.15	1.045 - 1.258	0.004
	Breed type, DLH	3.58	1.006 - 12.700	0.003
	Diet, PB	0.69	0.152 - 3.162	0.636
	Diet, PB+MB/H	-		
Dental	Age	1.11	1.070 - 1.152	< 0.001
	Diet, PB	0.84	0.495 - 1.434	0.528
	Diet, PB+MB/H	0.51	0.287 - 0.891	0.018
Dermatological	Age	1.08	1.038 - 1.129	< 0.001
	Diet, PB	0.79	0.415 - 1.512	0.480
	Diet, PB+MB/H	0.64	0.337 - 1.215	0.172
Endocrine	Age	1.37	1.234 - 1.519	< 0.001
	BCS, 3	0.13	0.030 - 0.561	< 0.001
	BCS, 5	0.07	0.018 - 0.272	< 0.001
	BCS, 7	0.07	0.015 - 0.315	0.001
	BCS, 9	0.05	0.008 - 0.359	0.003
	Diet, PB	0.57	0.156 - 2.113	0.248
	Diet, PB+MB/H	0.47	0.131 - 0.356	0.404
GI and hepatic	Age	1.09	1.049 - 1.140	< 0.001
	Diet, PB	0.16	0.051 - 0.530	0.003
	Diet, PB+MB/H	0.53	0.273 - 1.028	0.060
Lower urinary tract	Age	1.10	1.057 - 1.152	< 0.001
	Sex, male	2.88	1.836 - 4.518	< 0.001
	Diet, PB	0.96	0.508 - 1.827	0.908
	Diet, PB+MB/H	1.20	0.678 - 2.127	0.534
Neoplasia	Age	1.32	1.180 - 1.487	< 0.001
	Diet, PB	1.10	0.228 - 5.305	0.907
	Diet, PB+MB/H	1.83	0.543 - 6.194	0.329
Neurological	Diet, PB	1.14	0.326 - 4.014	0.834
	Diet, PB+MB/H	-		
Obesity	BCS, 9	8.50	1.064 - 67.940	0.041
	Diet, PB	0.84	0.360 - 1.977	0.695
	Diet, PB+MB/H	0.74	0.347 - 1.582	0.438
Ocular	Breed type, mix	3.66	1.609 - 8.321	0.003
	Diet, PB	0.64	0.218 - 1.849	0.406
	Diet, PB+MB/H	0.11	0.015 - 0.923	0.027
Renal	Age	1.30	1.196 - 1.411	< 0.001
	Diet, PB	0.16	0.021 - 1.250	0.081
	Diet, PB+MB/H	0.27	0.061 - 1.168	0.080

Referent categories: Breed type = domestic shorthair, Diet = meat-based, BCS = 1, sex = female.

DLH = domestic longhair, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt, BCS = body condition score. Odds ratios for categories with insufficient numbers could not be computed (-).

Table 4.6: Responses to seven Likert item questions asking respondents to rank indices of cat wellness, with comparison between cats fed different diets.

Wellness Indicator		Total n = 1025		MB n = 665		PB+MB/H n = 138		PB n = 117	
		n	%	n	%	n	%	n	%
Frequency of vomiting	Not at all	629	61	395	59	100	72	76	65
	A little	369	36	256	39	37	27	38	32
	Quite a bit	24	2.3	14	2.1	1	0.7	3	2.6
Frequency of inactivity	Not at all	783	76	492	74	115	83	103	88
	A little	208	20	147	22	19	14	13	11
	Quite a bit	34	3.3	28	4.2	4	2.9	1	0.9
Happy appearance	Not at all	3	0.3	1	0.2	0	0	1	0.9
	A little	31	3.0	23	3.5	1	0.7	3	2.6
	A moderate amount	316	31	206	31	39	28	34	29
	A great deal	675	66	437	66	98	71	79	68
Distress vocalization	Not at all	826	81	530	80	112	82	99	85
	A little	174	17	119	18	22	16	17	15
	A moderate amount	19	1.9	14	2.1	2	1.5	1	0.9
	A great deal	4	0.4	3	0.5	1	0.7	0	0
Demonstration of affection	Not at all	7	0.7	6	0.9	0	0	0	0
	A little	54	5.3	26	3.9	6	4.4	8	6.8
	A moderate amount	309	30	217	33	38	28	24	21
	A great deal	654	64	417	63	94	68	85	73
Contact avoidance	Not at all	735	72	762	70	114	82	92	79
	A little	236	23	170	26	18	13	23	20
	A moderate amount	43	4.2	28	4.2	5	3.6	1	0.9
	A great deal	7	0.7	4	0.6	1	0.7	1	0.9
Curious behaviour	Not at all	10	1.0	8	1.2	2	1.5	0	0
	A little	94	9.2	64	9.6	8	5.9	7	6.0
	A moderate amount	417	41	268	40	58	42	52	44
	A great deal	502	49	326	49	69	50	58	50

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

4.9 Figures

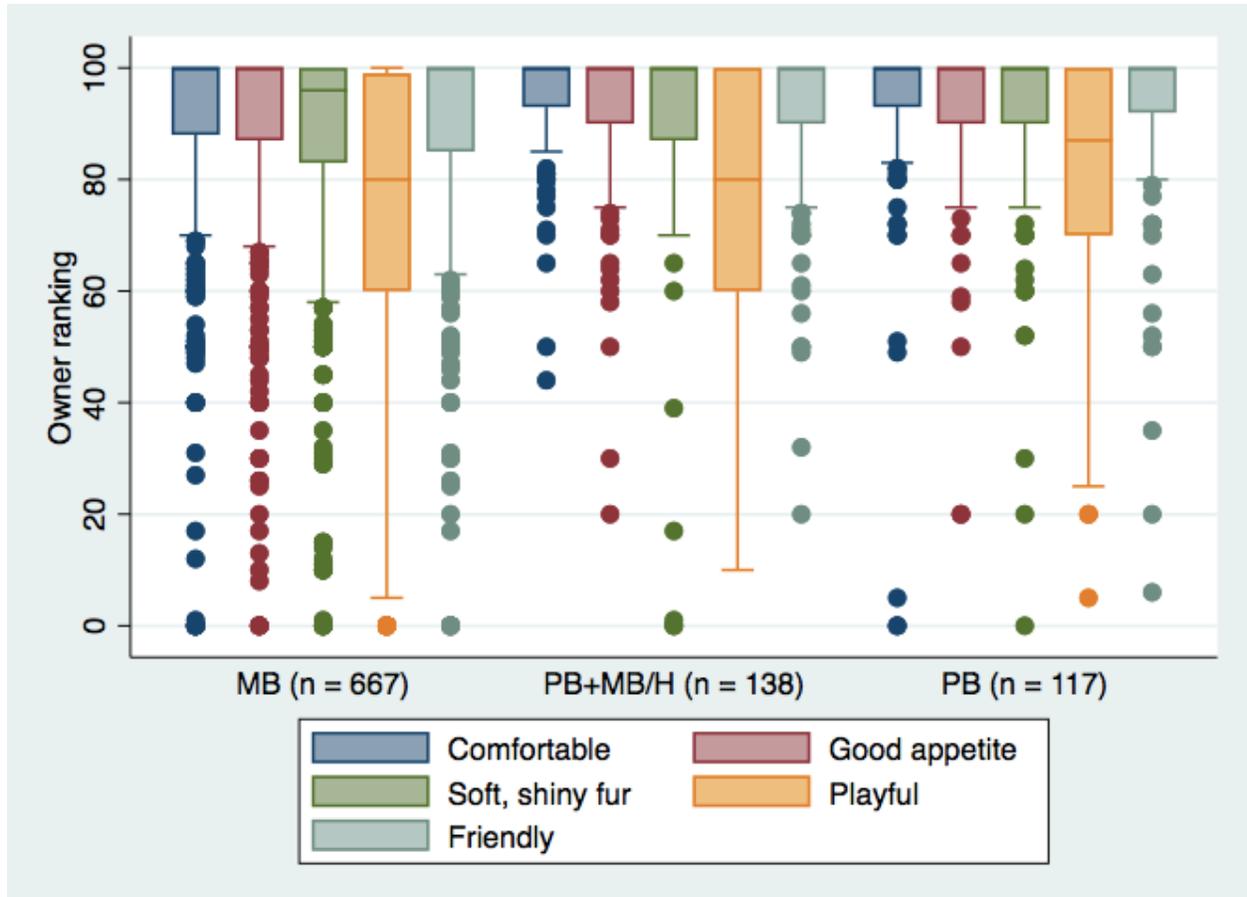


Figure 4.1: Owner ranking of positive cat wellness indicators based on visual sliding scale ranging from 0 (lowest) to 100 (highest), with comparison between cats fed different diets.

n = 1147

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

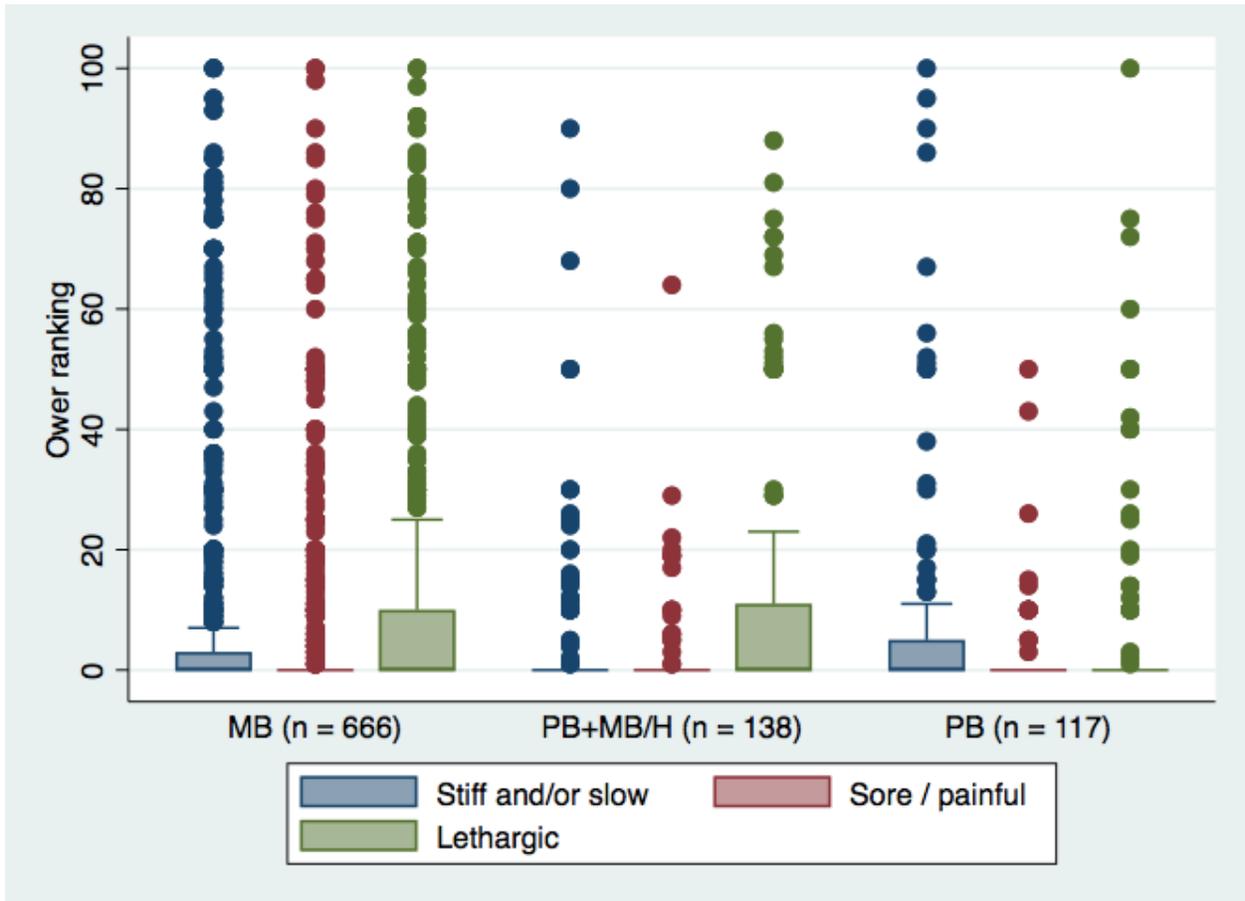


Figure 4.2: Owner ranking of negative cat wellness indicators based on visual sliding scale ranging from 0 (lowest) to 100 (highest), with comparison between cats fed different diets.

n = 1145

MB = meat-based, PB = plant-based, PB+MB/H = plant-based with animal-derived treats/snacks/supplements and/or ability to hunt.

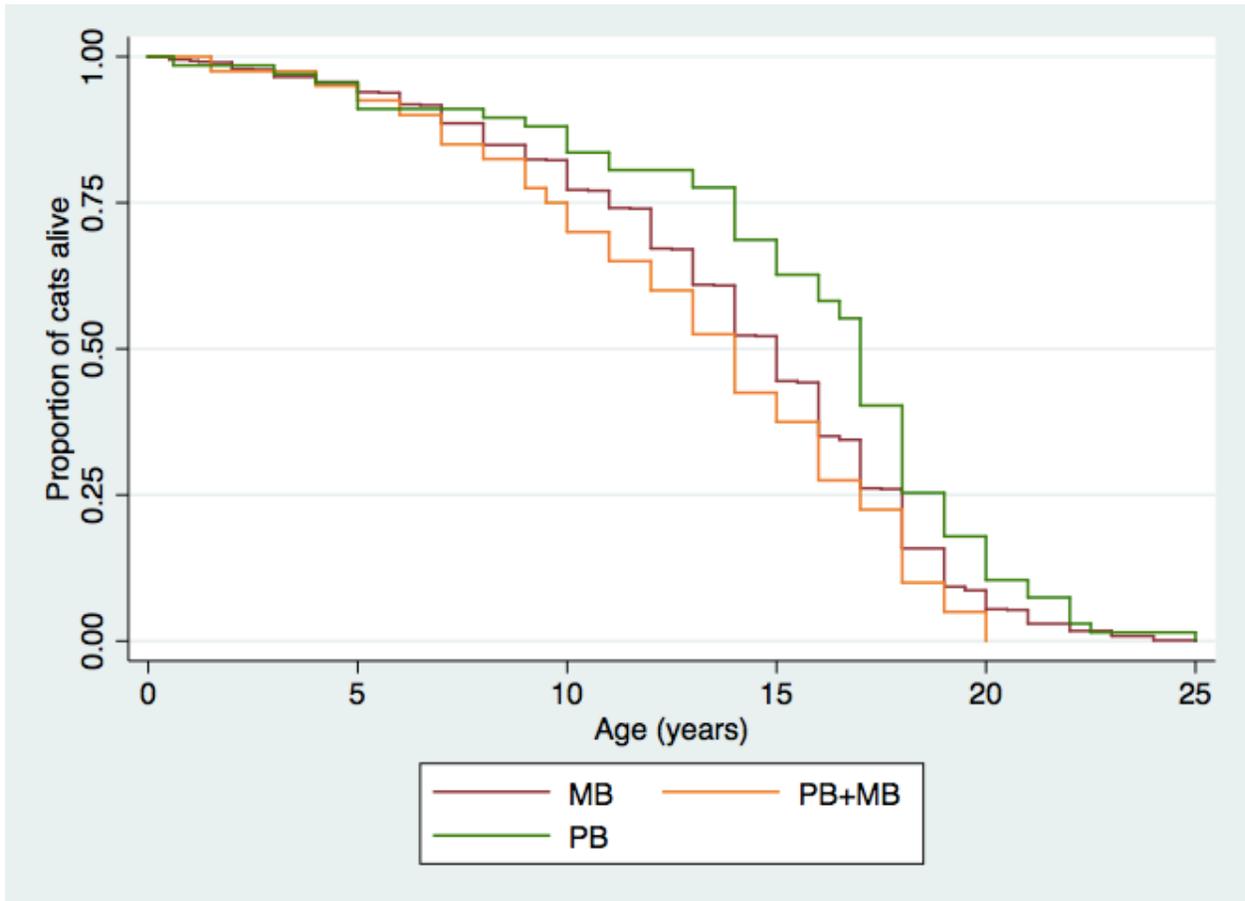


Figure 4.3: Kaplan Meier survival function of previous cats as reported by participants in the “Pet Health and Wellbeing” survey, with comparison between cats fed different diets.

No statistically significant differences were detected by log-rank test, $P = 0.192$.

MB = meat-based ($n = 807$), PB = plant-based ($n = 67$), PB+MB = plant-based with animal-derived treats/snacks/supplements ($n = 40$).

5 CHAPTER FIVE: A comparison of key essential nutrients in plant-based pet foods to canine and feline nutritional requirements and recommendations

Adapted from:



Article

A Comparison of Key Essential Nutrients in Commercial Plant-Based Pet Foods Sold in Canada to American and European Canine and Feline Dietary Recommendations

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A comparison of key essential nutrients in commercial plant-based pet foods sold in Canada to American and European canine and feline dietary recommendations.

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5.1 Abstract

Background: Plant-based foods intended for feeding dogs and cats are available in Canada, though few studies have examined the suitability of plant-based foods for dogs and cats.

Objectives: This study measured nutrients of key concern in plant-based diets and compared them to industry recommendations.

Methods: All commercial plant-based extruded and wet pet food products available in Ontario, Canada, in 2018 (n = 26) were acquired and analysed for energy, crude protein, crude fat, crude fibre, ash, amino acids, fatty acids, minerals and vitamins A, B12, D2 and D3. Results were compared with recommendations of the Association of American Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF).

Results: Thirteen products were labelled for adult canine maintenance, four for canine all life stages, one for puppy growth, two for adult feline maintenance, three for feline all life stages, one for adult maintenance of dogs and cats and two for all life stages of dogs and cats. Four products met AAFCO and one product met FEDIAF nutrient recommendations for canine maintenance. No diets met AAFCO or FEDIAF recommendations for feline maintenance or growth for either species. Nutrients most commonly found insufficient were: sulfur amino acids, taurine, arachidonic acid, EPA and DHA, calcium phosphorus and vitamin D. There were no nutrients unable to be provided from non-animal sources. Compliance with labelling guidelines was also poor, similar to other findings with commercial animal-based pet products.

Conclusions: The results from this study indicate areas where producers of plant-based pet foods must improve to meet the industry recommended nutrient profiles and labelling requirements.

5.2 Introduction

Commercial pet foods are often formulated to meet specific target nutrient profiles and exceed minimum nutrient requirements for the species and life-stage for which the product is intended.

In the USA, recommended nutrient profiles are published by the Association of American Feed Control Officials (AAFCO) (AAFCO, 2020). In European countries, the European Pet Food Industry Federation (FEDIAF) is the trade body representing European pet food manufacturers, which collaborates with European Union regulatory authorities to play a similar role as AAFCO within the pet food industry (FEDIAF, 2020). Canada does not have a regulatory agency overseeing pet food specifically, instead, Canadian manufacturers may voluntarily join the Pet Food Association of Canada and adopt AAFCO recommendations (PFAC, 2015).

Plant-based pet foods, fed to around 10% of dogs and 3% of cats (Dodd et al., 2019b), may be at particular risk of nutrient insufficiencies. One US study detected amino acids below AAFCO recommendation in two commercially available plant-based feline foods (Gray et al., 2004). One of the foods also had multiple mineral and vitamin insufficiencies, the other had one analysed vitamin below recommendation (Gray et al., 2004). More recently, another study in the USA found that 75% of 24 examined vegetarian pet foods met all minimum AAFCO amino acid recommendations, but only one third met labelling regulations (Kanakubo et al., 2015). Of note was that all canine, but few feline foods, met minimum adult maintenance amino acid recommendations. Four Brazilian products met minimum AAFCO and FEDIAF macronutrient recommendations, though only two met recommendations for all analysed amino acids, fatty

acids and minerals (Zafalon et al., 2020). All products tested did not meet the appropriate calcium to phosphorus ratio.

Though vitamin D has not been a focus of previous nutrient analyses studies, provision of this vitamin in particular warrants specific consideration. Vitamin D can be provided in two dietary forms: cholecalciferol (vitamin D3), found predominantly in animal tissues, or ergocalciferol (vitamin D2), found exclusively from non-animal sources (NRC, 2006). In cats, it has been demonstrated that dietary vitamin D2 does not have the same efficiency as vitamin D3 to maintain plasma 25-hydroxyvitamin D (Morris, 2002a), the main circulating vitamin D metabolite (Hazewinkel and Tryfonidou, 2002, Boyan et al., 2001, Weidner and Verbrugghe, 2016). In dogs, there is little comparable research, with some confusion remaining regarding the suitability of ergocalciferol as a source of vitamin D for dogs (Weidner and Verbrugghe, 2016). Nevertheless, plant-based diets may rely on non-animal derived ergocalciferol. Cholecalciferol has been detected in lichen species, which may serve as a source of dietary vitamin D3 in plant-based pet foods (Wang et al., 2001).

Considering the comparatively high prevalence of veganism reported within Canada (Dodd et al., 2019b), feeding plant-based diets may be more common than in other countries. However, the nutritional sufficiency of plant-based foods commercially available in Canada has not been investigated. Considering the nutrients identified to be potentially problematic in previous studies, the objective of this study was to measure essential nutrients of particular concern, including protein, amino acids, fat, fatty acids, minerals and vitamins A, B12 and D2 or D3 in all the plant-based pet foods commercially available in Canada and compare to AAFCO

and FEDIAF nutrient recommendations for dogs and cats; as well as to assess compliance with pet food label requirements. It was hypothesized that products would comply with the labelling regulations and that they would meet or exceed all nutrient recommendations of the industry organisations. Furthermore, it was expected that North American products may contain vitamin D2, though European products would only contain vitamin D3 as that is required to meet FEDIAF recommendations.

5.3 Materials and Methods

All twenty-six plant-based pet foods commercially available in Ontario, Canada in 2018 were included in the study. Manufacturers (V-Planet, Petcurean, Halo, Natural Balance and VGRRR) and a retail distributor (Vecado) were contacted by email with an invitation to participate. The letter included a description of the analyses to be performed and a request for donation of products for analyses. Participating companies received a nutrient analysis report for their own products prior to submission for publication. All products were procured at the same time. Six products were donated, 18 provided at cost and two purchased outright. Dry samples were collected from a single bag, wet samples were collected from a single can, all from the same batch. Bags were opened, samples immediately collected from multiple parts of the bag and combined, then analysed or shipped to analytical laboratories within 24 h. Additional samples were acquired at the time of bag opening, vacuum sealed, and frozen at -20°C . Cans were transported to the laboratories unopened and opened and sampled immediately prior to analyses. Additional cans were left unopened and stored at room temperature.

5.3.1 Proximate Analyses

Product samples were sent to the Central Testing Laboratory in Winnipeg, Manitoba, Canada. Dry matter, gross energy, crude protein, crude fibre, crude fat and ash were measured, moisture, metabolizable energy, and nitrogen-free extract (an approximation of carbohydrates), were calculated. Protein was determined by the Dumas method (Wiles and Gray, 1998, Simonne et al., 1997). Crude fat was determined by ether extract (AOAC 954.02)(AOAC, 2005). Crude fibre, a measure of most insoluble fibres, was determined by acid (sulfuric acid) and alkaline (potassium hydroxide) digestion (AOAC 962.09)(AOAC, 2005). Nitrogen-free extract was calculated by subtraction of crude protein, crude fat, crude fibre and ash from the total dry matter, and expressed as %DM. Gross energy (GE) was calculated by the equation: $GE = (5.7 * \text{protein}) + (9.4 * \text{fat}) + 4.1(\text{NFE} + \text{fibre})$ (NRC, 2006). Metabolizable energy (ME) for dogs was calculated by the equation: $ME = 575 + 0.816 * GE(\text{kcal/kg}) + 12.08 * \text{fat} - 52.76 * \text{crude fibre} - 20.61 * \text{protein} - 6.06 * \text{moisture}$. For cats, $ME = (GE * \text{energy digestibility}/100) - (0.77 * \text{protein})$ (NRC, 2006). According to the laboratory, the analyses had a variance of 0.3% for crude protein and 0.5% for crude fat.

5.3.2 Amino Acid Analyses

Food samples were sent to the Amino Acid Laboratory at the School of Veterinary Medicine of the University of California, Davis, in Davis, California, USA. Detailed description of methodology can be found for amino acid analysis (other than tryptophan) by AOAC official method 994.12, and for tryptophan by AOAC official method 988.15 (AOAC, 2005). Norleucine was used as an internal standard. With acidic hydrolysis, recovery of cystine was about 50% and results were corrected by multiplication factor. Methionine recovery was between 98–102%.

According to the laboratory, the amino acid analyses had a coefficient of variation within 5. All essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, taurine (essential within the diet for cats, not dogs), threonine, tryptophan and valine were included in the study.

5.3.3 Fatty Acid Analyses

Food samples were analyzed at the University of Guelph for fatty acids. Samples were prepared as-is, with dry kibbles being processed into a fine powder and wet foods being homogenized prior to analysis. Briefly, lipids were extracted by organic solvent in chloroform and methanol, then fatty acids were saponified and methylated using boron trifluoride. Methylated fatty acids were separated and analyzed by gas chromatography (Ma et al., 1999). C17:0 was added as an internal standard for quantification. Samples were analysed in duplicate and the mean of the two results reported. Dietary contents of all essential fatty acids: linoleic acid, α -linolenic acid (ALA), and long chain metabolites including arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were included in the study. In addition, results for γ -linolenic acid (GLA) are also reported, as it has been demonstrated that dietary GLA is effective at maintaining plasma and red blood cell arachidonic acid in adult cats fed arachidonic acid-deficient diets (Trevizan et al., 2012, Trevizan and Kessler, 2009).

5.3.4 Mineral Analyses

Food samples were sent to the Central Testing Laboratory in Winnipeg, Manitoba, Canada. Mineral evaluation was performed by multi-element inductively coupled plasma optical emission spectrometry (ICP-OES) aka atomic emission spectroscopy (ICP-AES) dry ash method

(AOAC 985.01) (AOAC, 2005). Food samples were sent to the Central Testing Laboratory in Winnipeg, Manitoba, Canada. Mineral evaluation was performed by multi-element inductively coupled plasma optical emission spectrometry (ICP-OES) aka atomic emission spectroscopy (ICP-AES) dry ash method (AOAC 985.01) (AOAC, 2005).

5.3.5 Vitamin Analyses

Food samples were sent to Bureau Veritas in Mississauga, Ontario, Canada. Detailed methodology for Vitamin A, retinol and beta-carotene analysis can be found in AOAC official method 992.04 and 992.06, for vitamin B12 in AOAC official method 986.23, and vitamin D3 in AOAC official method 982.29 (AOAC, 2005). For canine products, vitamin A content was determined from vitamin A measured in the product as retinol as well as beta-carotene, as dogs are able to cleave carotenoid precursors into active vitamin A. Thus, both retinol and beta-carotene contribute to total vitamin A intake (NRC, 2006). For feline products, vitamin A content was determined from vitamin A measured in the product as retinol only, as cats are unable to cleave carotenoid precursors into active vitamin A (NRC, 2006). Similar to vitamin A, vitamin D2/3 was also measured by HPLC-UV. Mean variance was 12% of the reported values. In addition to the total dietary vitamin D (the sum of D2 + D3), the values of ergocalciferol and cholecalciferol are also presented individually, as it is not known if ergocalciferol is utilized with the same efficacy as cholecalciferol to maintain vitamin D status (Morris, 2002a).

5.3.6 Label Evaluation

Each product was visually examined and photographed (SD), for all elements legally required by the Canadian Consumer Packaging and Labelling Act, as well as the AAFCO

Labelling Guidelines (Canada, 2019b, AAFCO, 2020). Consumer Packaging and Labelling Act requirements include: common or generic name, net weight, manufacturer's or importer's contact information. AAFCO Labelling Guidelines include: manufacturer contact information, product name, intended species, list of ingredients, feeding instructions, calorie content, guaranteed analysis, nutritional adequacy statement and intended life stage for which the product is suitable. The guaranteed analysis printed on the label was compared to the analyzed values. The list of ingredients was used to determine the source of vitamin D.

5.3.7 Categorization of Plant-Based Pet Foods

The companies were categorized by region of origin, either North America or Europe. For Canadian companies, membership with PFAC was determined by searching the list of PFAC members published on their website (PFAC, 2020). Products were categorized as canine or feline foods, according to the intended species mentioned on the pet food label; products labelled as intended for both species are included in results for both species. Products were also categorized for adult maintenance if labelled for adult maintenance or all life stages; or categorized for growth if labelled for puppy or kitten growth or all life stages. Thus, products labelled for all life stages were represented in both maintenance and growth groups.

5.3.8 Statistical Analyses

Descriptive statistics were reported as median and range of each dietary nutrient. All foods were compared to both AAFCO and FEDIAF nutrient profiles, reported on a unit per 100 g dry matter (AAFCO, 2020, FEDIAF, 2020). Products labelled for adult maintenance, or all life stages were compared to the adult recommendations, and products labelled for growth, or all life

stages were compared to the growth recommendations. Thus, products labelled for all life stages were represented in both maintenance and growth comparisons. The FEDIAF nutrient profiles for adult maintenance vary in accordance with energy intake, with the lower predicted energy intake requiring a higher nutrient density. The FEDIAF profiles for puppy growth differ between early growth, less than 14 weeks of age, and late growth from 14 weeks of age and older—both were included in the analyses performed. Calcium recommendations vary also based on predicted adult weight of 15 kg or less, and greater than 15 kg. The AAFCO nutrient profile for puppy growth differs in calcium recommendation for growth of puppies with a predicted adult weight of 31.75 kg or less, and greater than 31.75 kg. Comparisons to AAFCO nor FEDIAF could not be performed for: ergocalciferol, cholecalciferol and GLA, regardless of species or lifestage; taurine for dogs; and arachidonic acid and EPA + DHA for adult dogs; as these nutrients are not considered essential for the species and/or life stage.

For comparison to the labelled guaranteed analysis, the analyzed values for each product were compared to the guaranteed value on the product label and reported as percentage of guarantee fulfilled, with allowance for the analytical variations published by AAFCO (AAFCO, 2020). For nutrients with a guaranteed minimum content, percentages of label fulfilment should be equal to or greater than 100% to meet or exceed the guaranteed minimum. For nutrients with a guaranteed maximum content, percentages of label fulfilment should be equal to or below 100% to meet or stay below the guaranteed maximum.

The nutrient content of pet food products is not regulated within Canada, thus comparison and discussion of nutrients is with respect to the industry (AAFCO or FEDIAF)

recommendations. Government regulation strictly pertains to food safety and labelling. Discussion of labelling compliance is compared to the Canadian Consumer Packaging and Labelling Act.

5.4 Results

5.4.1 Description of Analysed Plant-Based Pet Foods

A summary of the plant-based pet food products is shown in Table S1. In total, 26 plant-based pet food products from eight different companies were included. Of the six North American companies, three were Canadian while three were American. One of the Canadian companies was a member of PFAC while the other two were not. Two companies were European. Eighteen products were labelled specifically for dogs (13 extruded, 5 wet), five specifically for cats (5 extruded), and three for both dogs and cats (3 wet). Of the canine foods, thirteen were labelled for adult maintenance (8 dry, 5 wet), four were labelled for all life stages (4 extruded) and one for puppy growth (1 extruded). Of the feline foods, two were labelled for adult maintenance (2 dry), three were labelled for all life stages (3 extruded) and none for kitten growth. Of the three combination canine/feline foods, all were wet products; one was labelled for adult maintenance and two were labelled for all life stages.

5.4.2 Nutrients in Canine Maintenance Products

Of the 20 products labelled for canine adults or all life stages, 12 were produced in North America and eight in Europe. Four products met all AAFCO recommendations for adult maintenance, one met all FEDIAF recommendations for adult maintenance at a lower energy

intake (95 kcal/kg^{0.75}) and two met all FEDIAF recommendations for adult maintenance at a higher energy intake (110 kcal/kg^{0.75}) (Table 5.1).

In comparison to AAFCO, almost every product (19/20, 95%) exceeded all crude protein recommendations. One (1/20, 5%) had a crude protein concentration below the industry recommended minimum, but within the analytical variation published by AAFCO (AAFCO, 2020). One extruded product (1/12, 8%) had a single amino acid (methionine + cystine) below AAFCO recommendation, while one or more amino acids were low in all eight wet products (8/8, 100%). Of those eight wet products, all (8/8, 100%) were low in methionine + cystine, three (3/8, 25%) in methionine and methionine + cystine, and one (1/8, 13%) in lysine, methionine, methionine + cystine and tryptophan. All products met AAFCO crude fat and essential fatty acid (linoleic acid) recommendations. Fifteen (15/20, 75%) met all mineral requirements; two extruded products (2/12, 25%) contained sodium below AAFCO recommendations, one (1/12, 8%) had sodium, chloride and calcium below AAFCO recommendations and an inverse calcium to phosphorus ratio. One extruded product (1/12, 8%) had a calcium to phosphorus ratio exceeding the recommended maximum (2:1). Two wet products (2/8, 25%) contained insufficient zinc, and one (1/8, 13%) had insufficient calcium and an inverse calcium to phosphorus ratio (0.8:1). Twelve (12/20, 60%) foods were replete in all vitamins measured. Two extruded products (2/12, 17%) had a single vitamin below AAFCO recommendations (A or D) and one (1/12, 13%) contained insufficient vitamins A, B12 and D. Two wet diets (2/8, 25%) were below AAFCO recommendations in one vitamin (B12 or D) and three (3/8, 38%) in vitamins B12 and D.

In comparison to FEDIAF recommendations for dogs with a lower energy consumption of 95 kcal/kg^{0.75}, seventeen (17/20, 85%) products met the total protein recommendation, and seven (7/20, 35%) met all amino acid recommendations. Two extruded products (2/12, 17%) contained insufficient methionine + cystine, while two (2/12, 17%) contained insufficient methionine and methionine + cystine and one (1/12, 8%) contained insufficient methionine + cystine and tryptophan. All wet products (8/8) contained insufficient methionine + cystine, five (5/8, 63%) contained insufficient methionine and methionine + cystine, and one (1/8, 13%) contained insufficient methionine, methionine + cystine, and tryptophan. All products met FEDIAF crude fat and fatty acid recommendations. Fourteen foods (14/20, 70%) met all FEDIAF mineral minimum recommendations. Four extruded products (4/12, 33%) contained insufficient sodium and one (1/12, 8%) insufficient sodium, chloride, calcium, and an inverse calcium to phosphorus ratio. One extruded product had a calcium to phosphorus ratio greater than the FEDIAF recommendation. Two wet products (2/8, 25%) contained insufficient zinc and one (1/8, 8%) insufficient calcium and an inverse calcium to phosphorus ratio. Of the European manufactured diets, three (3/8, 38%) contained minerals in excess of the EU legal limit. Six (6/20, 30%) products contained all vitamins measured above the FEDIAF minimum recommended content. Six extruded products (6/12, 50%) had a single vitamin below FEDIAF recommendations (A, B12 or D), one (1/12, 8%) contained insufficient vitamin A and D, and one (1/12, 8%) contained insufficient vitamin A, B12 and D. Two wet diets (2/8, 25%) contained a single vitamin (B12 or D) below FEDIAF recommendations and three (3/8, 38%) contained both vitamins B12 and D below recommendations.

In comparison to FEDIAF recommendations for dogs with a higher energy consumption of 110 kcal/kg^{0.75}, nineteen (19/20, 95%) products met the total protein recommendation, and ten (10/20, 50%) met all amino acid recommendations. One extruded (1/12, 8%) and all wet (8/8, 100%) products contained insufficient methionine + cystine. Four (4/8, 50%) wet products also contained insufficient methionine. All products met FEDIAF crude fat and fatty acid recommendations. Fourteen foods (14/20, 70%) met all FEDIAF mineral minimum recommendations. One extruded product (1/12, 8%) contained insufficient sodium, one (1/12, 8%) contained insufficient sodium and an excess calcium to phosphorous ratio, and one (1/12, 8%) insufficient sodium, chloride, calcium, and an inverse calcium to phosphorus ratio. Two wet products (2/8, 25%) contained insufficient zinc and one (1/8, 8%) insufficient calcium and an inverse calcium to phosphorus ratio. Of the European manufactured diets, three (3/8, 38%) contained minerals in excess of the EU legal limit. Nine (9/20, 45%) products contained all vitamins measured above the FEDIAF minimum recommended content. Five extruded products (5/12, 42%) had a single vitamin below FEDIAF recommendations (A, B12 or D) and one (1/12, 13%) contained insufficient vitamins A, B12 and D. Two wet diets (2/8, 25%) were below FEDIAF recommendations in one vitamin (B12 or D) and three (3/8, 38%) in vitamins B12 and D.

5.4.3 Nutrients in Canine Growth Products

Of the seven products labelled for canine growth or all life stages, six were made in North America, one in Europe. No product met all AAFCO or FEDIAF recommendations for growing dogs (Table 5.2).

In comparison to AAFCO recommendations, all products (7/7, 100%) met total protein recommendations, though only four (4/7, 57%) products met all amino acid recommendations. One extruded product (1/5, 20%) contained insufficient methionine + cystine, one wet product (1/2, 50%) contained insufficient methionine + cystine while the other (1/2, 50%) contained insufficient methionine + cystine and lysine. All products contained sufficient total fat, but no product met AAFCO EPA + DHA recommendations. Two products (2/7, 29%) met all AAFCO mineral recommendations. One extruded product (1/5, 20%) had only chloride below AAFCO recommendations, one (1/5, 20%) contained insufficient calcium and phosphorus, while one (1/5, 20%) had calcium, phosphorus and zinc below AAFCO recommendations. In both wet products (2/2, 100%) calcium and phosphorus were below AAFCO recommendations, while one wet product (1/2, 50%) also had insufficient sodium and an inverse calcium to phosphorus ratio. No products exceeded the AAFCO maximum recommended concentration of calcium for large or non-large breed dogs. Four (4/7, 57%) products met all AAFCO vitamin recommendations. One extruded product (1/5, 20%) contained insufficient vitamin A, another (1/5, 20%) insufficient vitamin D. One wet product (1/2, 50%) contained insufficient vitamins B12 and D.

In comparison to FEDIAF recommendations for growth of puppies less than 14 weeks of age, all products met total protein recommendations, though only four (4/7, 57%) met all amino acid recommendations. One extruded product (1/5, 20%) and both wet products (2/2, 100%) contained insufficient methionine + cystine (2/5, 40%). All products contained sufficient total fat, but no product (0/7, 0%) met FEDIAF EPA + DHA or arachidonic acid recommendations. Two products (2/7, 29%) met all FEDIAF minimum mineral recommendations. Two extruded products (2/5, 40%) contained a single mineral insufficiency (chloride or phosphorus), while one

(1/5, 20%) contained both insufficient phosphorus and zinc. Both wet products (2/2, 100%) contained insufficient calcium and phosphorus, with one (1/2, 50%) having an inverse calcium to phosphorus ratio. The single European diet contained copper in excess of the EU legal limit. Four products (4/7, 57%) met all FEDIAF vitamin recommendations. Two extruded products (2/5, 40%) had a single vitamin below FEDIAF recommendations (A or D) while one wet product (1/2, 50%) contained insufficient vitamins B12 and D.

In comparison to FEDIAF recommendations for growth of puppies over 14 weeks of age, all products met total protein and all amino acid requirements. All products met FEDIAF total fat recommendations, but no product (0/7, 0%) contained sufficient EPA + DHA or arachidonic acid. Three products met all FEDIAF mineral recommendations; one extruded product (1/5, 20%) contained insufficient chloride, while one (1/5, 20%) contained insufficient phosphorus and zinc. Both wet foods (2/2, 100%) contained insufficient calcium and phosphorus, with one (1/2, 50%) having an inverse calcium to phosphorus ratio. One extruded product (1/5, 20%) and both wet foods (2/2, 100%) contained insufficient calcium in comparison to FEDIAF recommendations for large-breed puppies, though none of the products were specifically labelled for large-breed puppy growth. Four products met all FEIDAF vitamin recommendations. Two extruded products (2/5, 40%) had a single vitamin below FEDIAF recommendations (A or D) while one wet product (1/2, 50%) contained insufficient vitamins B12 and D.

5.4.4 Selected Essential Nutrients in Feline Maintenance Products

Of the eight products labelled for feline adult or all life stages, five were manufactured in North America and three in Europe. No product met all AAFCO or FEDIAF recommendations for adult maintenance (Table 5.3).

In comparison to AAFCO recommendations, all (8/8, 100%) feline products labelled for maintenance or all life stages exceeded crude protein recommendations. Few products (2/8, 100%) met all amino acid recommendations. Two extruded (2/5, 40%) and no wet (0/3, 0%) products met the AAFCO recommended taurine concentrations. All other amino acid recommendations were met by all (8/8, 100%) products. Six (6/8, 75%) products met AAFCO crude fat recommendations, two wet products (2/3, 67%) did not do so. AAFCO Linoleic acid recommendations were surpassed by all products, though few (2/8, 25%) met arachidonic acid recommendations—both were wet products. Six (6/8, 75%) products met all AAFCO mineral recommendations, with one extruded product (1/5, 20%) containing insufficient sodium and one wet product (1/3, 33%) insufficient calcium. Five products (5/8, 63%) met all AAFCO vitamin recommendations. Two extruded products (2/5, 40%) had a single vitamin below AAFCO recommendations (A or D) and one wet product (1/3, 33%) had both vitamins B12 and D below recommendations.

In comparison to FEDIAF recommendations for cats fed a lower energy intake of 75 kcal/kg^{0.67}, few (2/8, 25%) feline products labelled for maintenance or all life stages exceeded crude protein recommendations. Two extruded products (2/5, 40%) and two wet products (2/3,

67%) contained only taurine below FEDIAF recommendations. One extruded product (1/5, 20%) contained insufficient arginine, phenylalanine + tyrosine, and taurine, and one wet product contained insufficient methionine + cystine and taurine. Six (6/8, 75%) products met FEDIAF crude fat recommendations, two wet products (2/3, 67%) did not do so. Most (6/8, 75%) diets met all FEDIAF fatty acid recommendations, with one extruded (1/5, 20%) and one wet (1/3, 33%) product failing to so. Five (5/8, 63%) products met all FEDIAF minimum mineral recommendations, with one extruded product (1/5, 20%) having a single mineral insufficiency (potassium), and one (1/5, 20%) having three minerals below recommendation (potassium, iron and zinc). One wet product (1/3, 33%) contained insufficient calcium and an inverse calcium to phosphorus ratio. Two European manufactured diets contained minerals in excess of the EU legal limit: one extruded product (1/5, 20%) contained excess copper and one wet product (1/3, 33%) contained excess zinc. Five products (5/8, 63%) met all FEDIAF vitamin recommendations. One extruded product (1/5, 20%) had a single vitamin below FEDIAF recommendations (D) and one (1/5, 20%) had two (vitamins A and B12). One wet product (1/3, 33%) had both vitamins B12 and D below FEDIAF recommendations.

In comparison to FEDIAF recommendations for cats fed a higher energy intake of 100 kcal/kg^{0.67}, all (8/8, 100%) feline products labelled for maintenance or all life stages exceeded crude protein recommendations, and all amino acid requirements, with the exception of taurine. Only two products, both extruded, met taurine requirements. Six (6/8, 75%) products met FEDIAF crude fat recommendations, two wet products (2/3, 67%) did not do so, but all (8/8, 100%) diets met all fatty acid recommendations. Seven (7/8, 88%) products met all FEDIAF minimum mineral recommendations, with one wet product (1/3, 33%) containing an inverse

calcium to phosphorus ratio. Sufficiency of vitamin provision was the same as for the recommendations for cats with a lower intake, described in the section above.

5.4.5 Selected Essential Nutrients in Feline Growth Products

Of the eight products labelled for feline growth or all life stages, all produced by two North American companies, none met all AAFCO and FEDIAF recommendations for feline growth (Table 5.4).

In comparison to AAFCO recommendations, most (4/5, 80%) feline foods labelled for growth or all life stages exceeded crude protein recommendations for kitten growth and development, though only one product (1/6, 17%) met all amino acid recommendations. One extruded product (1/3, 33%) had a single amino acid (methionine + cystine) below AAFCO recommendations and one (1/3, 33%) had four amino acids (methionine, methionine + cystine, phenylalanine + tyrosine, and taurine) below recommendation. Both wet products contained methionine, methionine + cystine and taurine below AAFCO recommended concentrations. Most (4/5, 80%) diets met AAFCO crude fat recommendations, with one wet product (1/2, 50%) failing to do so. No product met all AAFCO fatty acid recommendations, with all three extruded products containing insufficient arachidonic acid, and all five diets containing insufficient EPA + DHA. Two extruded products (2/3, 67%) met all AAFCO mineral recommendations while one extruded product (1/3, 33%) and both wet products (2/2, 100%) contained insufficient calcium and phosphorus. One extruded product also did not meet the AAFCO minimum copper recommendation Two diets (2/5, 40%) met all AAFCO vitamin recommendations. Two extruded

products (2/3, 67%) had a single vitamin (vitamin A or D) insufficiency, while one wet product (1/2, 50%) had both vitamins B12 and D below AAFCO recommendation.

In comparison to FEDIAF recommendations, all feline foods labelled for growth or all life stages exceeded crude protein recommendations for kitten growth and development. Two products (2/5, 40%) met all FEDIAF amino acid recommendations. One extruded product (1/3, 33%) had four amino acids (methionine, methionine + cystine, phenylalanine + tyrosine, and taurine). Both wet products contained methionine + cystine and taurine below FEDIAF recommended concentrations. One wet product (1/3, 20%) contained insufficient fat. All extruded products contained insufficient arachidonic acid, though both wet products contained sufficient arachidonic acid. No products met FEIDAF EPA+DHA recommendations. Two extruded products (2/3, 67%) met all FEDIAF minimum mineral recommendations while one extruded product (1/3, 33%) and both wet products (2/2, 100%) contained insufficient calcium and phosphorus and one wet product (1/2, 50%) had an inverse calcium to phosphorus ratio. None of the products were made in Europe, so European legal limits for minerals were not considered. One product (1/5, 20%) met all FEDIAF vitamin recommendations. Two extruded products (2/3, 67%) had a single vitamin insufficiency (vitamin A), while one extruded (1/3, 33%) product contained insufficient vitamins A and D. One wet product (1/2, 50%) had vitamins B12 and D below FEDIAF recommended concentrations.

5.4.6 Source of Vitamin D

Based on pet food label information, an equal number of products claimed to include vitamin D2 as claimed to included vitamin D3. Two wet foods (2/8, 25%), produced by the same

company, claimed to include a vitamin D supplement, but did not specify if it was vitamin D2 or vitamin D3. Despite FEDIAF recommendations being specifically for vitamin D3, six (6/11, 55%) European products listed vitamin D2 in the ingredient deck, five (5/11, 45%) listed vitamin D3. Six (6/15, 40%) North American diets listed vitamin D2 in the ingredient deck, seven (7/15, 47%) listed vitamin D3, and two (2/15, 13%) listed a non-specific vitamin D supplement.

When analysed, five products (4 extruded, 1 wet) contained vitamin D2 while seventeen (14 extruded, 3 canned) contained vitamin D3. Six products (2 extruded, 4 wet) contained no detectable vitamin D. Six (6/12, 50%) of the products claiming vitamin D2 had no detectable D2 but did contain D3, while two (2/12, 17%) contained both D2 and D3. Three (3/12, 25%) contained D2 only. One of the products bearing a claim for an unspecified vitamin D supplement contained D3, the other contained no detectable vitamin D. Two European diets claiming vitamin D2 content (2/6, 33%) contained vitamin D2, both also contained vitamin D3. Three North American diets claiming vitamin D2 (3/6, 50%) contained vitamin D2, none contained both vitamin D2 and vitamin D3.

5.4.7 Labelling

All but two products met all Canadian legal labelling requirements according to the Consumer Packaging and Labelling Act (Canada, 2019b). The two products failing to meet requirements did not include the contact details of the manufacturer. Both products were manufactured by the same company within Canada. Nine products (9/26, 35%) from five companies (5/8, 63%) met AAFCO Labelling Guidelines. Fifteen products (15/26, 58%) from three companies (3/8, 38%) did not meet AAFCO Labelling Guidelines as they did not include a

statement of calorie content. Six (6/15, 40%) foods manufactured in the USA bore a statement of caloric density. Only 3 (3/11, 27%) foods manufactured in Europe bore a statement of caloric density. All products produced in North America (15/15, 100%) included a statement of nutritional adequacy for the species and life stage, as substantiated by formulation to meet the targeted nutrient profile. No diet bore a statement of substantiation by feeding trial. The 11 products manufactured in Europe (11/26, 42%) did not have a nutritional adequacy statement as described by AAFCO, but did include a descriptor of the product being “Complete” for the animal species and life stage intended, in compliance with FEDIAF requirements. In comparison to the labelled guaranteed analysis, seven canine products (7/20, 33%) and no feline products (0/7, 0%) met or exceeded all labelled maxima; 16 canine (16/20, 80%) and six feline (6/7, 86%) complied with labelled minima. One canine and one feline product, both manufactured by the same Canadian company, bore no guaranteed analysis on the label. Table 5.5 shows the percentage of labelled value measured for each nutrient.

5.5 Discussion

While plant-based diets are shown to support health in humans (Craig, 2009, Appleby and Key, 2016, Dinu et al., 2017, Le and Sabaté, 2014), the nutritional requirements of obligate carnivorous cats and facultative carnivorous dogs are quite different. As a result of their evolution consuming most or part of their diet as prey, both cats and dogs have greater dietary requirements for protein and micronutrients typically found in higher concentrations in animal tissues in comparison to humans. Cats have additional nutritional idiosyncrasies common to carnivorous animals (Morris, 2002b, Zoran, 2002). Provision of these nutrients in appropriate quantities for dogs and cats without utilising animal-derived ingredients can be a challenge. The

formulation and production of commercial extruded and wet plant-based pet foods containing all essential nutrients required by dogs and cats is possible. Of the 26 plant-based foods commercially available in Canada, few (3/26, 12%) were found to meet all nutrient recommendations for the life stage they were intended, when compared to AAFCO or FEDIAF nutrient profiles. Only adult canine maintenance requirements were met, no product met feline or growth recommendations. Each essential nutrient measured could be detected in the plant-based foods, though the products did not all meet the recommended quantities or proportions. Though outside the scope of this study, digestibility and bioavailability of nutrients must also be considered, in addition to provision in appropriate quantities.

In adult canine foods, the most problematic nutrients, those most often failing to meet minimum recommendations, were the sulfur amino acids methionine + cystine and vitamin D. In products labelled for canine growth, calcium, phosphorus and the omega 3 fatty acids EPA + DHA were most limiting. For the feline adult products, sulfur amino acids were sufficient but taurine was not, particularly in the wet products. Additionally, provision of arachidonic acid was often below recommendations. Feline growth products proved the most problematic, commonly demonstrating insufficiencies in methionine, methionine + cystine, taurine, arachidonic acid, EPA + DHA, calcium and phosphorus. Dietary deficiency of most amino acids may be subclinical and go undetected for long periods of time. Though methionine and cysteine play critical roles in protein synthesis and function, as well as methyl donation and antioxidant generation, the signs of deficiency are non-specific and relate to reduction in food intake, weight loss, poor growth and dermatitis (Hirakawa and Baker, 1985, Milner, 1979b). Reduced intake and/or bioavailability of methionine and cysteine in dogs may also predispose to insufficient

taurine synthesis and result in dilated cardiomyopathy and retinal changes (Backus et al., 2006). In cats, taurine deficiency has been clearly associated with dilated cardiomyopathy (Pion et al., 1987). Calcium, phosphorus and vitamin D are all involved in skeletal metabolism and thus deficiencies and/or imbalances can predispose to skeletal deformities and pathological fractures, particularly in young, developing animals (Dodd et al., 2019a, Tal et al., 2018, Verbrugge et al., 2011). Arachidonic acid is considered an essential fatty acid for adult cats, kittens, and, in Europe, for puppies (AAFCO, 2020, FEDIAF, 2020). The essentiality of this omega-6 fatty acid in adult cats, however, has been questioned. With dietary provision of sufficient linoleic acid, with or without GLA, cats may be able to generate sufficient dihomo-GLA to arachidonic acid via delta-5 desaturase (Bauer, 2006). Adult cats fed diets devoid of arachidonic acid but rich in linoleic acid show no signs of arachidonic acid deficiency unless mated (MacDonald et al., 1984). Even during growth, males appear unaffected, but females demonstrate reliance on dietary arachidonic acid to maintain healthy reproductive activity (Morris, 2004). It has been concluded that arachidonic acid may not be essential for maintenance, growth, or reproduction in male cats, only for reproduction in female cats (Bauer, 2006). Non-reproducing cats fed plant-based diets devoid of arachidonic acid may thus show no overt clinical signs of arachidonic acid deficiency, particularly if the diets are rich in linoleic acid and/or GLA. Nevertheless, without addition of arachidonic acid the products did not meet industry nutrient guidelines.

These findings share some commonalities with other nutrient analyses of plant-based foods performed previously around the world. In Brazil, a limited sample of three plant-based dog foods were noted to have insufficiencies of methionine (1/3, 33%) while the single plant-based cat food evaluated contained no detectable arachidonic acid (Zafalon et al., 2020). In the USA, a

focused evaluation of amino acids in 24 plant-based and vegetarian products detected amino acid insufficiencies in six (25%) of the products, with three (3/6, 50%) of those having insufficiencies of methionine, methionine + cystine and/or taurine (Kanakubo et al., 2015). Five of the six products were labelled for cats, one was for dogs and cats. Eleven of the products analysed in that study were included in this current study, though direct comparison of results was not possible as means and ranges for all products were presented and individual product results were not published.

An older study in the USA examined amino acids as well as fatty acids and select vitamins and minerals in two feline products (Gray et al., 2004). Findings shared with the present study included insufficiencies in methionine (2/2, 100%), methionine + cystine (2/2, 100%), taurine (2/2, 100%), arachidonic acid (2/2, 100%), calcium (1/2, 50%), phosphorus (1/2, 50%), vitamin A (1/2, 50%), and vitamin B12 (1/2, 50%). In that study, the products were named and one of the products was also evaluated in the present study. In comparison to the previous findings, the product currently contained sufficient methionine, methionine + cystine, vitamin A and vitamin B12. However, the product was still below the recommended inclusion concentrations for taurine and arachidonic acid, and a new insufficiency in threonine was detected. Given that over 10 years have passed between studies, changes to the formulation and manufacturing of the product are likely to have occurred. Between the results of previous studies and the findings of the present study, it is clear that although formulation of foods meeting all recommended nutrient targets for both dogs and cats is possible without inclusion of animal products, there is great variability in the nutritional sufficiency of commercially available plant-based pet foods. Failure to meet industry nutrient recommendations has been demonstrated before in Canadian pet foods

(Burdett et al., 2018), though not to the same magnitude as demonstrated in this study. Of the commercial foods analyzed in that study, 93% (25/27) met or exceeded AAFCO nutrient recommendations, in comparison to 12% (3/26) in this investigation. Comparison to labelled guaranteed analyses was more consistent, with 33% (9/27) in the previous study meeting all nutrient content claims, compared to 27% (7/26) of the plant-based products. Given that there were no nutrients identified that were unable to be provided from non-animal sources and that there were products that met all nutrient recommendations, it is unclear if the failure to meet industry recommendations is due to the lack of animal ingredients in the products or if this is a reflection of inadequate formulation or manufacturing processes. A range of practice standards for quality assurance and quality control of pet foods exist. A recent study of pet food manufacturers, including 19 meat-based and 10 plant-based pet foods reported that most manufacturers had standards considered acceptable by the authors, with practices for plant-based diets considered slightly superior (Knight and Light, 2021). The dietary requirements of animals have been established based on essentiality of nutrients, not ingredients, and there are no known nutrients contained exclusively in animal products that cannot be provided from non-animal sources. Trends in the pet food industry tend to be based on ingredients, however, as a result of marketing and trends in human nutrition and philosophies. It is important to keep in mind that the nutritional requirements of dogs and cats differ from those of humans.

Concerningly, there was great discrepancy in this study between the labelled source of vitamin D and the type of vitamin D measured. Though twelve of the diets included a vitamin D2 supplement in their ingredient list, only five were found to contain vitamin D2. Two also contained vitamin D3, which was not listed in the ingredients. In those diets, no vitamin D3-rich

ingredients (animal or lichen) or vitamin D3 supplements were included in the ingredient list. Cross-contamination could be possible if animal-based diets had been manufactured in the same facility, but this would be insufficient to explain the concentrations of vitamin D3 in those diets, as they contained the full recommended quantity.

With respect to the most common nutrient insufficiencies detected, those being sulfur amino acids, taurine, arachidonic acid, EPA + DHA, calcium, phosphorus and vitamin D, correction of the insufficiencies would be expected to be relatively simple (Dodd et al., 2018) (Table 5.6).

Though non-animal sources of nutrients exist, there may be restrictions on the use of these ingredients in pet food products. For example, ergocalciferol is a non-animal derived source of vitamin D in the form of vitamin D2. Ergocalciferol is an approved ingredient for use in pet food formulation in North America (AAFCO, 2020), though in Europe it is not a registered feed additive, necessitating the addition of cholecalciferol, vitamin D3 (FEDIAF, 2020). Similarly, although EPA and DHA from algal sources have been demonstrated as safe and efficacious for pregnancy, lactation and growth for cats and dogs (Vuorinen et al., 2019, Dahms et al., 2019), despite this, current regulation limits the use of marine microalgae as a source of DHA and other omega-3 fatty acids for canine adult maintenance in North America (AAFCO, 2020).

A case report was recently published demonstrating adverse health outcome in cats maintained on a plant-based diet. Fantinati and colleagues identified multiple nutrient deficiencies in two cats fed a commercial plant-based product in Toulouse, France (Fantinati et

al., 2021). The cats' clinical signs were attributed mostly to the marked deficiency of folic acid detected on serum analysis, consistent with insufficiency of folate in the diet. Investigations of health status of dogs and cats fed plant-based diets have been unable to detect any clinical abnormalities associated with the unconventional diet (Brown et al., 2009, Wakefield et al., 2006). With plant-based feeding being a relatively novel trend and the proportion of pets being fed plant-based diets being low (Dodd et al., 2019b). With plant-based feeding being a relatively novel trend and the proportion of pets being fed plant-based diets being low (Dodd et al., 2019b), it is possible that adverse outcomes have occurred, but simply have not yet been reported.

In most US states, the AAFCO nutritional and labelling model guidelines are adopted by State Feed Control Officials and all pet foods sold within that state must register products with consideration of the recommendations. In Canada, pet food falls under federal regulation, with labelling and advertising regulated by the Consumer Packaging and Labelling Act (Canada, 2019b) and the Competition Act (Canada, 2019a). There is no enforcement through product registration, which includes typical analytical results, regarding the nutrient content of pet food products manufactured within the country, with the exception of federal safeguards for specific risk materials outlined in the Safe Food for Canadians Regulations (Canada, 2019c). Nonetheless, the Pet Food Association of Canada (PFAC) (PFAC, 2015), a voluntary industry association, requires pet food manufacturer and company members to adopt the AAFCO model bill. Products being imported into Canada must meet the Canadian importation requirements, which primarily focus on the Consumer Acts aforementioned. Pet foods produced and sold exclusively within Canada have been suggested to potentially be at risk of inadequate formulation and misleading labelling (Burdett et al., 2018). Similar findings have been reported

within the USA, with significant differences measured between labelled and analysed nutrient content (Hill et al., 2009). This is consistent with the findings reported here, with all foods imported into Canada meeting Canadian regulatory requirements, but two products manufactured and sold exclusively within Canada did not do so.

5.6 Conclusions

The findings of the present study suggest that formulation of plant-based foods meeting all nutrient requirements of dogs and cats for all life stages are possible, though current industry practices sometimes fall short of published nutritional recommendations. Concern for the provision of essential nutrients warrants consideration and testing of products for these common nutrients of concern and/or evaluating their status in pets fed plant-based may be justified. Inclusion of a statement of caloric content is a requirement to meet AAFCO labelling guidelines and would improve compliance of most commercial plant-based pet foods.

5.7 References

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5.8 Tables

Table 5.1.: Measured nutrient content in 20 plant-based canine foods commercially available in Canada and labeled for adult or all life stages compared to the AAFCO and FEDIAF recommended essential nutrient concentrations for canine adult maintenance on a dry matter basis (unit per 100 g dry matter).

Nutrient (Unit/100 g DM)	Median Range		AAFCO *	FEDIAF	
				95 kcal/kg 0.75 **	110 kcal/kg 0.75 ***
Crude protein (g)	27.6	17.3–36.6	18.0	21.00	18.00
Arginine (g)	1.62	1.03–2.57	0.51	0.60	0.52
Histidine (g)	0.60	0.36–0.76	0.19	0.27	0.23
Isoleucine (g)	1.40	0.88–1.65	0.38	0.53	0.46
Leucine (g)	2.41	1.49–5.26	0.68	0.95	0.82
Lysine (g)	1.26	0.59–2.09	0.63	0.46	0.42
Methionine (g)	0.52	0.22–1.50	0.33	0.46	0.40
Methionine + Cystine (g)	0.83	0.25–1.97	0.65	0.88	0.76
Phenylalanine (g)	1.48	1.03–2.24	0.45	0.63	0.54
Phenylalanine + Tyrosine (g)	2.56	1.61–3.89	0.74	1.03	0.89
Taurine (g)	0.11	0.00–0.21	n/a		
Threonine (g)	1.30	0.85–1.56	0.48	0.60	0.52
Tryptophan (g)	0.29	0.15–0.38	0.16	0.20	0.17
Valine (g)	1.53	0.96–1.90	0.49	0.68	0.59
Crude fat (g)	13.8	8.5–25.1	5.5		
Linoleic acid (g)	6.41	1.40–17.88	1.1	1.32	1.53
ALA (g)	0.70	0.16–2.13	n/a		
Arachidonic acid (g)	0.01	0.00–0.03	n/a		
EPA + DHA (g)	0.00	0.00–0.05	n/a		
GLA (g)	0.01	0.00–0.07	n/a		
Calcium (g)	1.07	0.38–1.90	0.5–2.5	0.58–2.50	0.50–2.50
Phosphorus (g)	0.8	0.5–1.5	0.4–1.6	0.46–1.6	0.4–1.6
Ca:P ratio (g)	1.3	0.8–2.5	1:1–2:1		
Potassium (g)	1.11	0.73–1.72	0.6	0.58	0.50
Sodium (g)	0.34	0.02–1.27	0.08	0.12	0.10
Chloride (g)	0.93	0.09–2.47	0.12	0.17	0.15
Magnesium (g)	0.15	0.10–0.23	0.06	0.08	0.07
Iron (mg)	22.76	10.71–75.04	4.0	4.17–68.18 (L)	3.60–68.18 (L)
Copper (mg)	2.34	0.89–5.47	0.73	0.83–2.80 (L)	0.72–2.80 (L)
Manganese (mg)	4.13	1.39–7.39	0.50	0.67–17.00 (L)	0.58–17.00 (L)
Zinc (mg)	15.52	4.46–35.93	8.0	8.34–22.70 (L)	7.20–22.70 (L)
Vitamin A (IU)	1001	274–3973	500–25,000	702.00–40,000	606.00–40,000
Vitamin D2 (IU)	0	0–152	n/a		

Vitamin D3 (IU)	73	0–172	n/a		
Total vitamin D (IU)	91	0–172	50–300	63.90–227.00 (L)	55.20–227.00 (L)
Vitamin B12 (mg)	0.00434	<u>0.0000–</u> <u>0.69074</u>	0.0028	0.00387	0.00335

* AAFCO nutrient profile for adult maintenance of dogs (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for adult maintenance of sedentary dogs, for an expected daily energy intake of 95 kcal/kg0.75 (minimum or minimum–maximum) (FEDIAF, 2020). *** FEDIAF recommendations for adult maintenance of active or working dogs, for an expected daily energy intake of 110 kcal/kg0.75 (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range for dogs with an energy intake of 95 kcal/kg0.75. Underlined values are outside of the FEDIAF recommended range for dogs with an energy intake of 110 kcal/kg0.75. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation, (L) = Legal limit.*

AAFCO nutrient profile for adult maintenance of dogs (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for adult maintenance of sedentary dogs, for an expected daily energy intake of 95 kcal/kg0.75 (minimum or minimum–maximum) (FEDIAF, 2020). *** FEDIAF recommendations for adult maintenance of active or working dogs, for an expected daily energy intake of 110 kcal/kg0.75 (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range for dogs with an energy intake of 95 kcal/kg0.75. Underlined values are outside of the FEDIAF recommended range for dogs with an energy intake of 110 kcal/kg0.75. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation, (L) = Legal limit.

Table 5.2.: Measured nutrient content in seven plant-based canine foods commercially available in Canada and labeled for puppies or all life stage compared to the AAFCO and FEDIAF recommended essential nutrient concentrations for canine growth on dry matter basis (unit per 100 g dry matter).

Nutrient (Unit Per 100 g)	Median Range		AAFCO *	FEDIAF	
				Early Growth **	Late Growth ***
Crude protein (g)	32.66	28.75–36.64	22.5	25.00	20.00
Arginine (g)	1.69	1.49–1.83	1.0	0.82	0.74
Histidine (g)	0.62	0.57–0.75	0.44	0.39	0.25
Isoleucine (g)	1.41	1.21–1.65	0.71	0.65	0.50
Leucine (g)	2.39	2.05–5.26	1.29		0.80
Lysine (g)	1.30	0.88–1.81	0.90	0.88–2.80	0.70–2.80
Methionine (g)	0.56	0.41–1.50	0.35		0.26
Methionine + Cystine (g)	0.86	0.59–1.97	0.70		0.53
Phenylalanine (g)	1.51	1.23–2.24	0.83	0.65	0.50
Phenylalanine + Tyrosine (g)	2.50	2.13–3.89	1.30		1.00
Taurine (g)	0.14	0.00–0.20	NR		
Threonine (g)	1.38	1.14–1.53	1.04	0.81	0.64
Tryptophan (g)	0.31	0.26–0.38	0.20	0.23	0.21
Valine (g)	1.54	1.37–1.90	0.68		0.56
Crude fat (g)	13.87	8.54–24.48	8.5		
Linoleic acid (g)	9.04	2.35–13.89	1.3	1.30–6.50	1.30
ALA (g)	0.70	0.17–2.13	0.08		
Arachidonic acid (g)	0.01	0.011–0.026	NR	0.030	
EPA + DHA (g)	0.00	0.00–0.01	0.05		
GLA (g)	0.03	0.00–0.05	NR		
Calcium (g)	1.12	0.48–1.45	1.2–2.5 a 1.2–1.8 b	1.00–1.60	0.80 a–1.80 1.00 b–1.80
Phosphorus (g)	0.88	0.58–1.12	1.0–1.6	0.90	0.70
Ca:P ratio	1.3	0.8–1.4	1:1–2:1	1:1–1.6:1	1:1–1.8:1 a 1:1–1.6:1 b
Potassium (g)	1.21	0.73–1.72	0.6	0.44	
Sodium (g)	0.36	0.29–0.69	0.3	0.22	
Chloride (g)	1.12	0.23–1.41	0.45	0.33	
Magnesium (g)	0.18	0.10–0.67	0.06	0.04	
Iron (mg)	41.53	12.77–61.64	8.8	8.80–68.18 (L)	
Copper (mg)	3.25	1.43–3.49	1.24	1.10–2.80 (L)	
Manganese (mg)	4.83	1.39–8.26	0.72	0.56–17.00 (L)	
Zinc (mg)	15.61	9.32–35.93	10.0	10.00–22.70 (L)	
Vitamin A (IU)	965.78	423.55– 2319.29	500.0– 25,000.0	500.00–40,000	

Vitamin D2 (IU)	0	0–62	NR		
Vitamin D3 (IU)	128	0–165	NR		
Vitamin D (IU)	137.78	0–190.04	50.0–300.0	50.00–227.00 (L)	55.20–227.00 (L)
Vitamin B12 (mg)	0.00424	<u>0.00000–</u> <u>0.69074</u>	0.00280		

* AAFCO nutrient profile for puppy growth (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for growth of puppies up to 14 weeks of age (minimum or minimum–maximum) (FEDIAF, 2020). * AAFCO nutrient profile for puppy growth (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for growth of puppies up to 14 weeks of age (minimum or minimum–maximum) (FEDIAF, 2020). *** FEDIAF recommendations for puppies over 14 weeks of age (minimum or minimum–maximum). a. Non-large breed puppies (predicted adult weight up to 31.75 kg [AAFCO] or 15 kg [FEDIAF]). b. Large breed puppies (predicted adult weight over 31.75 kg [AAFCO] or 15 kg [FEDIAF]). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range for early growth. Underlined values are outside of the FEDIAF recommended range for late growth. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation.

Table 5.3.: Measured nutrient content in eight plant-based feline foods commercially available in Canada and labeled for adult or all life stage compared to the AAFCO and FEDIAF recommended essential nutrient concentrations for feline adult maintenance on a dry matter basis (unit per 100 g dry matter).

Nutrient (Unit Per 100 g)	Median Range		AAFCO *	FEDIAF	
				75 kcal/kg ^{0.67} **	100 kcal/kg ^{0.67} ***
Crude protein (g)	31.61	29.16–35.31	26.0	33.30	25.00
Arginine (g)	1.59	1.27–2.45	1.04	1.30	1.00
Histidine (g)	0.63	0.40–0.75	0.31	0.35	0.26
Isoleucine (g)	1.40	0.81–1.61	0.52	0.57	0.43
Leucine (g)	2.39	1.40–4.30	1.24	1.36	1.02
Lysine (g)	1.26	0.88–2.07	0.83	0.45	0.34
Methionine (g)	0.47	0.27–0.85	0.20–1.5	0.23	0.17
Methionine + Cystine (g)	0.68	0.42–1.16	0.40	0.45	0.34
Phenylalanine (g)	1.54	0.98–1.87	0.42	0.53	0.40
Phenylalanine + Tyrosine (g)	2.52	1.55–3.19	1.53	2.04	1.53
Taurine (g)–extruded	0.09	0.01–0.21	0.10	0.13	0.10
Taurine (g)–canned	0.13	0.00–0.14	0.20	0.27	0.20
Threonine (g)	1.30	0.73–1.46	0.73	0.69	0.52
Tryptophan (g)	0.33	0.19–0.40	0.16–1.7	0.17	0.13
Valine (g)	1.59	0.94–1.82	0.62	0.68	0.51
Crude fat (g)	10.3	8.5–14.6	9.0		
Linoleic acid (g)	5.64	1.40–15.80	0.6	0.67	0.50
ALA (g)	0.68	0.17–1.83	NR		
Arachidonic acid (g)	0.009	0.006–0.026	0.02	0.008	0.006
EPA + DHA (g)	0.00	0.00–0.01	NR		
GLA (g)	0.01	0.00–0.04	NR		
Calcium (g)	0.84	0.48–1.42	0.6	0.53	0.40
Phosphorus (g)	0.79	0.56–1.07	0.5	0.35	0.26
Calcium to phosphorus ratio	1.1	0.8–1.4	NR	1:1–2:1	
Potassium (g)	1.06	0.68–1.72	0.6	0.80	0.60
Sodium (g)	0.33	0.17–0.73	0.2	0.10	0.08
Chloride (g)	0.95	0.38–1.46	0.3	0.15	0.11
Magnesium (g)	0.17	0.09–0.19	0.04	0.05	0.04
Iron (mg)	26.47	10.49–42.24	8.0	10.70–68.18 (L)	8.00–68.18 (L)
Copper (mg)	2.49	1.43–3.44	0.5	0.67–2.80 (L)	0.50–2.80 (L)
Manganese (mg)	4.11	1.31–6.72	0.76	0.67–17.00 (L)	0.50–17.00 (L)

Zinc (mg)	18.69	8.90–35.93	7.5	10.00–22.70 (L)	7.50–22.70 (L)
Vitamin A (IU)	907.5	251.4–2319.3	333.2– 33,330.0	444.00– 40,000	333.00–40,000
Vitamin D2 (IU)	0	0–26	NR		
Vitamin D3 (IU)	103	0–155	NR		
Vitamin D (IU)	111.4	0–154.5	28.0–3008.0	33.30–227 (L)	25.00–227 (L)
Vitamin B12 (mg)	0.00587	<u>0.00000–</u> 0.71690	0.0020	0.00235	0.00176

* AAFCO nutrient profile for adult maintenance of cats (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for adult maintenance of indoor and/or neutered cats, for an expected daily energy intake of 75 kcal/kg0.67 (minimum or minimum–maximum) (FEDIAF, 2020). *** FEDIAF recommendations for adult maintenance of active cats, for an expected daily energy intake of 100 kcal/kg0.67 (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range for cats eating 75 kcal/kg0.67. Underlined values are outside of the FEDIAF recommended range for cats eating 100 kcal/kg0.67. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation, (L) = Legal limit.* AAFCO nutrient profile for adult maintenance of cats (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for adult maintenance of indoor and/or neutered cats, for an expected daily energy intake of 75 kcal/kg0.67 (minimum or minimum–maximum) (FEDIAF, 2020). *** FEDIAF recommendations for adult maintenance of active cats, for an expected daily energy intake of 100 kcal/kg0.67 (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range for cats eating 75 kcal/kg0.67. Underlined values are outside of the FEDIAF recommended range for cats eating 100 kcal/kg0.67. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation, (L) = Legal limit.

Table 5.4: Measured nutrient content in five plant-based feline foods commercially available in Canada and labeled for growth or all life stages compared to the AAFCO and FEDIAF recommended essential nutrient concentrations for feline growth on a dry matter basis (unit per 100 g dry matter).

Nutrient (unit per 100 g)	Median	Range	AAFCO *	FEDIAF **
Crude protein (g)	31.61	29.16–35.31	30.0	28.00
Arginine (g)	1.54	1.27–2.45	1.24	1.07–3.50
Histidine (g)	0.61	0.40–0.75	0.33	
Isoleucine (g)	1.39	0.81–1.61	0.56	0.54
Leucine (g)	2.38	1.40–2.77	1.28	
Lysine (g)	1.14	0.88–2.07	1.20	0.85
Methionine (g)	0.47	0.36–0.85	0.62–1.5	0.44–1.30
Methionine + Cystine (g)	0.60	0.42–1.16	1.10	0.88
Phenylalanine (g)	1.48	0.98–1.71	0.52	0.50
Phenylalanine + Tyrosine (g)	2.49	1.55–2.79	1.92	1.91
Threonine (g)	1.24	0.73–1.40	0.73	0.65
Tryptophan (g)	0.33	0.19–0.38	0.25–1.7	0.16–1.70
Valine (g)	1.54	0.94–1.82	0.64	0.64
Taurine–extruded (g)	0.19	0.01–0.21	0.10	
Taurine–canned (g)	0.13	0.00–0.14	0.20	0.25
Crude fat (g)	9.56	8.50–14.57	9.00	
Linoleic acid (g)	5.65	1.40–15.80	0.6	0.55
α -linolenic acid (g)	0.68	0.17–1.83	0.02	
Arachidonic acid (g)	0.01	0.01–0.03	0.02	
EPA + DHA (g)	0.00	0.00–0.01	0.012	0.01
GLA (g)	0.02	0.00–0.04	NR	
Calcium (g)	0.96	0.48–1.39	1.00	
Phosphorus (g)	0.76	0.56–1.07	0.8	0.84
Calcium to phosphorus ratio	1.24	0.83–1.39	NR	1:1–1.5:1
Potassium (g)	1.44	0.68–1.72	0.60	
Sodium (g)	0.33	0.29–0.73	0.2	0.16
Chloride (g)	1.03	0.79–1.46	0.3	0.24
Magnesium (g)	0.18	0.09–0.19	0.08	0.05
Iron (mg)	24.59	10.49–41.39	8.00	8.00–68.18
Copper–extruded (mg)	3.14	1.46–3.44	1.5	1.00–2.80
Copper–canned (mg)	2.15	1.43–2.83	0.84	
Manganese (mg)	4.10	1.31–5.55	0.76	1.00–17.00
Zinc (mg)	21.46	8.90–35.93	7.50	7.50–22.70
Vitamin A (IU)	959.5	251.4–2319	666.8–33,333	900–33,333
Ergocalciferol (IU)	0.00	0.00–0.00	NR	
Cholecalciferol (IU)	115	0–155	NR	
Vitamin D (IU)	114.7	0–154.5	28.0–3008	28.00–227
Vitamin B12 (mg)	0.00464	0.00000–0.71690	0.002	0.00180

*AAFCO nutrient profile for kitten growth (minimum or minimum–maximum) (AAFCO, 2020).
** FEDIAF recommendations for kitten growth (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation.*AAFCO nutrient profile for kitten growth (minimum or minimum–maximum) (AAFCO, 2020). ** FEDIAF recommendations for kitten growth (minimum or minimum–maximum) (FEDIAF, 2020). Bolded values are outside of the AAFCO recommended range. Italicized values are outside of the FEDIAF recommended range. AAFCO = Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, NR = No recommendation.

Table 5.5: Percentage (%) of label guaranteed fulfilled for each nutrient of the guaranteed analysis in plant-based canine and feline foods commercially available in Canada (n = 26).

	Energy		Crude Protein (min)		Crude Fat (min)		Crude Fibre (max)		Moisture (max)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Canine extruded	114	102–123	104	82–118	97	66–150	62	22–87	81	56–115
Canine wet	107	102–112	120	92–124	138	21–177	32	21–140	93	90–102
Feline extruded	109	109–109	102	97–119	78	65–86	69	40–102	87	64–120
Feline wet	NR	NR	104	99–106	58	43–82	24	21–27	96	93–98

Min = minimum, Max = maximum, NR = value not reported on product label.

Table 5.6: Provision of key essential nutrients from non-animal sources.

Nutrient	Major source(s)
Methionine	Synthetic free amino acid (AAFCO, 2020)Synthetic free amino acid (AAFCO, 2020)
Taurine	Synthetic (AAFCO, 2020, Pion et al., 1987)Synthetic (AAFCO, 2020, Pion et al., 1987)
Arachidonic acid	Algae extract (Garcia-Vaquero and Hayes, 2016, Dawczynski et al., 2007, van Ginneken et al., 2011)
EPA	Algae extract (Garcia-Vaquero and Hayes, 2016, Dawczynski et al., 2007, van Ginneken et al., 2011)
DHA	Algae extract (Garcia-Vaquero and Hayes, 2016, van Ginneken et al., 2011)Algae extract (Garcia-Vaquero and Hayes, 2016, van Ginneken et al., 2011)
Calcium	Inorganic minerals (AAFCO, 2020)Inorganic minerals (AAFCO, 2020)
Phosphorus	Inorganic minerals (AAFCO, 2020) *Inorganic minerals (AAFCO, 2020) *
Vitamin D	Ergocalciferol (AAFCO, 2020); lichen-derived cholecalciferol (Boland et al., 2003, Jäpelt and Jakobsen, 2013) Ergocalciferol (AAFCO, 2020); lichen-derived cholecalciferol (Boland et al., 2003, Jäpelt and Jakobsen, 2013)

* Digestibility and bioavailability of inorganic phosphorus should be considered (Lineva et al., 2019, Alexander et al., 2019) and bioavailability of inorganic phosphorus should be considered (Lineva et al., 2019, Alexander et al., 2019).

6 CHAPTER SIX: Body composition, bone mineralization and vitamin D status of dogs fed a plant- or meat-based diet

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6.1 Abstract

Background: Dogs are considered omnivores or facultative carnivores based on evolutionary adaptation to a diet consisting of a large proportion of tissue from prey or scavenged animal carcasses. Few feeding trials to evaluate nutritional suitability of plant-based diets to meet canine requirements have been published, and efficacy of vitamin D2 to maintain canine serum vitamin D levels has not been determined.

Objectives: This study compared indices of wellness, body composition and vitamin D metabolism in dogs fed a plant-based or meat-based diet.

Methodology: A longitudinal trial including 61 dogs was performed, with 31 dogs fed an experimental extruded plant-based (PLANT) diet and 30 dogs fed a commercial extruded meat-based (MEAT) diet for three months. Dogs were screened for health and wellness via veterinary examination, CBC and serum biochemistry prior to enrolment into the study. At baseline and exit timepoints, examination, CBC and serum biochemistry were repeated, urinalysis was performed, and body composition was measured by dual-energy x-ray absorptiometry. Blood was also collected for serum amino acid concentrations and vitamin D profiling.

Results: Few differences were detected between dogs fed the PLANT or MEAT diet, with all dogs maintaining consistent body composition throughout the study. Of note, dogs maintained on the PLANT diet demonstrated a slight reduction in platelet count, lower serum levels of branched-chain amino acids, creatinine, blood urea nitrogen and cholesterol, though all blood values stayed within normal reference range. Dogs fed the PLANT diet demonstrated a shift

from vitamin D₃ metabolites to vitamin D₂ metabolites, with total vitamin D analogues and bone mineral content and density unaffected by diet.

Conclusion: Dogs fed a plant-based diet for 3 months maintained reference level health parameters. Vitamin D₂ was efficacious at maintaining serum total vitamin D concentrations and bone mineralization. Further research is required to elucidate whether plant-based diets may impact branched chain amino acid metabolism, protein metabolism and renal function, platelet production and function, and long-term maintenance.

6.2 Introduction

The modern domestic dog, *Canis lupus familiaris*, descend from wolves (*Canis lupus*), but deviate from their wild-type ancestor as a result of coadaptation with humans (Freedman et al., 2014). Both dogs and wolves fall within the family Canidae, order Carnivora (Wozencraft, 2005), and are considered omnivores or facultative carnivores based on their feeding strategies and gastrointestinal anatomy and physiology (Bosch et al., 2015). Though the diets of free-ranging wolves and dogs are characterised by intake of prey (Zlatanova et al., 2014), the domestic dog adapted to consume a diet richer in carbohydrates (Axelsson et al., 2013), and show some preference for starch as a dietary energy source (Hall et al., 2018). Domestication led dogs to rely on human counterparts for their dietary intake. In many communities where dogs are kept as companions, their diet often includes foods specifically prepared for pets (Dodd et al., 2020a, Seneviratne et al., 2016, Bekova and Makenov, 2018, Thomson et al., 2008). Most commercial dog foods contain animal ingredients; indeed, the pet food industry relies heavily on by-products from the slaughter of animals for human consumption (Meeker and Meisinger, 2015, Swanson et al., 2013). Homemade foods for dogs also typically have a high inclusion of animal-derived ingredients, due to the perception that dogs require meat as a main component of their diet (Remillard, 2008, Stockman et al., 2013).

In contrast, some dog owners elect to feed their dog an exclusively plant-based diet, devoid of animal-derived ingredients (Dodd et al., 2019b). Nevertheless, in North America, the industry guidelines for pet food formulation are based on profiles of indispensable nutrients, not ingredients (AAFCO, 2018), and commercial plant-based diets have been produced meeting all of these nutrient recommendations (Dodd et al., 2021b). Provision of some key essential

nutrients, however, has proven challenging as methionine, methionine + cystine, taurine, calcium, phosphorus, and vitamin D content have been reported below industry recommendations (Dodd et al., 2021b, Kanakubo et al., 2015, Zafalon et al., 2020). Given these findings, it would be expected that dogs fed plant-based diets may be at increased risk of nutritional deficiencies and clinical manifestations of irregularities in amino acid, calcium, phosphorus, and vitamin D metabolism.

A dearth of knowledge exists on the link between plant-based diets and canine health. Previous focus has been on physical examination, haematological and serum biochemical characteristics (Brown et al., 2009, Semp, 2014), and/or some additional serum nutrient evaluations (Semp, 2014). Impacts on digestibility, fecal microbiota, and glycemic and insulinemic responses have recently been investigated in dogs (El-Wahab et al., 2021, Hankel et al., 2020, Rankovic et al., 2020). To the authors' knowledge, body composition changes in response to plant-based diet have not been investigated. Given the differences in amino acid provision from plant- or animal-derived protein sources (Dodd et al., 2018, Reilly et al., 2020, Yamada et al., 1987), it is possible that increased mobilization of amino acids from body stores could evoke changes in lean tissue (Wakshlag et al., 2003). While suitable non-animal-derived dietary sources of calcium and phosphorus are plentiful (AAFCO, 2018), bone metabolism in dogs fed plant-based diets may be affected by the source of dietary vitamin D (Weidner and Verbrugghe, 2016). In most mammals, vitamin D can be produced in the skin with sufficient UV light exposure, though it has been demonstrated that endogenous synthesis is minimal in dogs (How et al., 1994). Dietary vitamin D can be provided as two different compounds: cholecalciferol, or vitamin D₃, predominantly derived from animal sources; or ergocalciferol,

vitamin D₂, derived from non-animal sources (NRC, 2006). In cats, dietary vitamin D₂ was less effective compared to vitamin D₃ in maintaining serum vitamin D (Morris, 2002a). Based on existing research, in dogs, the efficacy of D₂ in maintaining serum vitamin D has not been well known established. If vitamin D metabolism were impaired, this could impact bone mineral content and bone mineral density (Corbee, 2020).

The objectives of this study were thus to compare body composition, serum amino acid concentrations, bone mineralization and serum markers of vitamin D metabolism in dogs maintained on either a plant- or animal-based diet.

6.3 Methodology

This study was conducted at the University of Guelph with the approval of the Research Ethics Board (Research Ethics Approval number 19-02-036) and Animal Care Committee (Animal Use Protocol #4129), ensuring the research protocol was in line with institutional, provincial, and national guidelines and policies for humans participating in research as well as for the care and use of animals in research. The sample size chosen for this study was based on previous vitamin D-related nutrition studies which have utilized a similar number of dogs (Gerber et al. 2003, Gow et al. 2011, Hazewinkel and Tryfonidou, 2002, Osuga et al. 2015, Spoo et al. 2015, Weidner et al. 2017) and justified with sample size calculations based on previously reported average bone mineral density of dogs (Santarossa et al., 2020). For sample size calculation, confidence was set at 0.95 and power to 0.8. The calculated minimum sample size per treatment group was 27 dogs, with an expectation of 10% dropout, resulting in a target sample size of 30 dogs per group.

6.3.1 Participant Enrolment

Recruitment for trial participants was initiated June 3, 2019 and terminated July 5, 2020. The primary researcher (SD) was responsible for recruitment and enrolling of all participants. An eSurvey was designed on the Qualtrics (Provo, Utah, USA) platform to collect data regarding patient suitability. This survey was promoted locally through printed advertisements around the university campus and surrounding community, and shared virtually on social media to local dog-related groups. The first page of the questionnaire provided information on the study and served to provide informed consent for participation in the recruitment survey. Participants could only continue the questionnaire if they confirmed they understood their rights regarding participation in the survey and ability to exit the survey at any time. Potential participants were informed that questions were required to be answered honestly and sensitive personal details would be accessible to the research team, as well as provided with a description of the expectations of their participation. Expectations of participation included that the respondents would bring their dogs for a total of three visits to the Ontario Veterinary College Central Clinical Research Facility, would feed the study diet and follow the study protocol, and consent to their dogs undergoing physical examinations, blood draws and dual-energy x-ray absorptiometry (DEXA). There were no incentives for study participation, though benefits included complimentary veterinary examination, blood analyses, urinalysis, DEXA measurement of body composition and provision of the study diet for four months – one month of adaptation and then three months of trial. Information collected included: dog age, sex, weight and body condition score (BCS, evaluated by selection of WSAVA BCS image (WSAVA, 2013b) most closely resembling their dog, ordered randomly to attempt to avoid bias); the main diet fed;

details of treats and snacks; provision of supplements and medications; number of adults in household; presence of children in the household and interaction between children and dog feeding; presence of other pets in the household and access to other pets' food, dog housing (indoors vs outdoors); feeding management; access to unmonitored food sources; dog activity; and dog medical history. Dogs were excluded from consideration if they were reproductively intact, weighed less than 5kg, had an owner-reported BCS > 5, fed a homemade or raw diet, housed outdoors without supervision, had access to unmonitored food sources, had current medical problems, received medication other than parasite preventatives, had previous medical problems that could affect them currently (e.g. previously diabetic but in remission, recurrent ear infections, etc.) or had known dietary allergies. Dogs in households without children or other pets were prioritised for inclusion in the study as it was considered that the dietary intakes of dogs with potential access to other pets' foods or unattended with children would potentially be less reliable than those living in households with adult humans only.

A total of 569 surveys were undertaken and 280 were completed in full. Of these, 132 survey participants met inclusion criteria and were selected for invitation into the study. An email was sent with more detailed information regarding participation in the study and invitation to attend an enrollment appointment. Replies were received from 78 potential participants, with 72 enrollment appointments scheduled for 87 dogs (some participants had more than one dog).

Enrollment appointments involved discussion of the study procedures, collection of a signed informed consent form for participation in the study and a wellness examination conducted by a veterinarian. In order to minimize variability, the primary researcher (SD)

assessed all dogs at all visits. The examination included collection of medical and dietary history, a physical examination, bodyweight (BW) was measured using a weigh scale, and blood was collected for complete blood count (CBC) and serum biochemistry. Figure 6.1 depicts recruitment and enrollment. Participating dog characteristics are in Appendix II.

6.3.2 Diets

Two isocaloric diets, one commercial animal-based diet^a (MEAT) and one experimental plant-based diet (PLANT), were formulated by the research team to meet or exceed nutrient recommendations for adult maintenance (AAFCO, 2018). The diets were packaged into identical sealed white bags and labelled as Control and Diet 2 for the MEAT or Diet 1 for the PLANT. The investigators and participants were blinded to the identity of the diets. Diet identities were kept by a third person employed at the University of Guelph, who was not involved in data collection, statistical analysis and data interpretation, until statistical analyses were complete.

Nutrient analyses were performed on the diets post-manufacturing (Appendix II). Proximate analyses (moisture, protein, fat, ash, crude fibre) and minerals were performed at a commercial laboratory (Bureau Veritas, Mississauga, Ontario, Canada). Moisture, crude protein, crude fat, ash and minerals were measured by AOAC methodology (AOAC 992.15, 996.06, 923.03, 084.27, 935.29). Crude fibre was measured by AOCS Ba 6a-05 and iodine by neutron activation. Carbohydrate content, approximated as nitrogen-free extract (NFE), was calculated by the equation: $NFE = 100 - \text{crude protein} - \text{crude fat} - \text{crude fibre} - \text{ash}$ (AAFCO, 2018). Metabolizable energy was calculated as calories per kg as fed, using the equation: $ME \text{ (kcal/kg)} = 10[(3.5 \times \text{crude protein}) + (8.5 \times \text{crude fat}) + (3.5 \times \text{NFE})]$, with crude protein, fat and NFE values

as g/100g as fed (AAFCO, 2018). Samples underwent hydrolysis, oxidized hydrolysis and alkaline hydrolysis before amino acid profiling was performed by ultra high performance liquid chromatography coupled with mass spectrometry (UPLC-MS) (Cargo-Froom et al., 2019). Lastly, vitamins were measured at a commercial laboratory (DSM, Belvedere, New Jersey, USA). Vitamins A and D were measured by AOAC methodology (AOAC 974.29.45.102 mod and 2011.12 (modified), respectively), B vitamins were measured by QDa (Waters, 2008), with the exception of biotin while cobalamin was outsourced to a second commercial laboratory (Covance, Princeton, New Jersey, USA).

Food quantity was calculated based on the dog's current dietary intake to match calories and maintain current BW and a gram scale was provided to each household to precisely measure out the recommended quantity of food per day. Participants were instructed not to feed their dogs any other food for the duration of the study, with exceptions for some pieces of fruit or vegetables or plant-based treats, which were required to be recorded. Participants were given a list of treats that could be fed for the duration of the study (plant-based treats, without added micronutrients); an acceptable treat dose was given for each dog to avoid exceeding 10% of their daily energy intake from sources other than the trial diet.

6.3.3 Diet Trial

Originally, the diet trial was scheduled to be completed before the end of September, 2020. However, as a result of the global COVID-19 pandemic, the University of Guelph closed down for research and the diet trial was paused in March, 2020, until research was allowed to resume in July, 2020 with implementation of appropriate public health protocols. During the

lockdown, dogs participating in the trial were maintained on the diet, either MEAT or PLANT depending on their phase of the study (acclimatisation or trial) and experimental group, in order to allow immediate resumption of data collection when the facilities were made available again. This resulted in variability in the duration of dogs participating in the trial, with some dogs consuming the baseline diet for more than 4 weeks, and some dogs consuming the experimental diet for more than 12 weeks.

Figure 6.2 depicts the timeline of the trial. An initial adaptation period of 4 weeks was performed between the screening and baseline appointments to ensure all dogs started the trial from the same diet and mitigate variation due to differences in pre-trial diets. Although all dogs enrolled in the study were fed commercial diets prior to their participation, there was variation of ingredients and nutrient profiles of these diets, including six dogs previously fed commercial plant-based diets. During the adaptation period, all dogs were fed MEAT, packaged in bags labelled “Control”. Upon completion of the 4-week adaptation phase, the dogs were scheduled baseline appointments and randomly allocated to treatment groups (MEAT or PLANT) prior to the baseline evaluation. Baseline evaluations consisted of a veterinary wellness examination (medical and dietary history, physical examination, CBC, serum biochemistry and urinalysis), body composition analysis by DEXA, and blood collection for vitamin D and amino acid profiles. Bodyweight, BCS, CBC, serum biochemistry and urinalysis were analyzed as measures of general health and wellness, as typically performed for routine veterinary wellness examinations (AAHA, 2021). Participants were advised to fast their dogs for 12 hours prior to the appointment. Bodyweight, BCS, CBC, serum biochemistry and urinalysis were analyzed as measures of general health and wellness, as typically performed for routine veterinary wellness

examinations (AAHA, 2021). Participants were advised to fast their dogs for 12 hours prior to the appointment.

After the baseline evaluation, dogs were transitioned to their respective experimental diet – either continuing on the MEAT, labelled as “Diet 2” or starting the PLANT, labelled as “Diet 1”. Dogs were fed the trial diets for 12 weeks, then returned at the end of the trial for their exit evaluation. Exit evaluations were the same as the baseline evaluations. Throughout the study, including the 4-week adaptation and the 12-week experimental period, participants were asked to record quantity of food offered, quantity of food eaten, amount and type of snacks or treats provided, frequency of defaecation, faecal condition score, BCS, BW, duration of walks or play activity, and any other notable events in a daily diary. Faecal condition score and BCS charts were provided (WSAVA, 2013b, Anonymous, 2017).

6.3.4 Body Composition Analysis

For determination of body composition, dogs were sedated with dexmedetomidine (2-10 ug/kg BW; Dexdomitor®, Zoetis, Parsipanny, New Jersey, USA) and butorphanol (0.2mg/kg BW; Torbugesic®, Zoetis, Parsipanny, New Jersey, USA) administered together in the same syringe, intravenously (IV) via the cephalic or lateral saphenous vein or intramuscularly (IM) in epaxial muscle, depending on the behaviour of the dog. If a top up of sedation was required during the procedure, only dexmedetomidine dose was repeated and administered IV, starting at 50% of the initial delivered dose.

Body composition was measured by DEXA (Prodigy Advance, GE Healthcare, Madison, Wisconsin, USA). Dogs were placed in ventral recumbency and positioned with forelimbs

extending caudally, adjacent to but not touching the body wall, and hindlimbs extending caudally away from the body, as previously described (Bjørnvad et al., 2017). Total Body scanning mode was utilized within the scanner's software (enCORE Version 16, GE Healthcare, Madison, Wisconsin, USA), with the software selecting thick, standard or thin settings according to the dog's BW. Body fat, lean soft tissue, bone mineral content (BMC) and total tissue were measured in grams, from which proportions of body fat (BF%), lean soft tissue (LST%) and bone (B%) were calculated. Proportions were calculated as tissue mass divided by total mass, multiplied by 100%. Bone mineral density was measured as radiation energy per pixel converted into area density, measured in g/cm² (Berger, 2002). Dogs were scanned in duplicate and values from the two scans were averaged. Upon completion of the body composition analysis, sedation was reversed with atipamezole (0.02-0.1 mg/kg IM; Antisedan, Zoetis, Parsippany, New Jersey, USA), administered IM in the epaxial muscles in a volume matching the administered dexmedetomidine volume. During sedation, heart rate, respiratory rate and temperature were monitored in each dog prior to and immediately after administering sedation, prior to and after moving and positioning dogs on the DEXA table, prior to, in between scans, and after completion of DEXA scanning, and prior to and after administering the sedation reversal until the dog recovered. Recovery was established once dogs were able to stand and ambulate unassisted. Sedation doses were recorded, and the same dosing as administered at the exit appointment was administered at the baseline appointment, as suitable.

6.3.5 Blood and Urine Collection

Blood analyses were performed at screening, baseline and exit timepoints. while urinalysis was performed at baseline and exit timepoints only.

At the screening evaluation, 6mL of blood were collected by venipuncture of the saphenous or cephalic vein using a 22G BD Vacutainer® needle into a 3mL BD lavender-top EDTA Vacutainer® tube and a 3mL BD red-top plain Vacutainer® tube. At the baseline and exit timepoints, blood was collected while the dog was sedated for body composition analysis. Once reversal (atipamezole) was administered, dogs were positioned in lateral recumbency for blood collection. Venipuncture was performed via the saphenous, or jugular vein, depending on dog size, using a 22G BD Vacutainer® needle (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Fifteen mL of blood was collected into five 3mL BD red top Vacutainer® blood collection tubes with no additives and 6mL was collected into two 3mL BD lavender top EDTA Vacutainer® blood collection tubes. After clotting for 30 minutes at room temperature, serum was separated from whole blood in plain red top tubes by centrifugation at 1,500G in a refrigerated centrifuge set to 4°C. Samples for same-day testing, including samples for CBC and serum biochemistry, were submitted immediately for analysis. Remaining sera aliquots were immediately frozen at -80°C in 1.5mL Eppendorf tubes and stored until analyzed.

At baseline and exit timepoints, dogs were walked prior to their examination and sedation, and free-catch urine collected into plain 12mL tubes. In a small number of dogs for which free catch urine collection was not possible prior to body composition analysis, urine was collected via ultrasound-guided (Clarius C3, 2-6 MHz frequency; Clarius, Vancouver, BC, Canada) cystocentesis performed after completion of DEXA scans. Urine collection method was recorded for each dog. Urine samples were submitted immediately for analysis.

6.3.6 Complete Blood Count, Serum Biochemistry and Urinalysis

Complete blood count, serum biochemistry and urinalyses were performed at the Animal Health Lab of the Ontario Veterinary College, University of Guelph (Guelph, Ontario, Canada) on the same day as sample collection. Complete blood count with machine differential was performed on whole blood mixed with EDTA using a Siemens ADVIA 2120 hematology analyzer (Siemens AG, Munich, Germany). Photometric serum biochemistry was analysed by Roche Cobas 6000 c501 (Roche Holding AG, Basel, Switzerland). Urinalysis was performed by Siemens Multistix urine dipstick read on the Siemens Clinitek Plus urine strip reader (Siemens AG, Munich, Germany), with a microscopic examination of the urine sediment by an experienced laboratory technician.

6.3.7 Serum Amino Acid Profiles

Amino acids were measured as described previously (Cargo-Froom et al., 2019). Briefly, serum samples were thawed and 100 μL of serum was deproteinated by adding 100 μL of 10% sulfosalicylic acid (Sigma Aldrich, Oakville, ON) solution and centrifuged using a Fisherbrand accuSpin Micro 17 (Thermo Fisher Scientific, Waltham, MA) at 12,000 rpm for 5 min. After centrifuging, 10 μL of supernatant was sampled for derivatization. Amino acid standards and serum were derivatized using an AccQ-Tag Ultra derivatization kit (Waters Corporation, Milford, MA, USA). Derivatized AA (1 μL injection volume) were separated in a column (2.1 \times 200 mm, 1.7 μL) maintained at 55 $^{\circ}\text{C}$ using UPLC (Waters Corporation, Milford, MA) with ultraviolet detection at a wavelength of 260 nm. Amino acid peak areas were integrated using Waters Empower 2 Software (Waters Corporation, Milford, MA, USA).

6.3.8 Vitamin D and Analogues

Frozen sera were shipped on dry ice to external analytical laboratories. Vitamin D profile, including 25-hydroxyvitamin D (25-OHD), parathyroid hormone (PTH) and ionized calcium was performed by the Veterinary Diagnostics Laboratory of the Michigan State University (Lansing, Michigan, USA). Analysis of 25-OHD and PTH were performed by commercial radioimmunoassays (Diasorin, Stillwater, Minnesota, USA; Scantibodies, Santee, California, USA), while ionized calcium was measured with an ion-sensitive electrode (NOVA 8 bioanalyzer, Nova Medical, Waltham, Massachusetts, USA).

Serum vitamin D analogues were analyzed by Heartland Assays (Ames, Iowa, USA). Vitamin D₂ (25(OH)D₂), vitamin D₃ (25(OH)D₃), calcitriol (1,25(OH)₂D₂, 1,25(OH)₂D₃), 24,25(OH)₂D₂ and 24,25(OH)₂D₃ were measured by liquid chromatography di-mass spectrometry (LC/MS/MS). Vitamin D₂ and D₃ as well as 24,25(OH)₂D₂ and 24,25(OH)₂D₃ were extracted and quantified by the same method. Serum samples along with standard curve and controls were precipitated with 0.2M ZnSO₄ then vortexed. Methanol was added and all samples were vortexed again. Then, d₃-vitamin D₃/d₃-25(OH)D₂/d₃-25(OH)D₃/d₆-24,25(OH)₂D₃ internal standards were added and vortexed. Hexanes were added to all samples and controls, then vortexed, followed by centrifugation. The organic layer was transferred and dried, then all standards, controls and samples were reconstituted with LCMS grade methanol and water containing 0.1% formic acid, before loading onto the auto-sampler for LC/MS/MS analysis (Agilent 1290 infinity HPLC coupled to Agilent 6400 MS/MS with ESI source).

For extraction and quantification of 1,25(OH)₂D, samples, controls and standards were first spiked with d6-1,25(OH)₂D₃ internal standard before protein precipitation. Samples and controls were purified by liquid-liquid extraction, followed by solid phase extraction. Samples, controls and standards were then derivatized using 4-phenyl-1,2,4-triazoline-3,5-dione, followed by a second solid phase extraction. Samples and controls were reconstituted in LC/MS/MS mobile phase before injection and analysis (Agilent 1290 HPLC system using a Zorbax Eclipse Plus C-18 column coupled to 6460 Agilent triple tandem MS/MS in positive ion mode using an ESI source).

6.3.9 Statistical analyses

All analyses were performed using commercial statistical software (StataIC, StataCorp, College Station, Texas, USA). Independent variables (dog sex, age, weight, BCS and season) were tested for normality of distribution using Shapiro-Wilk normality test and visual evaluation of frequency histograms and normal probability plots. Differences in distribution of independent variables (age, sex, BCS) between diet groups after randomization was tested using *t* test for parametric and Wilcoxon rank-sum test for non-parametric data. Dependent variables (CBC, biochemistry, urinalysis, body composition, serum amino acids, vitamin D metabolites) were tested for normality of distribution using Shapiro-Wilk normality test and visual evaluation of frequency histograms and normal probability plots. Non-parametric data were log transformed prior to analyses (blood cell counts, MCV, MPC, plateletcrit, CO₂, albumin, globulin, albumin:globulin, urea, glucose, conjugated bilirubin, ALP, steroid-induced ALP, GGT, ALT, CK, amylase, lipase, osmolality, all urinalysis variables, alanine, arginine, aspartate, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine,

tryptophan, tyrosine, 25(OH)D₂, 25(OH)D₃, total 25(OH)D, 1,25(OH)₂D₃, 24,25(OH)₂D₃, total 24,25(OH)₂D). Categorical data like urine colour and clarity were coded into ordinal scales from 0-3, with 0 being pale yellow or clear, respectively, and 3 being dark yellow or cloudy, respectively. Ordinal scales of 0-3 represented, negative, trace, and 1-3+ values reported by the laboratory for the presence of: protein, glucose, ketones, bilirubin, blood, epithelial cells, crystals, fat and bacteria. Blood cell counts of none, rare and 1-100+ cells per high power field were translated into ordinal scales of 0-6. Ordinal data were treated as non-parametric.

Differences in dependent variables between dogs of different age, sex and BCS were tested using ANOVA and post-hoc Tukey test, as indicated. Univariate analyses were performed via repeated measures mixed model, using residual maximum likelihood, with time and treatment as fixed effects and dog ID as the variable on which data were repeated. As differences were detected between groups at baseline for some dependent variables (serum leucine, lysine and valine, urine epithelial cells), baseline was included as a variable for all analyses. Post hoc analyses were performed by contrasting main effects and graphing the interactions. As multiple comparisons were made, the critical cut-off for P-values were adjusted to control for false discovery rate of less than or equal to 10% according to the Benjamini Hochberg procedure as recommended for bioinformatics data (Jafari and Ansari-Pour, 2019).

6.4 Results

6.4.1 Group randomization and trial completion

The independent variables considered for each group were sex, age, BW, BCS and the seasons during which the dog was participating in the study. After randomization, the

distribution of independent variables between groups did not differ (Supplementary Table 2). Breeds and breed mixes did not differ between the two groups (Supplementary Table 1). During the adaptation phase, when all dogs were fed the MEAT diet, five dogs were dropped from the study as they did not like or tolerate the food - one dog refused to consume it, two showed signs consistent with adverse food reaction, with one dog producing loose faeces while the other exhibited episodes of bilious vomiting, and two dogs developed anal gland complications. A further four dogs were discontinued from the study during the adaptation phase as a result of the COVID-19 forced lockdown and when the research project resumed the participants declined to continue. Of the 67 dogs completing the adaptation phase, 66 underwent baseline evaluations. One dog was excluded due to excessive weight gain during the adaptation period resulting in obese habitus. Of the 66 dogs completing baseline evaluations, 61 completed the trial and underwent exit evaluations. Three dogs were lost to follow-up during the trial, one specifically as a result of the COVID-related delays. One dog developed a urinary tract infection, was managed medically including prescription of a therapeutic diet and was discontinued from the study. Another dog developed gastric ulceration after administration of a non-steroidal anti-inflammatory, underwent medical management and was discontinued from the study.

6.4.2 General health and wellness

Bodyweight and BCS were consistent between groups and between timepoints. There were no differences in CBC or biochemistry variables between groups after baseline.

On CBC, dogs fed MEAT demonstrated an increase in red cell parameters, including red cell count (RBC), haemoglobin and haematocrit, while dogs fed PLANT showed a decrease in

platelet count and corresponding increase in mean platelet volume (MPV) (Table 6.1, Figure 6.3). Serum biochemistry revealed differences in electrolytes, urea, creatinine, cholesterol and osmolality (Table 6.2, Figure 6.4).

Urine transitional epithelial cells, fatty casts, fine and coarse granular casts were too infrequent for statistical analysis. In both groups, presence of bacteria in the urine increased slightly over time (Table 6.3). At the exit timepoint, epithelial cells were lower in the urine of dogs fed PLANT than dogs fed MEAT (Figure 6.5).

6.4.3 Body composition

Body composition was not affected by sex, but differed according to age and BCS (Figure 6.6), as BF% increased and LST% decreased with age and overweight body condition. However as distribution of dependent variables did not differ between the two groups (Appendix IIV), further control was not considered necessary. Body composition did not differ between diet groups or within groups over time (Figure 6.6).

6.4.4 Serum amino acids

Over time, there were no changes in serum amino acid concentrations in the MEAT group, though changes in the PLANT group resulted in detectable differences by the exit timepoint (Table 6.4, Figure 6.7). Non-essential amino acids alanine and glutamine increased, while non-essential glycine decreased, and the essential amino acids isoleucine, methionine and valine decreased.

6.4.5 Vitamin D metabolites and bone mineralization

At the exit timepoint, differences between groups were detected in serum concentrations of 25(OH)D₂, 25(OH)D₃, 1,25(OH)₂D₂, 1,25(OH)₂D₃, 24,25(OH)₂D₂, 24,25(OH)₂D₃, total 24,25(OH)₂D₂ and iCa (Table 6.5, Figure 6.8). In the PLANT group, all serum D₃ metabolites (25(OH)D₃, 1,25(OH)₂D₃, and 24,24(OH)₂D₃) decreased, and all serum D₂ metabolites (25(OH)D₂, 1,25(OH)₂D₂, and 24,24(OH)₂D₂) increased. As well, total serum 24,25(OH)₂D concentrations decreased in the PLANT group. The only change in the MEAT groups was a slight increase in serum iCa concentrations. Despite the differences in vitamin D metabolism, there were no differences in bone proportion, bone mineral content or bone mineral density between diet groups at exit or within groups over time.

6.5 Discussion

The current study assessed the suitability of a plant-based extruded dry food formulated for adult maintenance in adult client-owned dogs compared to a conventional extruded diet containing animal-derived ingredients. The results support the hypothesis that a complete and balanced plant-based diet is able to support general health and wellness in dogs, with no apparent adverse effects due to diet over a 3-month period. Throughout the trial, dogs maintained BW, BCS, body composition parameters in both groups. The results align with those reported by Wakshlag and colleagues (2003), which demonstrated no differences in lean mass, fat mass, or total BW in female dogs fed a high protein diet rich in either plant- or animal-protein (Wakshlag et al., 2003). Indices of health were within normal limits after feeding PLANT or MEAT for 3 months. Urinalysis did not appear to be impacted by diet in the manner expected in this study. Though it has been hypothesized that a plant-based diet could result in increased urinary pH and

predispose to formation of struvite crystalluria and urinary tract infection (Knight and Leitsberger, 2016), urine pH and quantity of struvite crystals were no different between dogs fed PLANT or MEAT, and only the presence of epithelial cells in the urine were lower in the dogs fed PLANT.

A main focus of this study was the use of vitamin D₂ in place of vitamin D₃ in its ability to maintain serum vitamin D and its analogues. Presently, the relationship between dietary vitamin D intake and serum 25(OH)D is poorly understood, with many complicating factors including dog age, breed, body condition and health status (Weidner et al., 2020, Weidner et al., 2017). Nevertheless, it is known that a dietary source of vitamin D for dogs is required because of negligible cutaneous synthesis (How et al., 1994). Considering this dependency of dogs on diet as their vitamin D source, it was expected there would be significant differences in vitamin D metabolites between PLANT and MEAT-fed dogs, as the PLANT diet contained vitamin D₂ exclusively while the MEAT diet contained vitamin D₃ exclusively. Dietary levels of total vitamin D were slightly higher in the PLANT diet than the MEAT diet. In the dogs fed the PLANT diet, the most prevalent circulating vitamin D metabolite, 25(OH)D, was present predominantly as 25(OH)D₂. This occurred despite being negligible at baseline, with 25(OH)D₂ increasing to become the predominant metabolite by the exit timepoint, while concurrently, 25(OH)D₃ dropped. Comparatively, MEAT-fed dogs had negligible 25(OH)D₂ at both timepoints, with no change between baseline and exit. In both groups of dogs at exit, total 25(OH)D when calculated as the sum of 25(OH)D₂ and 25(OH)D₃ was comparable. Similar to 25(OH)D, the active vitamin D metabolite, 1,25(OH)₂D was also present either as 1,25(OH)₂D₂ or 1,25(OH)₂D₃, depending on the dietary vitamin D source. In the dogs fed the PLANT diet,

1,25(OH)₂D₂ levels were almost double 1,25(OH)₂D₃ by the exit timepoint, with no changes in the MEAT-fed dogs. Total 1,25(OH)₂D was comparable between groups. Most revealing, 24,25(OH)₂D, the metabolite long considered to be the inactive waste metabolite of vitamin D, differed between the two groups. 24,25(OH)₂D, while less potent than 1,25(OH)₂D, plays a role in bone mineralization, as well as being the precursor for 1,24,25(OH)₃D, which is the inactive form excreted in the urine (Norman et al., 2002, Brown et al., 1999). As with the other vitamin D metabolites, the D₂ version, negligible in both groups at baseline, increased in the PLANT group, though 24,25(OH)₂D₃ remained more prominent. Total 24,25(OH)₂D was reduced markedly in the dogs fed PLANT. The two triol metabolites, 1,25(OH)₂D and 24,25(OH)₂D are both synthesized from calcidiol, either via 1-alpha-hydroxylase or 24-hydroxylase (de Brito Galvao et al., 2013, Christakos et al., 2010). The decreased level of 24,25(OH)₂D may indicate an increased flux of 25(OH)D through to 1,25(OH)₂D, or increased excretion of 1,24,25(OH)₃D. In both groups of dogs, no alterations in PTH was detected, though an increase in ionized calcium was detected in the MEAT group. Considering that both groups were fed the MEAT diet for four weeks prior to the study, it was expected that the MEAT group would remain stable, and the increase in ionized calcium was unexpected. There was no difference in total calcium in the MEAT group, thus the increase in ionized calcium may be attributed to a shift from bound calcium to ionized calcium, not an increase in blood calcium levels altogether. Shifts from bound to free or ionized calcium can occur with changes in blood pH, PTH or albumin levels (Baird, 2011). As PTH and albumin levels were unchanged, it is possible that the increase in ionized calcium demonstrated by the dogs fed the MEAT diet may have been attributable to a shift in blood pH, though acid-base balance was not evaluated in this study. In contrast, though ionized

calcium levels were maintained by dogs fed PLANT, total serum calcium decreased. In addition, serum magnesium increased. Calcium provision in the PLANT diet was lower than the MEAT diet, while magnesium was higher (Appendix II), which may account for these variations.

Bone mineralization was comparable between both groups with no changes in bone mineral density, bone mineral content, and total body bone percent, with all parameters similar to those previously reported in healthy, young dogs (Lauten et al., 2001). Based on extrapolation from studies examining vitamin D and bone mineralization in rats and humans, the three month duration was considered sufficient to detect changes in bone mineralization, if vitamin D₂ were ineffective as a dietary vitamin D source for dogs (Tam et al., 1986, Lerchbaum et al., 2019). To the authors' knowledge, these findings present the first published data in dogs to support dietary supplementation with vitamin D₂ as a source of vitamin D, efficacious in maintaining active vitamin D metabolites and normal bone metabolism for at least 3 months. This could be used to update guidelines for pet food manufacturing, as presently industry recommendations have been generated exclusively from studies evaluating dietary vitamin D₃ (AAFCO, 2020, FEDIAF, 2020, NRC, 2006).

Though still within reference ranges, the observed changes in CBC, serum biochemistry and amino acid profile could provide a direction for further research on possible benefits and adverse effects of plant-based and meat-based diets in dogs. In comparison to baseline haematology, at the exit timepoint dogs fed PLANT had lower platelets counts, but increased platelet volume. Dogs fed the MEAT diet demonstrated increased red blood cell count, hemoglobin and haematocrit. The changes in the MEAT group were particularly unexpected,

considering the dogs were maintained on the same diet as they had been consuming when baseline haematology was performed. The lifespan of canine red blood cells is around 115 days (Kurata et al., 1993), though changes in red cell count can be expected to be detectable within two weeks in response to alterations in haematopoiesis (Yuki et al., 2007). In both diet groups, RBC and Hb fell from screening to baseline, then increased from baseline to exit (data not reported), though the increase was only significant in the MEAT group. It is thus possible that participation in the trial reduced RBC, given the drop from pre-participation (screening) to baseline, and the MEAT group was demonstrating a more rapid return to pre-trial levels, while the PLANT group was slower to do so. This potentially indicated an advantage in haematopoiesis in the MEAT-fed dogs. One possible explanation for this could be iron content and intake, as iron is a required component of the haem moiety of haemoglobin (McCown and Specht, 2011). Iron provision was higher in the PLANT than the MEAT diet and in both diets supplementation was in the form of ferrous iron. However, it is possible that other dietary components in the PLANT diet could impair uptake and availability of dietary iron, such as polyphenols and phytates present in the plant ingredients (Ariza-Nieto et al., 2007). Heat processing can destroy these anti-nutritional factors, however, and a recent study demonstrated comparable iron digestibility in dogs fed heat-processed diets with plant- or animal-based proteins (Cargo-Froom et al., 2019). Dietary provision of copper was lower in the PLANT diet, which could potentially reduce exportation of copper from enterocytes and thus impair iron status, as the enzyme responsible for this transportation, ferroportin, requires copper as a cofactor (McCown and Specht, 2011). As neither iron nor copper status were measured in the present study, it is unclear whether or not iron availability may have played a role in the apparent

superior haematopoiesis in the MEAT-fed dogs compared to those that were fed the PLANT diet. Other nutritional factors critical for haematopoiesis include the B vitamins cobalamin and folate. Dietary folate was higher in the PLANT than the MEAT diet, though cobalamin was lower. Both cobalamin and folate were measured in the sera of the study dogs (Chapter Eight), and were comparable, suggesting these were unlikely to contribute to these findings.

The findings of increased platelet volume and lower platelet count in dogs fed the PLANT diet may be of interest. Adult men consuming no animal products demonstrated greater platelet volume, as compared to ovo-lacto vegetarian, moderate meat-eating or high meat-eating adult males (Li et al., 2002). In that study, mean platelet volume was associated with platelet phospholipid fatty acid profile, with larger platelets having higher linoleic acid and lower dihomo- γ -linolenic acid, eicosapentaenoic acid and docosapentaenoic acid (DPA) concentrations. In the present trial, the PLANT diet had lower linoleic acid, eicosapentaenoic acid and docosapentaenoic acid. The lower intake of dihomo- γ -linolenic, and docosapentaenoic acids could have resulted in lower concentrations of those fatty acids in both serum and platelets, resulting in larger platelets. Although no adverse effects of the hematological changes were detected in the dogs fed PLANT, further research to determine the effect of plant-based diets on platelet count, morphology and function in dogs is indicated.

Though plants are rich sources of both omega-6 and omega-3 fatty acids, animal tissues contain predominantly omega-6 fatty acids, including arachidonic acid, which is not commonly found in plants. Correspondingly, the PLANT diet provided lower linoleic acid, dihomo- γ -linolenic acid, arachidonic acid, total omega-6 fatty acids and lower omega-6 : omega-3 ratio,

though there were no concerns regarding any fatty acid insufficiency or imbalance in either group. Linoleic acid is an essential fatty acid in the omega-6 family, a precursor for the long-chain PUFA arachidonic acid, among others. In addition to being the parent fatty acid of the omega-6 family, the main role of linoleic acid is within cell membranes, where it affects the structure, stability and function of the lipid bilayer (Wertz and Downing, 1982). As such, the most obvious clinical sign of linoleic acid deficiency is a loss of skin integrity and functional water barrier, resulting in dermatoses (Watson, 1998). Although linoleic acid was lower in the PLANT diet, it exceeded minimum recommendations, so adverse signs consistent with linoleic acid deficiency, such as poor coat condition, would not be expected and were not detected. Arachidonic acid, derived from linoleic acid, also plays a large role in cell membrane structure and function, as well as eicosanoid production (Schlotter et al., 2009). The eicosanoids produced from arachidonic acid are considered more inflammatory than those produced from EPA and DHA, so a slight reduction of this fatty acid may have provided a health benefit (Bauer, 2016, Bauer, 2011). The overall ratio of omega-6 to omega-3 fatty acids was also lower in the PLANT diet, supporting potential for anti-inflammatory effects (Kearns et al., 1999).

Reduced serum cholesterol concentrations were also observed in dogs fed PLANT. By the end of the trial, differences were observed between groups though serum cholesterol was within the laboratory reference range^b for both diet groups. This reduction of serum cholesterol concentrations may be explained by the lack of dietary cholesterol in PLANT, resulting in only endogenous cholesterol being measured. In comparison, dogs fed the MEAT diet had endogenous cholesterol supplemented with dietary cholesterol from the animal ingredients (primarily the chicken and egg ingredients) in the diet. Chicken tissues and eggs are known to

contain high cholesterol content, consumption of which has been demonstrated to increase serum cholesterol concentrations in humans (Ahmad et al., 2017, Chung et al., 1990, Rule et al., 2002). Lower serum cholesterol concentrations has been demonstrated in both men and women consuming no animal products, in comparison to vegetarians, meat-eaters and fish-eaters (Bradbury et al., 2014, Lütjohann et al., 2018). In humans, hypercholesterolaemia is most commonly associated with atherosclerosis and subsequent cardiovascular disease (Fernandez and Andersen, 2014, Stone, 1993, Klag et al., 1993). In dogs, however, although atherosclerosis can be experimentally induced, this is not a common naturally-occurring condition (Mahley et al., 1977). Instead, hypercholesterolaemia occurs in, and contributes, to a number of other chronic disease states in dogs, including diabetes mellitus, hyperadrenocorticism, hypothyroidism, obesity, pancreatitis, primary/idiopathic hyperlipidemia, primary hypercholesterolemia and protein-losing nephropathy (Barrie et al., 1993, Johnson, 2005, Xenoulis and Steiner, 2010). While there are no known benefits of dietary cholesterol restriction in healthy dogs, and no known health concerns for healthy dogs consuming cholesterol-containing diets, the findings of the present study suggest that a plant-based diet could be beneficial as a therapeutic aid to reduce hypercholesterolaemia in dogs and investigation in hyperlipidemic dogs may be warranted.

Other notable differences in serum biochemistry included reductions in creatinine and urea in dogs fed PLANT. Urea, measured as blood urea nitrogen (BUN), is a low-toxicity end product of protein metabolism and is eventually excreted in the urine (Steinbach et al., 2010). As such, changes in BUN can be expected with changes in dietary protein level, with diets high in protein associated with higher BUN and diets deficient in protein reducing BUN (Davenport et al., 1994, Prause and Grauer, 1998, Yamamoto et al., 2019). The PLANT and MEAT diets, however,

provided a similar quantity of dietary protein and were both in excess of the industry recommended minimum (AAFCO, 2018). Though the trial diets were formulated to be isonitrogenous, testing of the completed product revealed a difference of 1.1g/100kcal between the two diets, with PLANT having the lower protein content. All other markers of protein metabolism, such as BW, body condition, lean body mass, total protein and albumin were maintained. Furthermore, previous studies have demonstrated comparable digestibility of plant-based proteins in dogs fed plant- or chicken-based iso-nitrogenous diets (Carciofi et al., 2009, Clapper et al., 2001, Yamka et al., 2003, Zentek and Mischke, 1997). Soy, a plant-based protein, has been reported to be beneficial for dogs with hepatic disease, as the amino acid profile may positively influence protein and amino acid metabolism and decrease blood ammonia levels (Zentek and Mischke, 1997). It is thus unclear if the protein quantity or quality in PLANT contributed to the lower BUN in the dogs fed PLANT. In addition to protein metabolism, BUN is also a marker of renal function, along with serum creatinine, which also decreased significantly in dogs fed PLANT and was lower in these dogs than the dogs fed the MEAT diet. In both groups, BUN and creatinine were maintained within the normal laboratory reference range^b for the duration of the study. Creatinine can also be affected by muscle mass, as its precursor, phosphocreatine, is found in high concentrations in muscle (Médaille et al., 2004, Freeman III et al., 2003). However, all dogs in the trial maintained BW and LST mass, thus the reduction in creatinine is unlikely to be associated with any changes in muscle mass. In humans, plant-based proteins and plant-based diets have been suggested to be associated with kidney health, with improved outcomes associated with hypertension, glomerular filtration rate decline and risk of developing chronic kidney disease (Kim et al., 2019a, Joshi et al., 2018). Potentially, dogs fed

PLANT experienced either decreased protein catabolism, as a result of lower dietary protein intake or reduced endogenous protein catabolism, and thus reduced BUN and creatinine synthesis, and/or increased renal clearance of BUN and creatinine. Further research is required to determine if plant-based diets affect protein metabolism and renal function in dogs.

Despite being provided in a marginally higher proportion in PLANT, two of the branched-chain amino acids (BCAA), isoleucine and valine, decreased in the serum of the dogs fed PLANT. This may represent a difference in BCAA metabolism in dogs fed a plant-based diet. The main role of the BCAA is in protein synthesis and skeletal muscle development, with leucine being a critical regulator of the mammalian target of rapamycin (mTOR) protein translation pathway (Duan et al., 2016). In addition to this role, BCAA are also utilized as hormone secretagogues, particularly insulin (Nair and Short, 2005). Despite lower serum BCAA concentrations in PLANT as compared to MEAT dogs, serum levels in both groups were within the ranges that have been reported for apparently healthy adult dogs (Kathrani et al., 2018). Dogs fed PLANT showed no differences in indicators of energy metabolism or protein synthesis, with serum glucose and protein levels, body weight, and lean soft tissue mass all maintained and comparable to the MEAT-fed dogs. Leucine has been demonstrated to be a major donor of nitrogen to alanine synthesis in dogs (Galim et al., 1980). Possibly, the indispensable BCAA were utilised to generate the dispensable amino acid alanine, as alanine levels were lower in PLANT yet serum alanine levels increased from baseline to exit in the dogs fed PLANT. In humans, plasma BCAA have been demonstrated to be predictive indicators of obesity and type II diabetes, and a predominantly plant-based diet has been utilised to reduce plasma BCAA and has been hypothesized to potentially confer protection against these diseases (Eishorbagy et al.,

2017). The metabolism of BCAA in dogs fed plant-based diets thus warrants further investigation regarding potential beneficial or detrimental effects.

The sulfur amino acid methionine was also lower in the serum of dogs fed PLANT. The decrease may be attributable to dietary provision alone, as, although the diets were formulated to provide comparable sulfur amino acid content, when the products were tested the PLANT diet was found to contain lower levels of sulfur amino acids than the MEAT diet (Appendix II). Methionine is a ubiquitous methyl donor, critical in protein synthesis and the precursor of the most prevalent antioxidant in the body, glutathione (Brosnan et al., 2007, Khayati et al., 2017, Bin et al., 2017). Taurine is found in the body as a free amino acid, not incorporated into protein, with main roles pertaining to cardiac function and bile acid conjugation and secretion. Although taurine is considered a dispensable amino acid, in that dogs are capable of *de novo* synthesis provided adequate precursors, taurine deficiency has become a concern in some cases (Backus et al., 2006, Fascetti et al., 2003). In this study, both the MEAT and PLANT groups demonstrated serum sulfur amino acids and taurine within the ranges reported for apparently healthy adult dogs (Kathrani et al., 2018). However, the reduction in serum methionine in the PLANT group warrants consideration. Further evaluation using diets with more comparable sulfur amino acid content are indicated.

The dogs fed PLANT demonstrated an increase in serum glutamine and had higher glutamine levels at the end of the study than the MEAT-fed dogs. This has also been demonstrated in humans eating a predominantly plant-based diet (Eishorbagy et al., 2017). Glutamine is found in many protein-rich foods, including animal-derived and plant-based

proteins, with both trial diets containing multiple glutamine-rich ingredients. Unfortunately, glutamine levels had not been measured in the diets, and so the contribution of dietary glutamine is unknown. Glutamine is an anabolic amino acid and may reduce leucine oxidation and spare protein (Humbert et al., 2002). Possibly, as a result of lower dietary leucine intake, glutamine synthesis may have been upregulated. Additionally, glutamine is a preferred substrate for enterocytes, and may be beneficial for gut health (Gouttebel et al., 1992, Ohno et al., 2009). If dogs fed a plant-based diet have a higher glutamine intake or increased glutamine synthesis, this could potentially explain the lower prevalence of gastrointestinal disorders in dogs fed a plant-based diet, as reported by their owners (Dodd et al., 2020c) (Chapter Three). Further investigation into the glutamine metabolism of dogs fed a plant-based diet is indicated. The PLANT diet also contained a lower quantity of glycine, and dogs fed PLANT exhibited a reduction in serum glycine and had lower glycine levels than the MEAT-fed dogs. Considering the discrepancy in dietary provision, this is considered the most likely explanation.

The findings from this study may not be applicable to plant-based diets in general, as the diet used in this study was specifically formulated and tested to ensure nutritional sufficiency as compared to the nutrient profile recommended by AAFCO for maintenance of adult dogs, but other plant-based diets available commercially may not do so (Chapter 5)(Dodd et al., 2021b, Zafalon et al., 2020). Furthermore, the stipulations of participation in the trial required feeding of the trial diets exclusively and limited treats and snacks, which may otherwise form a greater proportion of a dog's daily food offering and could impact their nutrient intake. Given that privately-owned dogs were enrolled in the study, the heterogeneity of the sample population could potentially be a source of confounding. However, based on the lack of differences found in

the independent variables (sex, age, BW, BCS, season, and breed) and general health and wellness indices (CBC, serum biochemistry) between groups at baseline, randomization was considered successful with no predisposition to bias in either group. Another limitation presented by using privately-owned animals was the duration of the trial. Using data extrapolated from other species or from other canine trials, the 3-month timeline was decided upon and was sufficient to demonstrate some differences between groups. However, it is possible that a longer trial could reveal further differences, or that variables would stabilize over a greater time period, though compliance with study protocols and retention time could become issues with longer study duration in client-owned dogs. Serial testing (i.e., testing at intervals and not just baseline and exit timepoints) would also provide more information about the changes discovered in some variables. Lastly, the limitation of using only healthy, desexed dogs between the ages of 2-10 years of age limits extrapolation of these findings to a comparable proportion of the canine population.

6.6 Conclusion

For the duration of the study, all dogs maintained health, bodyweight and body composition. Although all health parameters were maintained, dogs fed PLANT exhibited changes in haematology, biochemistry, serum amino acids and vitamin D metabolites in comparison to dogs fed MEAT. Bone mineralization, serum vitamin D levels and ionized calcium did not differ between groups, suggesting that vitamin D₂ may be an efficacious dietary form of vitamin D for dogs. In addition, the effect of plant-based diets on platelet count, morphology and function, branched-chain amino acid and protein metabolism, as well as renal function warrant further investigation.

6.7 Foot Notes

- a. Petcurean Go! Solutions Skin + Coat Care Chicken Recipe
- b. Animal Health Laboratory, University of Guelph
- c. Veterinary Diagnostic Laboratory, Michigan State University

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6.9 Tables

Table 6.1: Significant results of repeated mixed model analysis of complete blood count between dogs fed a plant- (PLANT, n = 31) or animal-based (MEAT, n = 30) diet for 3 months.

Interaction	CBC analyte	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	WBC	PLANT	Exit	-0.74	-1.438- -0.038	0.001
	RDW	PLANT	Exit	0.27	0.034-0.508	0.025
Time*Group (referent = baseline timepoint)						
	RBC	MEAT	Exit	0.19	0.079-0.307	0.001
	Haemoglobin	MEAT	Exit	4.40	1.839-6.961	0.001
	Platelets	PLANT	Exit	-38.47	-57.055- -19.893	<0.001
	MPV	PLANT	Exit	2.64	1.969-3.307	<0.001
	Segmented neutrophils	PLANT	Exit	-0.63	-1.065- -0.966	0.002
Group*Time (referent = plant-based)						
	RBC	MEAT	Exit	0.17	0.053-0.280	0.004
	Haemoglobin	MEAT	Exit	4.69	2.150-7.231	<0.001
	Haematocrit	MEAT	Exit	0.01	0.005-0.020	0.001
	Platelets	MEAT	Exit	31.69	13.114-50.269	0.001
	MPV	MEAT	Exit	-2.13	-2.972- -1.281	<0.001

Table 6.2: Significant results of repeated mixed model analysis of serum biochemistry between dogs fed a PLANT (n = 31) or MEAT (n = 30) diet for 3 months.

Interaction	Biochemistry analyte	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	K	PLANT	Exit	0.13	0.039-0.212	0.004
	Na:K ratio	PLANT	Exit	-1.03	-1.646- -0.418	0.001
Time*Group (referent = baseline timepoint)						
	Calcium	PLANT	Exit	-0.04	-0.072—0.013	0.005
	Magnesium	PLANT	Exit	0.04	0.014-0.057	0.001
	Urea	PLANT	Exit	-0.77	-1.326—0.216	<0.001
	Creatinine	PLANT	Exit	-14.71	-19.302- -10.117	<0.001
	Cholesterol	PLANT	Exit	-1.00	-1.319- -0.678	<0.001
Group*Time (referent = plant-based)						
	Calcium	MEAT	Exit	0.05	0.022-0.082	0.001
	Magnesium	MEAT	Exit	-0.38	-0.060- -0.016	0.001
	Urea	MEAT	Exit	1.09	0.523-1.651	<0.001
	Creatinine	MEAT	Exit	17.87	13.209-22.524	<0.001
	Cholesterol	MEAT	Exit	1.12	0.797-1.446	<0.001
	Osmolality	MEAT	Exit	2.53	0.790 – 4.272	0.004

Table 6.3: Significant results of repeated mixed model analysis of urinalysis between dogs fed a plant- (PLANT, n = 31) or animal-based (MEAT, n = 30) diet for 3 months.

Interaction	Urinalysis analyte	Diet	Timepoint	Contrast	95% CI	P
Group*Time (referent = plant-based)						
	Epithelial cells	MEAT	Exit	0.68	0.284-1.083	<0.001

Table 6.4: Significant results of repeated mixed model analysis of serum amino acids between dogs fed a PLANT (n = 31) or MEAT (n = 30) diet for 3 months.

Interaction	Amino acid	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	Asparagine	PLANT	Exit	5.43	0.959-9.894	0.017
	Aspartic acid	PLANT	Exit	-2.67	-4.739- -0.603	0.004
	Taurine	PLANT	Exit	-0.18	-0.295- -0.062	0.003
Time*Group (referent = baseline timepoint)						
	Alanine	PLANT	Exit	60.38	17.423-103.328	0.005
	Glutamine	PLANT	Exit	82.12	43.622-120.618	<0.001
	Glycine	PLANT	Exit	-41.38	-56.850- -25.912	<0.001
	Isoleucine	PLANT	Exit	-7.40	-11.565- -3.227	<0.001
	Methionine	PLANT	Exit	-5.54	-8.919- -2.167	0.001
	Valine	PLANT	Exit	-16.53	-25.958- -7.097	0.001
Group*Time (referent = plant-based)						
	Alanine	MEAT	Exit	-60.74	-103.594- -17.876	0.003
	Glutamine	MEAT	Exit	-83.69	-121.913- -45.465	0.003
	Glycine	MEAT	Exit	41.07	25.542-56.594	<0.001
	Isoleucine	MEAT	Exit	10.70	6.498-14.902	<0.001
	Methionine	MEAT	Exit	5.63	2.278-8.987	0.001
	Valine	MEAT	Exit	21.18	11.542-30.824	<0.001

Table 6.5: Significant results of repeated mixed model analysis of vitamin D metabolites between dogs fed PLANT (n = 31) or MEAT (N =30) diet for 3 months.

Interaction	Vitamin D metabolite	Diet	Timepoint	Contrast	95% CI	P
Time*Group (referent = baseline timepoint)						
	25(OH)D2	PLANT	Exit	21.70	19.313-24.087	<0.001
	25(OH)D3	PLANT	Exit	-24.42	-27.271- -21.568	<0.001
	1,25(OH) ₂ D2	PLANT	Exit	52.513	46.073-58.952	<0.001
	1,25(OH) ₂ D3	PLANT	Exit	-24.12	-55.291- -32.942	<0.001
	24,25(OH) ₂ D2	PLANT	Exit	2.20	1.943-2.457	<0.001
	24,25(OH) ₂ D3	PLANT	Exit	-12.42	-14.512- -10.334	<0.001
	Total 24,25(OH) ₂ D	PLANT	Exit	-10.222	-12.270- -8.185	<0.001
	Ionized calcium	MEAT	Exit	0.03	0.013-0.049	0.001
Group*Time (referent = plant-based)						
	25(OH)D2	MEAT	Exit	-22.56	-24.974- -20.154	<0.001
	25(OH)D3	MEAT	Exit	24.09	21.215-26.970	<0.001
	1,25(OH) ₂ D2	MEAT	Exit	-52.51	-58.952- -46.073	<0.001
	1,25(OH) ₂ D3	MEAT	Exit	47.89	36.610-59.161	<0.001
	24,25(OH) ₂ D2	MEAT	Exit	-2.32	-2.579- -2.061	<0.001
	24,25(OH) ₂ D3	MEAT	Exit	12.46	10.348-14.565	<0.001
	Total 24,25(OH) ₂ D	MEAT	Exit	10.16	8.089-12.223	<0.001
	Ionized calcium	MEAT	Exit	0.04	0.022-0.058	<0.001

6.10 Figures

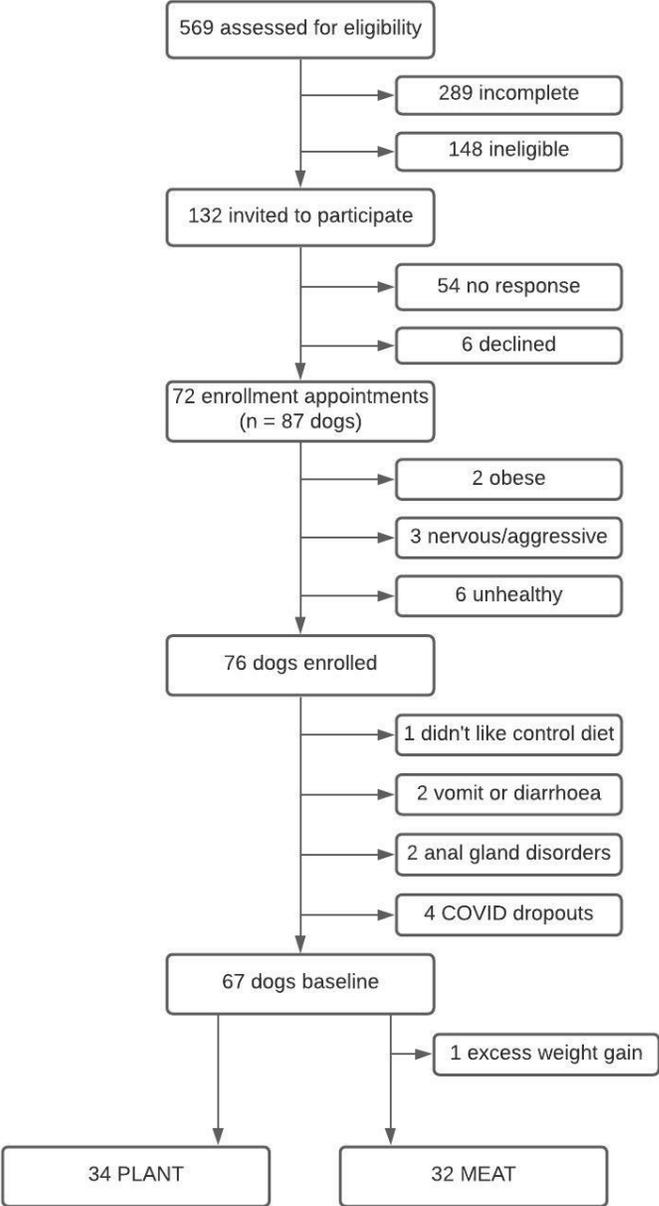


Figure 6.1: Recruitment and enrollment of dogs in the diet trial.

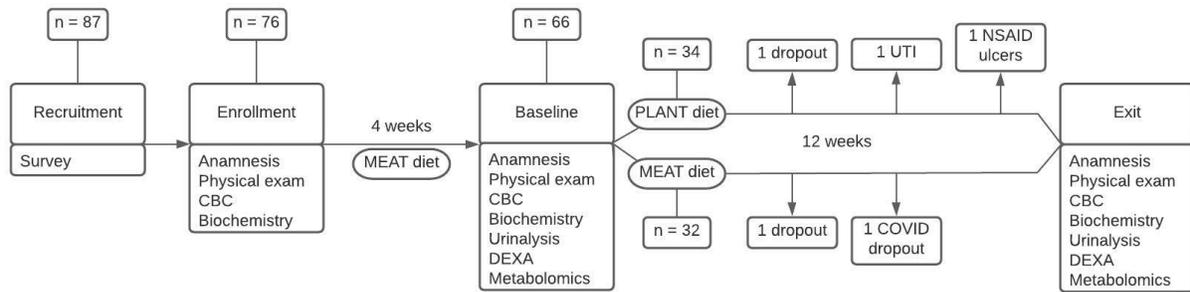


Figure 6.2: Diet trial timeline

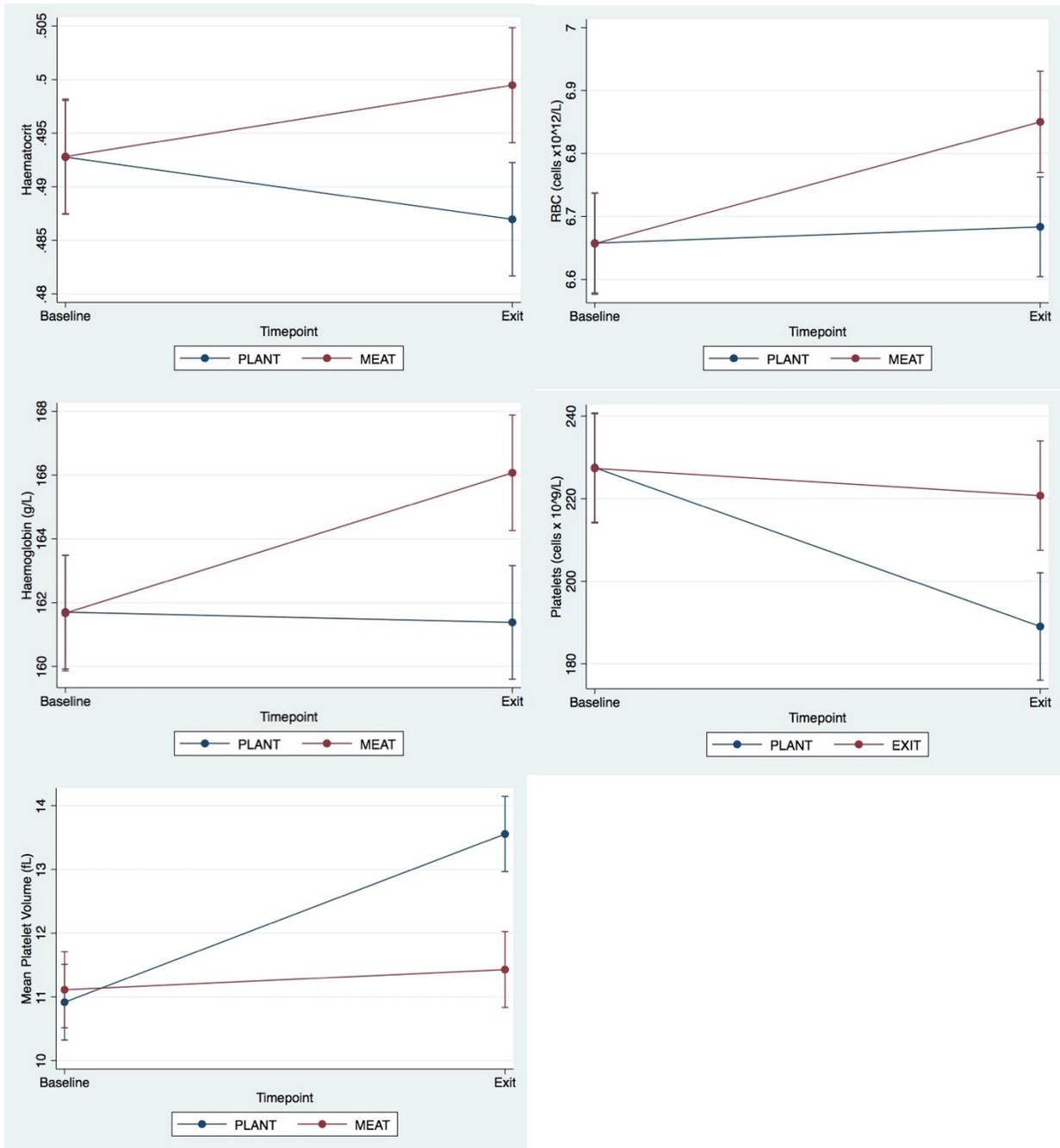


Figure 6.3: Significant differences in complete blood count between dogs fed plant- (PLANT n =31) and animal-based (MEAT n = 30) diets for 3 months.

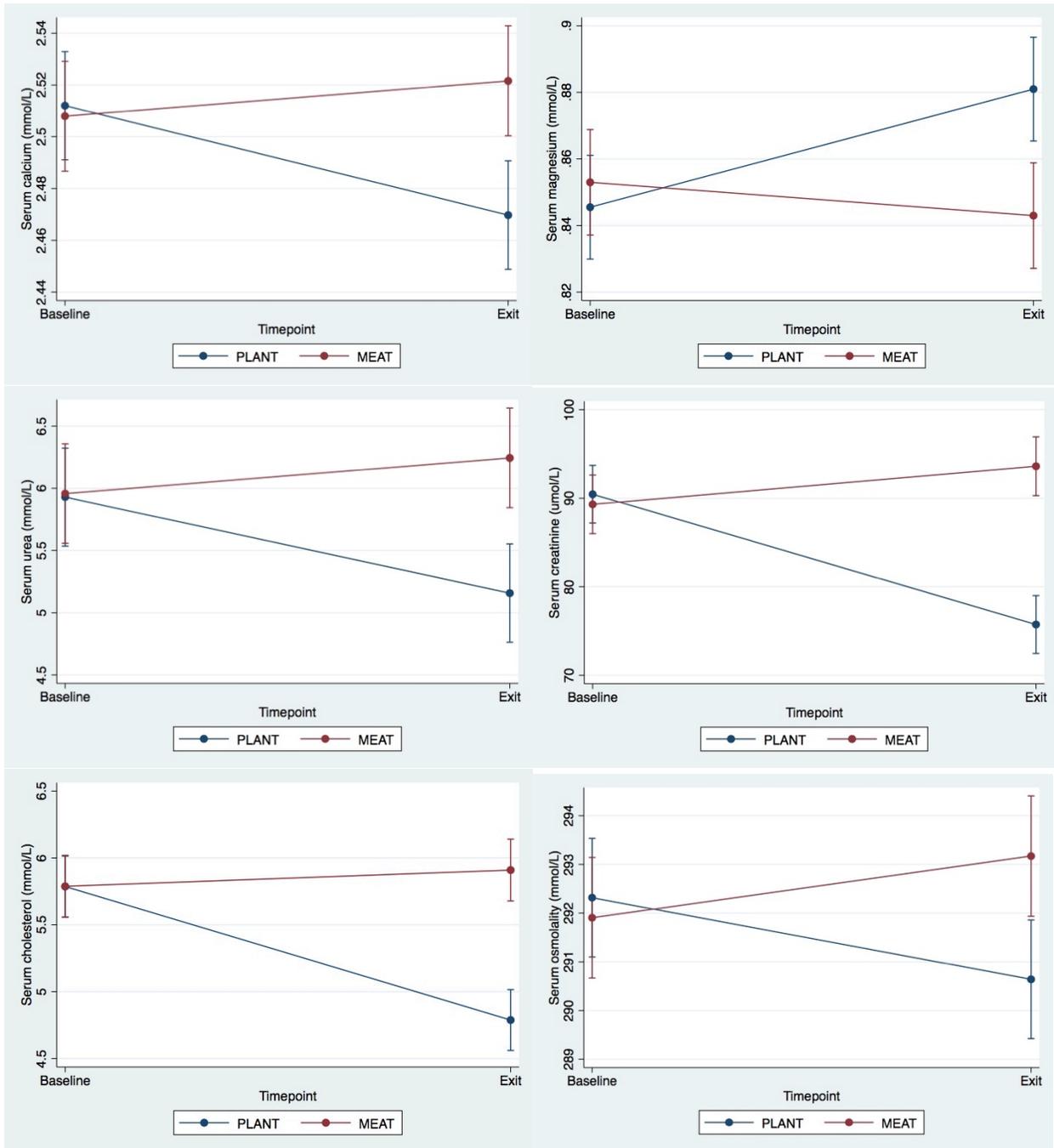


Figure 6.4: Significant differences in serum biochemistry between dogs fed PLANT (n = 31) or MEAT (n = 30) diets for 3 months.

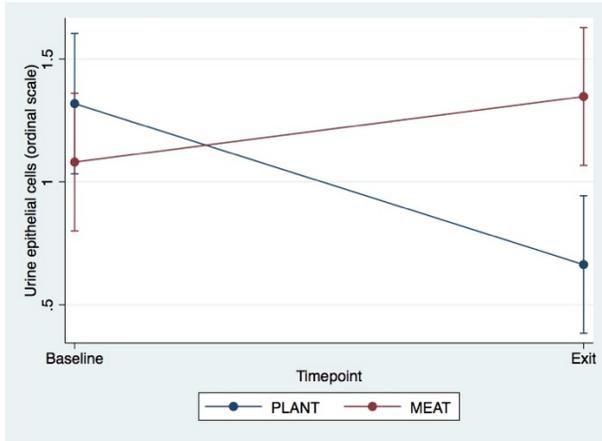


Figure 6.5: Epithelial cells in the urine of dogs fed PLANT (n = 31) or MEAT (n = 30) diets for 3 months.

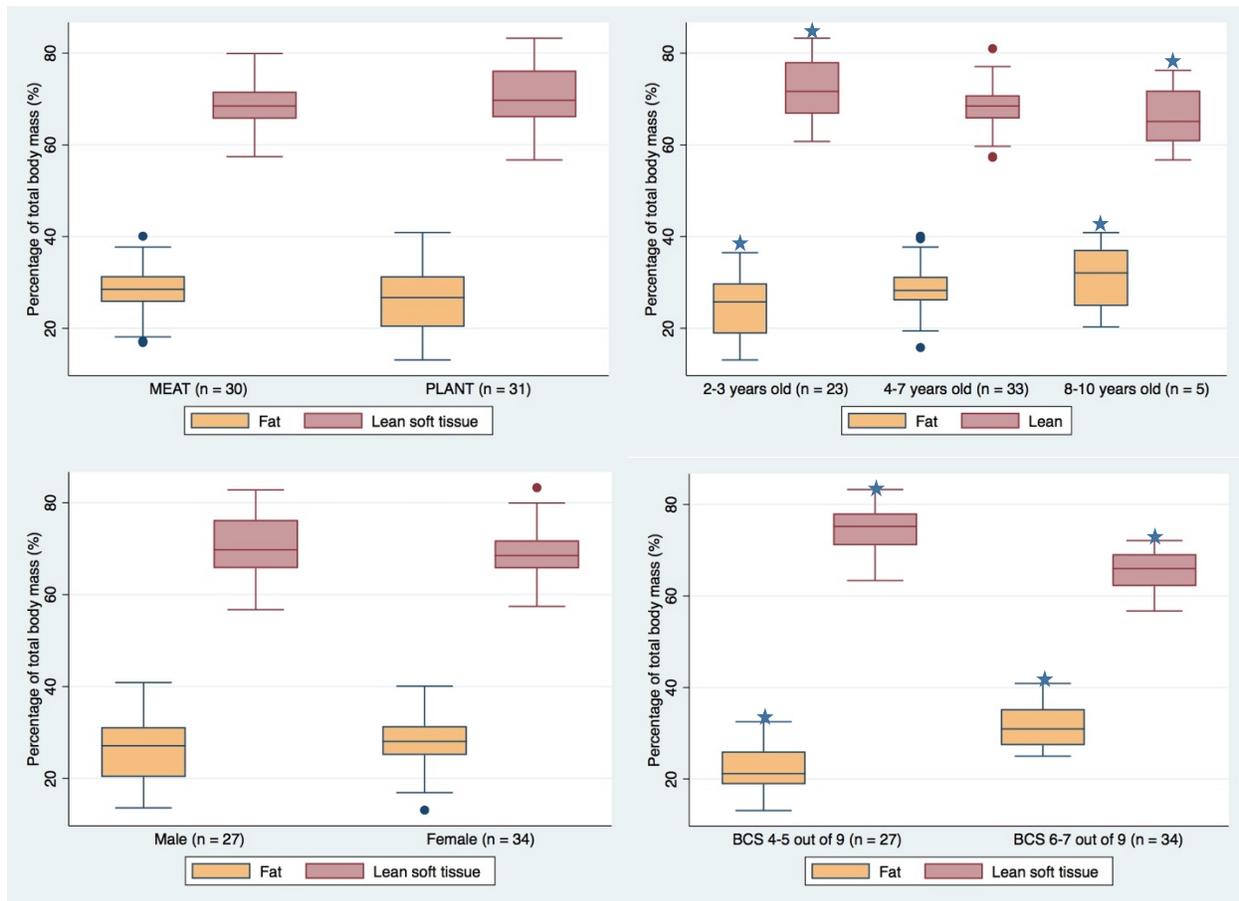


Figure 6.6: Dual energy x-ray body composition assessment in dogs fed a plant- (PLANT, n = 31) or animal-based (MEAT, n = 30) diet at the end of the 3-month diet trial.

Stars indicate significant differences ($P < 0.05$) in body composition between categories as detected by one-way ANOVA.

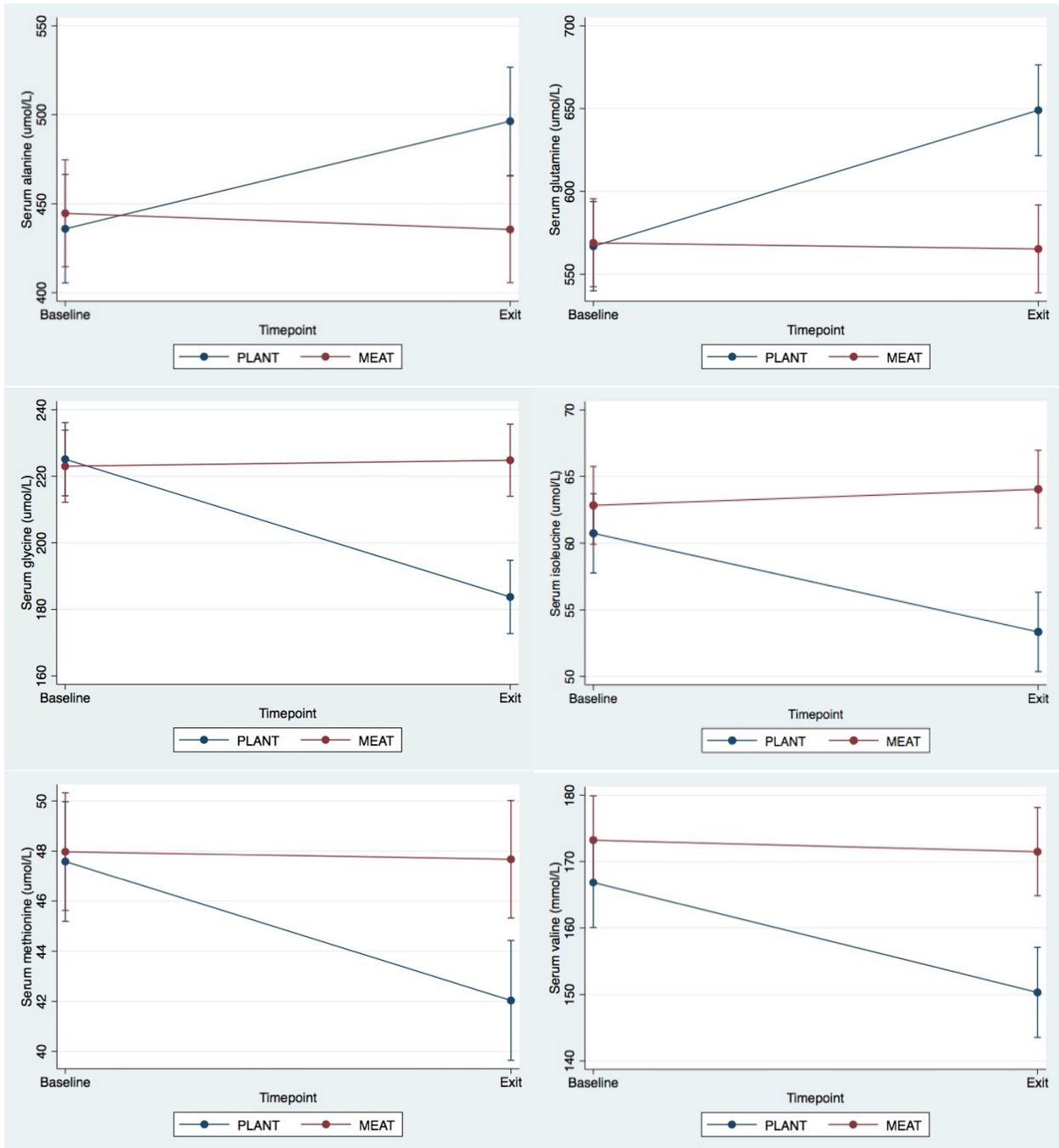
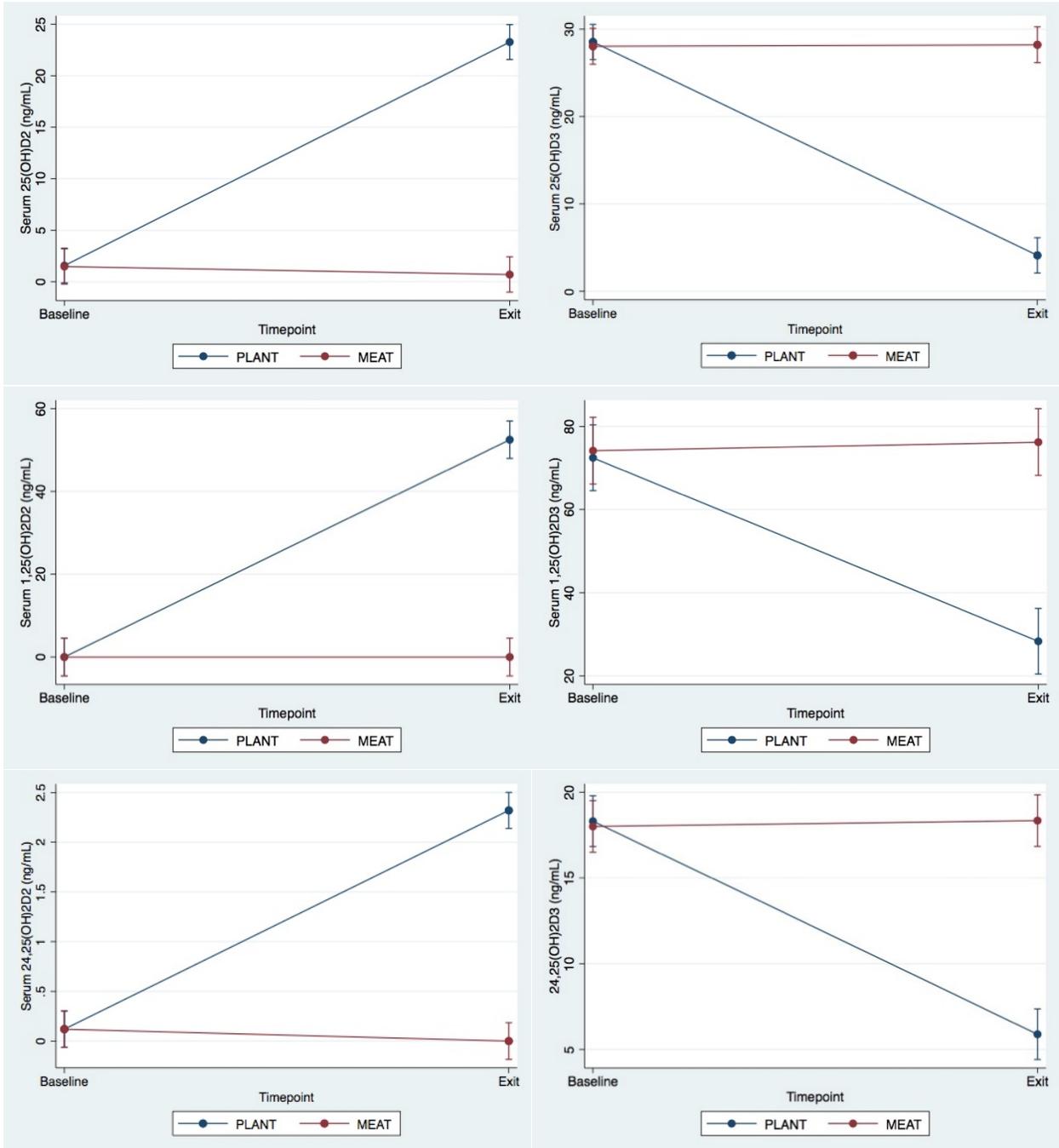


Figure 6.7: Significant differences in serum amino acids between dogs fed PLANT (n = 31) or MEAT (n = 30) diet for 3 months.



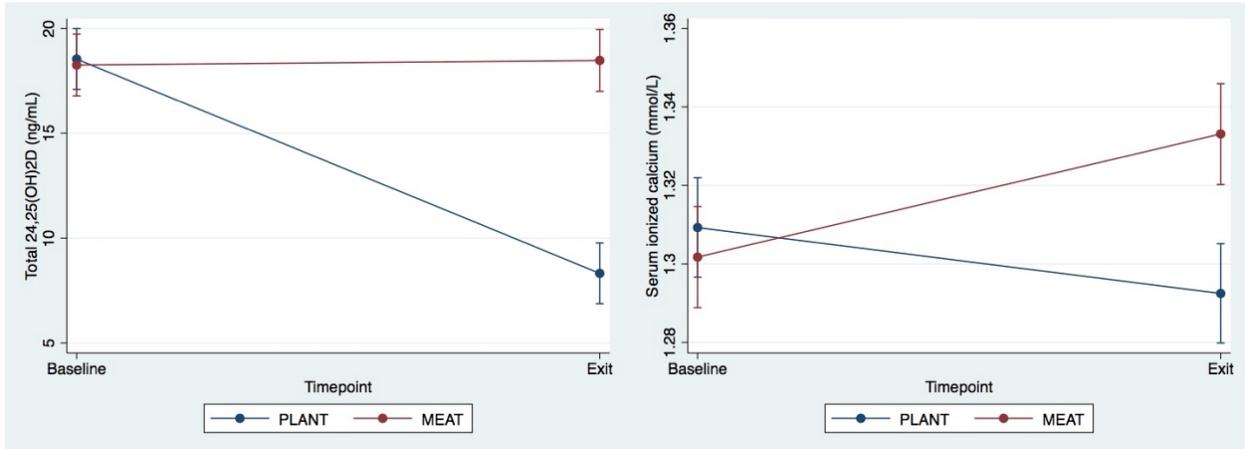


Figure 6.8. Significant differences in vitamin D metabolites between dogs fed PLANT (n = 31) or MEAT (n = 31) diet for 3 months.

7 CHAPTER SEVEN: Serum metabolomics, with a focus on protein and amino acid metabolism, in dogs fed a plant- or meat-based diet

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7.1 Abstract

Background: Metabolomics allow for the investigation of dietary influences on animal health beyond what may be revealed by routine healthcare assessments. Though dogs are omnivores, not herbivores, entirely plant-based diets can be formulated to meet the current known nutrient recommendations. However, little is known about the health effects of diets containing no animal-derived nutrients on the dogs to which they are fed.

Objectives: This study compared serum metabolomics in dogs fed a plant-based or meat-based diet.

Methodology: A longitudinal trial including 61 dogs was performed, with 31 dogs fed an experimental extruded plant-based (PLANT) diet and 30 dogs fed a commercial extruded meat-based (MEAT) diet for three months. Dogs were screened for health and wellness via veterinary examination, CBC and serum biochemistry prior to enrolment into the study. At baseline and exit timepoints, examination and serum metabolomics were performed.

Results: Few metabolomic differences were detected between dogs fed the PLANT or MEAT diet. The most marked differences were detected in fatty acid and lipid metabolites, with a decrease in serum fatty acids overall and a reduction of the omega-6 to omega-3 fatty acid ratio, attributed to differences in the fat composition of the diets. Notably, dogs maintained on the PLANT diet demonstrated a reduction in serum branched-chain amino acids (BCAA) despite higher dietary provision, though no evidence for differences in BCAA catabolism or muscle

metabolism were found. A reduction in serum creatinine without corresponding changes in creatine was also observed in the PLANT diet group.

Conclusion: Dogs fed PLANT for 3 months maintained health parameters and showed few metabolic differences compared with dogs fed MEAT. Further research is required to describe the impacts of plant-based diets of varying nutrient composition on canine metabolism and determine whether these diets impact branched chain amino acid metabolism and renal function.

7.2 Introduction

Metabolite concentrations measured in serum represent the combined effects of dietary composition and nutrient absorption, metabolic activity of gut microbiota and gene expression in body tissues of the animal from whom it is sampled (Corthésy-Theulaz et al., 2005). Serum metabolite analysis helps to provide a wholistic picture of the metabolic activity of the animal, including the effects of diet, microbiota and individual variability. Metabolomics is a cross-disciplinary study of metabolites, combining analytical chemistry with bioinformatic tools to detect patterns in metabolic profiles (Zhang et al., 2015). Serum metabolomic studies in dogs have included descriptive investigations (Puurunen et al., 2016a, Gamble et al., 2018), comparative studies between dogs with or without health conditions (Forster et al., 2018, Puurunen et al., 2016b, Söder et al., 2019, Kawabe et al., 2015, Ottka et al., 2021, O’Kell et al., 2020, Muñoz-Prieto et al., 2021), and trials to determine the effects of different dietary interventions (Ephraim et al., 2020, Allaway et al., 2019, Tamai et al., 2014, Li et al., 2020, Moore et al., 2020, Kim et al., 2019b, Ambrosini et al., 2020).

Though dogs are omnivores, capable of consuming, digesting and utilizing nutrients from both plant and animal sources (Wozencraft, 2005, Batchelor et al., 2011), to the authors’ knowledge, the metabolic effects of feeding a strictly plant-based diet to dogs have not yet been described. Indeed, few studies researching effects of plant-based diets on the health and wellbeing of dogs have been published. One study demonstrated comparable haematological parameters between dogs fed meat-free or meat-containing diets (Brown et al., 2009), and another found fewer owner-reported health disorders in dogs fed plant-based diets (Dodd et al., Submitted). When the nutrient composition of plant-based diets intended for feeding to dogs was

investigated, the provision of some essential nutrients was not adequate relative to canine nutrient requirements, despite the availability of non-animal sources of these nutrients (Dodd et al., 2021b, Zafalon et al., 2020, Kanakubo et al., 2015). Of particular concern was the possibility of insufficient provision and/or digestibility of sulfur amino acids, potentially contributing to sub-normal production or retention of taurine (Quilliam et al., 2021). Though taurine is not recognized as a dietary essential nutrient for dogs, it is possible that insufficient provision of sulfur amino acids, poor protein digestibility, amino acid bioavailability, and/or reduced taurine-conjugated bile acid recycling may contribute to taurine deficiency and its clinical manifestations of retinopathy and dilated cardiomyopathy (Backus et al., 2006, Fascetti et al., 2003).

The objective of this study was to compare the serum metabolome of dogs fed either a plant-based or conventional animal-based extruded diet formulated for adult maintenance. It was hypothesized that differences in protein and amino acid metabolic pathways may be detected, with increased endogenous flux of methionine to taurine synthesis in the dogs fed the plant-based diet.

7.3 Methodology

Samples for this study were collected as part of larger longitudinal study investigating the health effects of a plant-based (PLANT) versus an animal-based (MEAT) diet over a 3-month period (Chapters Six and Seven). The effect on completed blood count, serum biochemistry, serum vitamin D, amino acid profile and body composition are presented in Chapter Six. This study was conducted at the University of Guelph with the approval of the Research Ethics Board (Research Ethics Approval number 19-02-036) and Animal Care Committee (Animal Use

Protocol #4129), ensuring the research protocol was in line with institutional, provincial, and national guidelines and policies for humans participating in research as well as for the care and use of animals in research. Sample size determination can be found in Chapter Six.

7.3.1 Participants

Recruitment for trial participants was initiated June 3, 2019 and terminated July 5, 2020. The student researcher (SD) was responsible for recruitment and enrolling of all participants. An eSurvey was designed on the Qualtrics (Provo, Utah, USA) platform to collect data regarding patient suitability. Recruitment and survey details are described in Chapter 7. Briefly: information collected included: dog age, sex, weight and body condition score (BCS, evaluated by selection of WSAVA BCS image (WSAVA, 2013b) most closely resembling their dog, ordered randomly to attempt to avoid bias); the main diet fed, details of treats and snacks, provision of supplements and medications, number of adults in household, presence of children in the household and interaction between children and dog feeding, presence of other pets in the household and access to other pets' food, dog housing (indoors vs outdoors), feeding management, access to unmonitored food sources, dog activity, and dog medical history. Dogs were excluded from consideration if they were intact, weighed less than 5kg, had an owner-reported BCS > 5, fed a homemade or raw diet, housed outdoors without supervision, had access to unmonitored food sources, had current medical problems, received medication other than parasite preventatives, had previous medical problems that could affect them currently (e.g. previously diabetic but in remission, recurrent ear infections, etc.) or had known dietary allergies. Dogs in households without children or other pets were prioritised for inclusion in the study. The recruitment survey resulted in 72 enrollment appointments scheduled for 87 dogs

(some participants had more than one dog) during which an informed consent form for participation in the study was signed and a veterinary wellness examination was performed. Following collection of medical and dietary history, physical examination was performed, bodyweight (BW) measured and blood collected for analysis of complete blood count (CBC) and serum biochemistry. Description of the enrolled dogs is available (Appendix II).

7.3.2 Diets

Two isocaloric diets, one commercial animal-based diet^a (MEAT) and one experimental plant-based diet (PLANT), were formulated by the research team to meet or exceed nutrient recommendations for adult maintenance (AAFCO, 2018). The diets were packaged into identical sealed white bags and labelled as Control and Diet 2 for the MEAT or Diet 1 for the PLANT. The investigators and participants were blinded to the identity of the diets. Diet identities were kept by a third person employed at the University of Guelph, who was not involved in data collection, statistical analysis and data interpretation, until statistical analyses were complete.

Nutrient analyses, including proximates (moisture, crude protein, crude fat, ash, crude fibre) and profiles of amino acids, FAs, vitamins and minerals, were performed on the diets post-manufacturing (Appendix III). Description of nutrient analyses is provided in Chapter Six. Food quantity was calculated based on the dog's current dietary intake to match calories and maintain current BW and a gram scale was provided to precisely measure out the recommended quantity of food per day as well as any leftovers. Participants were instructed not to feed their dogs any other food for the duration of the study, with exceptions for some pieces of fruit or vegetables or plant-based treats, which were required to be recorded. Participants were given a list of treats

that could be fed for the duration of the study (plant-based treats, without added micronutrients), an acceptable treat dose was given for each dog to avoid exceeding 10% of their daily energy intake from sources other than the trial diet.

7.3.3 Diet Trial

An initial adaptation period of 4 weeks was performed between the screening and baseline appointments to ensure all dogs started the trial from the same diet and to mitigate variation due to differences in diets. Although all dogs enrolled in the study were fed commercial diets prior to their participation, there was variation of ingredients and nutrient profiles of these diets, including six dogs previously fed commercial plant-based diets. During this adaptation period, all dogs were fed MEAT, packaged in bags labelled “Control”. Upon completion of the 4-week adaptation phase, the dogs were randomly allocated to treatment groups (MEAT or PLANT) prior to the baseline evaluation. Baseline evaluations consisted of a veterinary wellness examination and blood collection for serum metabolomics. Other parameters measured are described in Chapter 7. Participants were advised to fast their dogs for 12 hours prior to the appointment.

After the baseline evaluation, dogs changed to their respective experimental diet – either continuing on the MEAT, labelled as “Diet 2” or starting the PLANT, labelled as “Diet 1”. As the diets were formulated to have matching macronutrient profiles, a slow transition was not performed and the diet was changed all at once. Dogs were fed the trial diets for 12 weeks, then returned at the end of the trial for their exit evaluation. Exit evaluations were the same as the baseline evaluations. Throughout the study, including the 4-week adaptation and the 12-week

experimental period, participants were asked to record quantity of food offered, quantity of food eaten, amount and type of snacks or treats provided, frequency of defaecation, faecal condition score, BCS, BW, duration of walks or play activity, and any other notable events in a daily diary. Owners were asked to weigh their dogs on scales. Faecal condition score and BCS charts were provided (WSAVA, 2013b, Anonymous, 2017).

Originally, the diet trial was scheduled to be completed before the end of September, 2020. However, as a result of the global COVID-19 pandemic, the University of Guelph closed down for research and the diet trial was paused in March, 2020, until research was allowed to resume in July, 2020 with implementation of appropriate public health protocols. Dogs participating in the trial were maintained on the diet, either MEAT or PLANT depending on their phase of the study (acclimatisation or trial) and experimental group, in order to allow immediate resumption of data collection when the facilities were made available again. This induced variation in the duration of the trial for some dogs.

7.3.4 Blood collection

At the baseline and exit timepoints, blood was collected from the saphenous or jugular vein. Venipuncture was performed via the saphenous or jugular vein, depending on dog size, using a 22G BD Vacutainer® needle (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Blood was collected for serum metabolomics into BD red top Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) with no additives. After clotting for 30 minutes at room temperature, serum was separated from whole blood in plain red top tubes by centrifugation at 1,500G in a refrigerated centrifuge set to 4°C.

Sera aliquots were immediately frozen at -80°C in 1.5mL Eppendorf tubes and stored until analyzed.

7.3.5 Serum metabolomics

All samples were batched once sample collection was complete. For organic acids and their derivatives, lipids, lipid-like molecules, one-carbon and folate pathway metabolites, sera aliquots were shipped frozen on dry ice to the Metabolomics Innovation Centre laboratories at the University of Alberta (Edmonton, Alberta, Canada) and the University of Victoria (Victoria, British Columbia, Canada). For FA, sera aliquots were analysed at the University of Guelph (Guelph, Ontario, Canada).

7.3.5.1 Organic acids and derivatives, lipids and lipid-like molecules

Detection of organic acids and their derivatives, lipids and lipid-like molecules was performed by DI/LC-MS/MS (TMIC PRIME) assay at the Metabolomics Innovation Centre laboratory at the University of Alberta, as previously described elsewhere (Foroutan et al., 2020). A 96 deep-well plate with a filter plate attached with sealing tape was used. First 14 wells were used for one blank, three zero samples, seven standards and three quality control samples. For all metabolites except organic acid, samples were thawed on ice and were vortexed and centrifuged at 13,000x g. Ten µL of each sample was loaded onto the center of the filter on the upper 96-well plate and dried in a stream of nitrogen. Subsequently, phenyl-isothiocyanate was added for derivatization. After incubation, the filter spots were dried again using an evaporator. Extraction of the metabolites was then achieved by adding 300 µL of extraction solvent. The extracts were obtained by centrifugation into the lower 96-deep well plate, followed by a dilution step with MS

running solvent. For organic acid analysis, 150 μL of ice-cold methanol and 10 μL of isotope-labeled internal standard mixture was added to 50 μL of sample for overnight protein precipitation. Then it was centrifuged at 13000x g for 20 min. 50 μL of supernatant was loaded into the center of wells of a 96-deep well plate, followed by the addition of 3-nitrophenylhydrazine (NPH) reagent. After incubation for two hours, BHT stabilizer and water were added. Next, targeted quantitative analysis of samples was performed using a combination of direct injection mass spectrometry with a reverse-phase liquid chromatography (LC) – tandem mass spectrometry (MS/MS) custom assay. Mass spectrometric analysis was performed on an ABSciex 4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with an Agilent 1260 series ultra-high performance liquid chromatography (UHPLC) system (Agilent Technologies, Palo Alto, CA). The samples were delivered to the mass spectrometer by a LC method followed by a direct injection (DI) method. A total of 143 metabolites were screened, data analysis was done using Analyst 1.6.2. The measured analytes included: amino acids (alanine, arginine, asparagine, aspartic acid, citrulline, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, sarcosine, serine, threonine, tryptophan, tyrosine, valine), amino acid derivatives and metabolites (alpha-amino adipic acid, alpha-ketoglutaric acid, asymmetric dimethylarginine, betaine, choline, creatine, creatinine, homovanillic acid, HPHPA, indole acetic acid, kynurenine, methionine-sulfoxide, methylhistidine, putrescine, total dimethylarginine, trans-hydroxyproline, serotonin, spermidine, spermine, taurine, uric acid), lipid metabolites (acylcarnitines C0-C18:2, phosphatidylcholines C32:2-C40:6, lysophosphatidylcholines C14:0-C28:1, sphingolipids C14:1-C24:1, beta-hydroxybutyric acid,

butyric acid, isobutyric acid and propionic acid) and carbohydrate and TCA cycle metabolites (beta-hydroxybutyric acid, citric acid, fumaric acid, glucose, lactic acid, methylmalonic acid, pyruvic acid and succinic acid). Analytes were categorized as protein and amino acid metabolites, lipid and lipid-like metabolites, or carbohydrate and TCA cycle metabolites, based on descriptions of each compound available via MetaboAnalyst^b pathway analysis, the Metabolomics Innovation Centre Human Metabolome Database^c, and the National Library of Medicine PubChem^d platforms.

7.3.5.2 One-carbon and folate pathway metabolites:

Detection of one-carbon and folate pathway metabolites was performed by LC-MRM/MS (TMIC One-carbon and Folate Metabolism Pathway) at the Metabolomics Innovation Centre laboratory at the University of Victoria. For cobalamin, folate, s-adenosylmethionine, s-adenosylhomocysteine, folate and derivatives, samples were thawed on ice, then 50uL of serum was mixed with 50uL of internal standard solution (containing SAH-D4, 200-mM ammonium formate and 10g/L ascorbic acid) and vortexed. 150uL of acetonitrile was added, then samples were vortexed again, followed by sonication in an ice-water bath for 1 minute, prior to centrifugation at 21,000g for 15 minutes. Supernatant was collected and then dried under nitrogen, then the residue resuspended in 50uL of 10% methanol. After centrifugation, 10uL of the supernatant and standard solutions containing reference standards of the targeted compounds were injected into a Waters Acquity UPLC system coupled to a Sciex XTRAP 65000 Plus mass spectrometer operated in the positive-ion mode. Liquid chromatography separation was performed on a C18 UPLC column (2.1 x 100mm, 1.8 um) with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as the binary solvents for gradient elution (2% to 25% B

in 12 min) at 40C and 0.3mL/min. Concentrations of the detected metabolites were calculated with internal standard calibration by interpolating the constructed linear-regression curves of individual compounds with the analyte to internal standard peak ratios measured from each sample solution. The measured analytes included: acetylcholine, betaine, cobalamin, choline, cystathionine, cystine, cysteine, dihydrofolic acid, folic acid, glutathione disulfide, glutamic acid, glutamine, glycine, homocystine, methionine, methylmalonic acid, niacin, pyridoxine, riboflavin, s-adenosylhomocysteine, s-adenosylmethioninamine, adenosylmethionine, serine, tetrahydrofolic acid (THF) and 5-methyltetrahydrofolic acid.

For taurine and homoserine, samples were thawed on ice, then 10uL were mixed with 25uL of acetonitrile. The mixture was vortexed then sonicated for 1 minute in an ice-water bath, followed by centrifugation at 21,000g at 5C for 15 minutes. 20uL of supernatant or standard solution (0.0005 to 100 uM reference standards of targeted compounds) was mixed with 20uL of the internal standard solution (containing ¹³C or deuterium-labelled targeted amino acids), 40 uL of 20 mM dansyl chloride solution and 40 uL of pH=9 borate buffer. The mixtures were allowed to react at 40C for 30 min, prior to mixing with 480 uL of 50% acetonitrile. 10 uL aliquots of each resultant solution and each standard solution were injected in a Waters Acquity UPLC system coupled to a Sciex QTRAP 4000 mass spectrometer operated in the positive-ion mode. LC separation was carried out on a C18 UPLC column (2.1 x 150mm, 1.8 um) with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile/isopropanol (B) as the binary solvents for gradient elution (20% to 50% B in 10 min) at 55C and 0.3mL/min. Concentrations of the detected metabolites were calculated with internal standard calibration by interpolating the

constructed linear-regression curves of individual compounds with the analyte to internal standard peak ratios measured from each sample solution.

For glutathione, homocysteine, betaine and choline, samples were thawed on ice, then 15 uL was mixed with 35 uL of acetonitrile and vortexed then sonicated for 10 seconds in an ice-water bath. Samples were centrifuged at 21,000 g at 5C for 15 min, then 20 uL of the supernatant were mixed with 180 uL of the internal standard solution (containing isotope-labeled glutathione, homocysteine, betaine, and choline in 25-mM N-ethylmaleimide). 10 uL aliquots of the resultant solutions and each standard solution were injected into an Agilent 1290 UHPLC system coupled to an Agilent 6495B QQQ mass spectrometer operated in the positive-ion mode. Liquid chromatography separation was carried out on a C18 UPLC column (2.1 x 100 mm, 1.9 um) with a 5-mM ammonium acetate solution (A) and methanol (B) as the binary solvents for gradient elution (0% to 60% B in 15 min) at 45C and 0.25mL/min. Concentrations of the detected metabolites were calculated with internal standard calibration by interpolating the constructed linear-regression curves of individual compounds with the analyte to internal standard peak ratios measured from each sample solution.

7.3.5.3 Serum fatty acids

Once sample collection was complete, sera were transported frozen to the University of Guelph Human Health and Nutrition Gas Chromatography Laboratory (Guelph, Ontario, Canada). Lipids were extracted by organic solvent in chloroform and methanol, then FA were saponified and methylated using boron trifluoride. Methylated FA were separated and analyzed by gas chromatography. C17:0 was added as an internal standard for quantification.

The measured FA included: saturated FA (SFA: 12:0 [lauric acid], 14:0 [myristic acid], 15:0 [pentadecylic acid], 16:0 [palmitic acid], 17:0 [margaric acid], 18:0 [stearic acid], 20:0 [arachidic acid], 21:0 [heneicosylic acid], 22:0 [behenic acid], 23:0 [tricosylic acid], 24:0 [lignoceric acid]), monounsaturated FA (MUFA: 14:1, 16:1c9, 18:1c9 [oleic acid], 18:1c11, 18:1c12, 20:1c5, 20:1c8, 20:1c11, 22:1n-9 [erucic acid], 24:1n-9 [nervonic acid]), polyunsaturated FA (PUFA: 18:2c/c [conjugated linoleic 1] 18:2c/c [conjugated linoleic 2], 18:2c9c14, 18:2c9c15, 20:3n-9 [mead acid]) omega-3 PUFA (18:3n-3 [alpha-linolenic acid], 20:3n-3 [eicosatrienoic acid], 20:5n-3 [eicosapentaenoic acid, EPA], 22:3n-3, 22:5n-3 [docosapentaenoic acid], 22:6n-3 [docosahexaenoic acid, DHA]), omega-6 PUFA (18:2n-6 [linoleic acid], 18:3n-6, [gamma-linolenic], 20:2n-6 [eicosadienoic acid], 20:3n-6 [dihomogamma-linolenic], 20:4n-6 [arachidonic acid], 22:2n-6 [docosadienoic acid], 22:4n-6 [adrenic acid], 22:5n-6 [n-6 docosapentaenoic acid]), and trans FA (16:1t9 [palmitoelaidic], 18:1t4, 18:1t5, 18:1t6-8, 18:1t9 [elaidic acid], 18:1t10, 18:1t11, 18:1t12, 18:1t13&14, 18:1t16, 18:2ct, 18:2tt [linoelaidic], 18:2tt [conjugated linoeleic acid], 18:2c9t11 [conjugated linoleic acid], 18:2c9t12, 18:2t9t12, 18:2c9t13, 18:2t9c12, 18:2t10c12 [conjugated linoleic acid], 18:2c11t13 [conjugated linoleic acid]).

7.3.6 Statistical analyses

All analyses were performed using commercial statistical software (StataIC, StataCorp, College Station, Texas, USA and MetaboAnalyst 5.0, www.metaboanalyst.ca).

Using Stata, independent variables (dog sex, age, weight, BCS and season) were tested for normality of distribution using Shapiro-Wilk normality test and visual evaluation of

frequency histograms and normal probability plots. Differences in distribution of independent variables (age, sex, BCS) between diet groups after randomization was tested using *t* test for parametric and Wilcoxon rank-sum test for non-parametric data. Dependent variables (metabolites) were tested for normality of distribution using Shapiro-Wilk normality test and visual evaluation of frequency histograms and normal probability plots. Non-normally distributed variables were log transformed prior to analyses. Differences in metabolites between dogs of different age, sex and body condition score were tested for using ANOVA and post-hoc Tukey test. Univariate analyses were performed via repeated measures mixed model, using residual maximum likelihood, with dog ID as the variable on which data were repeated. For some variables (leucine, tryptophan, TMAO, THF) there were differences detected between groups at baseline, thus baseline was included as a covariable in all analyses. Post hoc analyses were performed by contrasting main effects and graphing the interactions. As multiple comparisons were made, the critical cut-off for P-values were adjusted to control for false discovery rate of less than or equal to 10% according to the Benjamini Hochberg procedure as recommended for bioinformatics data (Jafari and Ansari-Pour, 2019).

Data with significant associations detected on repeated measures mixed model were uploaded to the Metaboanalyst platform and normalized for comparison by row-wise normalization (median or quantile normalization), mathematical transformation (log or square root) and/or data scaling (autoscaling) as appropriate. Visual inspection of density plots and box plots was performed to evaluate normalization technique. Overview of data was visualized using interactive principal component analysis and two-way heatmaps were generated with Euclidean distance measure and Ward clustering algorithm.

7.4 Results

7.4.1 Group randomization and trial completion

The independent variables considered for each group were sex, age, BW, BCS and the seasons during which the dog was participating in the study. After randomization, there distribution of independent variables between groups did not differ (Appendix IIV). Breeds and breed mixes did not differ between the two groups (Appendix II). Drop-outs from the trial are described in Chapter 7. A total of 61 dogs completed the trial: 31 in the PLANT group and 30 in the MEAT group.

7.4.2 Serum metabolites

143 serum metabolites were screened by DI/LC-MS/MS (TMIC PRIME) assay and 31 by LC-MRM/MS (TMIC One-carbon and Folate Metabolism Pathway). Thiamine, pyridoxamine, hypotaurine, histamine, phenylethylamine, cis-hydroxyproline, dopamine, DOPA, carnosine, nitro-tyrosine, diacetylspermine, tyramine, phosphocreatine and p-hydroxyhippuric acid were measured, but excluded from analyses as concentrations were below the detection limits.

7.4.2.1 Protein and amino acid metabolites

A total of 47 amino acids, amino acid derivatives and protein metabolites were analyzed at the University of Alberta. Based on univariate mixed modelling, amino acids and protein metabolites were determined to be affected by time, diet, or the interaction of time and diet (Table 7.1, Figure 7.1). All metabolites affected by time, diet or the interaction of time and diet as identified on mixed modeling were included for further analyses using the MetaboAnalyst

platform. A heatmap was generated to visualize differences between the diet groups and timepoints (Figure 7.3).

7.4.2.2 Folate pathway and 1-C metabolites

A total of 29 folate pathway and one-carbon metabolites were measured at the University of Victoria. Based on univariate mixed modelling, 9 analytes were determined to be affected by time, group, or the interaction of time and group (Table 7.2, Figure 7.4). A heatmap was generated including all folate pathways and one-carbon metabolites of interest identified by univariate repeated measures mixed modelling (Figure 7.5).

7.4.2.3 Carbohydrate metabolites and TCA cycle intermediates

Sixteen carbohydrate and TCA cycle intermediates were analyzed at the University of Alberta. Based on univariate mixed modelling, only three analytes were determined to be affected by time, group, or the interaction of time and group (Table 7.3). In both diet groups, alpha-ketoglutaric acid and succinic acid increased over time. Due to a dearth of significant effects identified by repeated mixed measures models, carbohydrate data were not further analysed using MetaboAnalyst.

7.4.2.4 Fatty acids

A total of 61 FA were measured at the University of Guelph. On univariate repeated measures mixed modelling, individual FA, omega 6:3 ratio, and total FA were found to be affected by time or the interaction of time and group (Table 7.4, Figures 7.7-7.10). All fatty acids affected by time, diet or the interaction of time and diet as identified on mixed modeling were

included for further analyses using the MetaboAnalyst platform. A heatmap was generated to visualize differences between the diet groups and timepoints (Figure 7.11).

7.4.2.5 Lipid metabolites

A total of 78 lipid metabolites and lipid-like molecules were measured at the University of Alberta. Based on univariate mixed modelling, phosphatidylcholines (PC), lysophosphatidylcholines (LPC), sphingomyelins (SM) and acylcarnitines, were determined to be affected by time, group, or the interaction of time and group (Table 7.5, Figures 7.12-7.15). In both diet groups, SM(OH) C14:1 and SM(OH) C16:1 decreased over time. All lipid metabolites affected by time, diet or the interaction of time and diet as identified on mixed modeling were included for further analyses using the MetaboAnalyst platform. A heatmap was generated to visualize differences between the diet groups and timepoints (Figure 7.16).

7.5 Discussion

Serum metabolomics, comprising 204 protein and amino acid derivatives, carbohydrate and TCA cycle intermediates, folate pathway and one-carbon donors, and fatty acid and lipid metabolites, were compared between dogs fed extruded PLANT or MEAT diets. Few differences were detected between diet groups in most pathways, with the exception of fatty acids and lipid metabolites. Most fatty acids and lipid differences coincided with differences in the fat composition of the diets.

Protein quantity and quality have been reported to be of concern in plant-based diets for dogs (Kanakubo et al., 2015, Dodd et al., 2018, Dodd et al., 2021b). However, in this study, dogs fed the PLANT diet demonstrated few differences in protein metabolism compared to the

MEAT-fed dogs. Over the duration of the trial, the dogs fed the PLANT diet demonstrated decreases in the amino acids ornithine, glycine, isoleucine and valine, as well as the amino acid metabolites 3-methylhistidine, trans-hydroxyproline, serotonin, creatinine and dcSAM. Serum acetylmethionine and alpha-aminoadipic acid increased in the same group. With the exception of isoleucine, serum concentrations of all amino acids, derivatives and metabolites were within the ranges reported for apparently healthy adult dogs^b (Kathrani et al., 2018).

The reduction in serum isoleucine demonstrated by dogs fed the PLANT diet was despite a higher provision of isoleucine in PLANT compared to the MEAT diet (0.27 vs 0.23 g/100kcal), suggesting components of decreased absorption, decreased bioavailability, increased intracellular flux, and/or increased consumption. Valine content of the PLANT diet was also higher than in the MEAT diet (0.33 vs 0.30 g/100kcal), yet serum valine levels also decreased in the PLANT dogs. Both isoleucine and valine are branched chain amino acids (BCAA), and these differences in serum concentrations may suggest a difference in BCAA metabolism in dogs fed the PLANT diet. Indeed, this was consistent with other findings (Chapter Seven). Isoleucine and valine's main roles in the body are as a constituent of proteins, with the documented signs of deficiency being depression in food intake, weight loss and, for isoleucine only, a negative nitrogen balance in immature beagles (Milner, 1979b, Burns et al., 1984, Milner, 1979a). In our study, as reported in chapter 7, dogs in both groups maintained food intake, body weight and body composition throughout the study, suggesting that, although serum concentrations were lower than the MEAT-fed dogs, BCAA metabolism seemed sufficient to maintain normal protein synthesis in the dogs fed the PLANT diet over the three month trial. Supporting this was the concurrent reduction in serum 3-methylhistidine, a metabolite formed from post-translational methylation of

histidine in muscle proteins, which is released when muscle protein is catabolized (Hill et al., 2001, Li et al., 2021). This, along with a lack of increase in beta-hydroxybutyric acid, a degradation metabolite of BCAA suggests no increase in muscle and/or BCAA catabolism. Nevertheless, further investigation into BCAA metabolism in dogs fed plant-based diets is warranted to determine the underlying cause for the reduction in serum BCAA.

Creatinine is a protein metabolite produced from creatine and phosphocreatine and excreted with little reabsorption or secretion through the kidneys (Yamamoto et al., 2019). Creatinine synthesis has been reported to be closely associated with lean body mass, especially skeletal muscle, with little influence of dietary protein intake (Borsook and Dubnoff, 1947, Yamamoto et al., 2019, Freeman III et al., 2003). Changes in serum creatinine concentrations thus indicate changes in muscle mass, protein catabolism, and renal clearance (Médaille et al., 2004, Yamamoto et al., 2019, Freeman III et al., 2003). In the dogs fed the PLANT diet, serum creatinine decreased to become significantly lower than the MEAT-fed dogs by the exit timepoint. Considering that bodyweight, BCS, MCS and body composition were maintained in both groups (see Chapter 7), the reduction in serum creatinine was not consistent with a reduction in muscle mass. This suggests that either dietary creatinine intake was reduced, protein catabolism was reduced and/or renal clearance was improved. Though levels of creatinine were not measured in the diet, it is likely that intake was lower in the dogs fed PLANT as opposed to MEAT. Creatinine can be formed from creatine, which, as mentioned, is contained in high concentrations in skeletal muscle, during heat processing. Ingestion of cooked meat, but not vegetarian meals, has been demonstrated to increase serum creatinine levels postprandially in humans (Pimenta et al., 2016). However, all dogs were fasted prior to blood collection at each

timepoint, negating acute post-prandial effects. This could suggest that reduced protein catabolism or increased renal clearance may contribute to the decreased serum creatinine concentration in dogs fed the PLANT diet. This would be in agreement with the concurrent reduction in 3-methylhistidine as mentioned above. Further research is thus warranted to investigate protein conservation and renal function in dogs fed a plant-based diet.

The sulfur amino acids methionine and cysteine have been a recent area of research in canine nutrition, spurred by concern for potential dietary-associated dilated cardiomyopathy secondary to insufficiency of taurine. There were minimal changes to the sulfur amino acid metabolites in the PLANT-fed dogs, and serum taurine concentrations were maintained.

Hydroxyproline, a derivative from post-translational modification of proline by prolyl hydroxylase, is the major component of collagen and plays an essential role in the quaternary triple helix structure (Hofman et al., 2011, Yamauchi and Sricholpech, 2012). Given the abundance of hydroxyproline in animal tissues (Li and Wu, 2018), lower serum levels of trans-hydroxyproline in the PLANT dogs can likely be explained by lower dietary intake, although hydroxyproline concentration in the trial diets was not measured. It is possible that conservation of tissue collagen with reduced degradation and/or improved renal clearance may also have contributed to the lower serum trans-hydroxyproline in dogs fed the PLANT diet, though lower dietary intake was likely a large contributing factor (Siddiqi et al., 2011, Siddiqi and Alhomida, 2006). Similarly, serum glycine concentrations also decreased in the PLANT group, though serum glycine concentrations were measured to be substantially lower in the PLANT diet than the MEAT diet (0.30 vs 0.60 g/100kcal), and thus likely also reflects lower dietary intake.

Conversely, serum acetylmethionine concentrations increased in dogs fed the PLANT diet. This may account for the reduction of ornithine, if a greater proportion of ornithine were acetylated. Acetylmethionine is a precursor substrate for arginine synthesis, though dogs cannot meet their requirements with *de novo* synthesis and have a requirement for dietary arginine. In this trial, arginine was provided in a slightly lower concentration in PLANT than in the MEAT diet (0.43 vs 0.48 g/100kcal). Potentially acetylation of ornithine to acetylmethionine was upregulated to increase *de novo* synthesis of arginine, since serum arginine levels were comparable between groups, despite a lower dietary intake in the PLANT group. Additionally, in humans, serum acetylmethionine has been demonstrated to be a marker of vegetable intake (Playdon et al., 2017). This could be the case in dogs as well, considering the vegetable content of the plant-based trial diet.

Concentrations of serum folate and most of the folate pathway metabolites did not differ between dogs fed the PLANT or MEAT diet, indicating little difference in one-carbon metabolism between the two groups. Only S-adenosylmethioninamine (dcSAM), betaine methylmalonic acid differed. dcSAM is the decarboxylated metabolite of S-adenosylmethionine (SAME), the main methyl donor in the body, and is the precursor for biosynthesis of the polyamines spermine and spermidine, from arginine, via ornithine and putrescine (Pegg, 1986, Soda, 2018). dcSAM is thus the link between one-carbon metabolism and polyamine synthesis. The main roles of polyamines are as intracellular anti-oxidants and anti-inflammatory agents, though they may also play a role in gene methylation (Soda, 2018). Polyamine synthesis is strictly regulated, with intracellular spermine concentrations negatively feeding back on the rate-limiting enzyme, ornithine decarboxylase, resulting in increased SAME and decreased dcSAM

concentrations. Decreases in dcSAM concentrations have been associated with suppression of aberrant methylation (Soda, 2018). It is possible that the dogs fed the PLANT diet had altered polyamine metabolism, resulting in lower decarboxylation of SAmE to dcSAM, though serum SAmE levels were unaffected. Microbes also possess dcSAM, with spermine and spermidine also associated with microbial degradation of proteins and free amino acids in food items (Montegiove et al., 2020). As lower levels of microbial protein degradation may be expected for a plant-based protein source than an animal-based one, it is possible that there was reduced intake of dcSAM directly in the PLANT fed dogs (Montegiove et al., 2020). Despite a lower concentration of serum dcSAM in the PLANT group, spermine and spermidine did not differ from those of the MEAT-fed dogs.

Methylmalonic acid (MMA) is a sensitive indicator of cobalamin deficiency, as it accumulates when cobalamin-dependent enzymatic reactions are impaired (Savage et al., 1994, Mason, 2003). Mean serum MMA in the PLANT-fed dogs increased by 20% in the PLANT group, and was 15% higher than the MEAT-fed dogs at the exit timepoint. Yet, the serum MMA concentration in the PLANT group was still within the low end of the reference interval for healthy dogs with normal cobalamin status (Berghoff et al., 2012). Furthermore, serum cobalamin levels were also within normal range for healthy dogs and did not differ between diet groups. Increased MMA concentrations have been demonstrated in dogs and cats with normal serum cobalamin concentrations, prompting a hypothesis of cellular cobalamin deficiency and impaired cobalamin-dependent metabolism in the face of normal serum cobalamin status (Berghoff et al., 2012, Ruaux et al., 2009). However, the levels of MMA reported in dogs with hypocobalaminaemia or cellular cobalamin deficiency were ten fold higher than the MMA

reported in the dogs fed the PLANT diet in this trial. Thus, cellular cobalamin deficiency is unlikely to explain the changes in MMA. The cobalamin content in the PLANT diet was half the concentration of the MEAT diet; potentially, the increase in serum MMA could be associated with lower dietary cobalamin intake.

Betaine, which can be derived from the diet or endogenously synthesized from choline, functions in the one-carbon pathway to re-methylate homocysteine to methionine, without utilising 5-methyl-tetrahydrofolate. Elevated levels of betaine have been found in dogs fed methionine-restricted diets (Harrison et al., 2020). As mentioned, sulfur amino acid metabolism appeared largely undisturbed in the PLANT-fed dogs, with no differences in methionine levels detected between groups. Potentially, the elevated betaine could indicate increased recycling of methionine via methylation of homocysteine, although homocysteine levels were unaffected, unlike dogs fed methionine-restricted diets, where elevated betaine was found in conjunction with decreased homocysteine (Harrison et al., 2020). This could possibly be explained as although the PLANT diet contained less methionine than the MEAT diet (Appendix III), dietary provision exceeded minimum recommendations and thus the shift in metabolism may have reflected the change in dietary provision without a need to compensate for inadequate intake.

Of all the classes of nutrients, FA and lipid metabolites were the most affected by diet in this trial, with one third of FA and one third of lipid metabolites differing between diet groups at the end of the trial. Many of the serum lipid profile changes could be explained by differences in the fatty acid composition of the diets (Appendix III). Dietary provision of shorter-chain SFA (14:0, 16:0, 18:0) were lower in the PLANT diet, while longer SFA (22:0 and 24:0) were higher,

corresponding mostly with the changes in the serum profile. One exception was tricosanoic acid (23:0), which was higher in the PLANT diet yet serum 23:0 decreased in the PLANT group. Total saturated FA were lower in the PLANT diet and lower in the serum of the PLANT dogs. Similarly, the serum profile of the *cis*-MUFA (16:1c9, 18:1c11 and 18:1c9) matched the dietary fatty acid profile. Dietary levels of 18:1t6-8 were lower in the PLANT diet, yet higher in the serum of the PLANT-fed dogs. Conversely, dietary 18:1t13&14 were higher in the PLANT, yet lower in the serum of the PLANT dogs.

Changes in serum n-6 and n-3 PUFA levels also matched dietary content, with the PLANT diet providing lower levels of n-6 and higher levels of n-3 PUFA. The serum FA profiles of the dogs changed accordingly, with the PLANT dogs demonstrating reductions in individual n-6 PUFA, increase in 22:6n3 (DHA) and an overall reduction in the serum n-6:n-3 ratio. In dogs, the long-chain n-3 PUFA, EPA and DHA, have been noted to provide important health benefits, including reduction of serum cholesterol and triglycerides, improved renal perfusion, reduced inflammation associated with osteoarthritis, obesity, cardiovascular, and inflammatory or immunologic skin disorders (Bauer, 2011, Streeter et al., 2015). Furthermore, low serum DHA and a higher n-6:n-3 ratio have been suggested to be associated with pathological aggression in male German Shepherd dogs (Re et al., 2008). The FA profile changes may represent a health benefit in this case for the PLANT compared to the MEAT. However, this is not necessarily a benefit due to the plant-based nature of the diet, *per se*, as EPA and DHA are not commonly found in plant-derived ingredients. In this trial, the PLANT diet included dried marine algae, a concentrated source of DHA, in comparison to MEAT, in which the only marine-derived ingredient was salmon meal. Salmon meal is a concentrated source of

protein while providing limited amounts of long-chain n-3 PUFA. Thus, the finding of increased DHA and the reduced n-6:n-3 ratio may not be a finding translatable to plant-based diets in general depending on the fatty acid sources used in the diet formulation.

In addition to serum fatty acid profile, three classes of lipid metabolites were investigated: phosphatidylcholines (PC) and lysophosphatidylcholines (LPC), sphingomyelins (SM), and acylcarnitines (AC). Phosphatidylcholines are a group of phospholipids composed of a choline 'head' and FA 'tails', and are commonly found in biological membranes, blood lipoproteins and natural surfactants. These phospholipids play roles in endogenous cholesterol and triacylglyceride transport and cellular signalling, growth and apoptosis (Hu et al., 2020, Quell et al., 2019). The FA composition of PC impacts cell membrane fluidity and may be influenced by nutrition as well as by disease states (Melchert et al., 1987, Jacobsson et al., 1990, Sanders et al., 1978, Quell et al., 2019). In this study, phosphatidylcholine aa C40:6 (18:1n-9/22:5n-6), with unsaturated FA in both positions, was found to be the most abundant. This was followed by the unsaturated/saturated PC aa 38:6 (22:6n-3/16:0), doubly saturated PC aa C36:0 (14:0/22:0), and saturated/unsaturated PC ae C40:6 (18:0/22:6n-3). The PLANT dogs had lower proportion of doubly unsaturated PC aa 32:2 (16:1n-7/16:1n-7) and higher proportion of double saturated PC ae 36:0 (16:0/20:0), PC aa 38:0 (16:0/22:0), doubly unsaturated PC aa 40:6 (18:1n-9/22:5n-6) and PC aa 40:2 (20:1n-9/20:1n-9), saturated/unsaturated PC ae 40:6 (18:0/22:6n-3) and unsaturated/saturated PC aa 40:1 (24:1n-9/16:0). Potentially, the increase in most of the phosphatidylcholine species may be attributable to a higher concentration of choline in the PLANT diet than the MEAT diet. As well, many of the FA species of PC elevated in the dogs fed the PLANT diet corresponded with higher concentrations of those FA in the PLANT diet as

compared to the MEAT diet, apart from the short chain saturated FA. In humans, PC concentrations of 16:0, 18:1, 20:5n-3, 22:5n-3, and 22:6n-3 FA were demonstrated to be lower in vegans, with higher proportions 18:2n-6, 20:2n-6, 20:4n-6 and 22:4n-6, and a lipid metabolite profile suggested to be protective against angina pectoris, hyperlipidemia and ischaemic heart disease (Sanders et al., 1978). These findings were not replicated in the dogs, as the PLANT dogs only had lower PC 16:1n-7, but had higher quantities of PCs with saturated FA 16:0, 18:0, 20:0, 22:0 and unsaturated FA 18:1n-9, 20:1n-9, 22:5n-6, 22:6n-3 and 24:1n-9.

Hydrolysis of phosphatidylcholines by lysophosphatidylcholine acyltransferase (LPCAT) enzymes result in production of LPC, which can in turn be reacylated by LPCAT back into PC (Kazachkov et al., 2008). These lipid molecules are in a fluctuant remodelling process, with PC FA constantly being freed and recombined. In our study, the most prevalent LPCs were 16:0 and 18:0. Lysophosphatidylcholine a 18:2 and 18:1 were also found in high concentrations. Interestingly, changes in LPC concentrations did not always match changes in PC concentrations. Despite higher concentration of PC 16:0 and 20:4, PLANT dogs had lower proportions of LPC16:0 and 20:4. This potentially may indicate reduced hydrolysis of these PC in particular. It is possible the increased PC 18:1 and simultaneously increased LPC 18:1 in PLANT dogs represented a greater abundance of monounsaturated 18 carbon FA available for PC production, as well as a preference for hydrolysis of PC 18:1 to LPC 18:1. Previously, it has been demonstrated that higher 18:1-CoA concentrations could inhibit the acylation activity of a particular LPCAT enzyme (Kazachkov et al., 2008), perhaps partially explaining the increase in LPC 18:1 corresponding with the increased PC 18:1.

Sphingomyelins are major components of cell membranes, comprised of a phosphatidylcholine or phosphatidyl ethanolamine linked to a sphingosine ceramide backbone and a FA (Li et al., 2020). Sphingomyelin roles in membrane fluidity and cellular signalling appear to be influenced by the level of saturation or desaturation of their FA chains (Bienias et al., 2016, Li et al., 2020). In other species, alterations of SM metabolism have been associated with insulin resistance, obesity, cardiovascular disease, neoplasia, and central nervous system diseases (Hu et al., 2020). Sphingomyelins with longer saturated FA chains have been associated with reduced risk of heart disease and heart failure in humans in comparison to those with shorter saturated FA chains (Peterson et al., 2018, Lemaitre et al., 2019). In the current study, SM 16:0 was the most abundant, followed by SM 18:0, SM(OH) 22:1, and SM 16:1 and 18:1. Eight SMs were decreased in the dogs fed the PLANT diet. In the present study, the dogs with the PLANT diet had lower levels of SMs with short-chain saturated FA C16:0 and 18:0, but also lower levels of SMs with unsaturated FA C16:1, 18:1, 20:2, 22:1 and 24:1. This corresponded closely with lower concentrations of 16:0, 18:0, 16:1, and 20:2 FA, though not with the higher 18:1 FA, in the PLANT diet. In a canine study, lower levels of SM 16:0 were associated with slowed progression of canine myxomatous mitral valve disease in dogs fed a cardioprotective diet (Li et al., 2020). However, those dogs also had elevated levels of SM 20:0, 22:0 and 24:0. The longer chain saturated SMs were unfortunately not measured in the current study.

Though carnitine is an amino acid derivative, it has been considered with the lipid metabolites due to its role in fatty acid catabolism. L-carnitine, the mammalian stereoisomer, functions intracellularly to shuttle free FA from the cytosol into the mitochondria for β -oxidation and energy production (Reuter and Evans, 2012). Endogenous carnitine exists in flux with a pool

of various acylcarnitines, the esterified product for fatty acid transportation. Within this pool, carnitine is the predominant metabolite. Carnitine and acylcarnitine homeostasis is carefully balanced by dietary intake, endogenous synthesis and renal tubular reabsorption (Reuter and Evans, 2012). Changes in carnitine and acylcarnitine concentrations may thus be a result of dietary changes, mitochondrial function and renal health. In the PLANT dogs, serum carnitine, individual acylcarnitines and total carnitine levels increased in comparison to the MEAT dogs. Both trial diets were formulated to contain the same added carnitine concentration, though carnitine content was not measured post-production. It would be expected that carnitine levels were higher in the MEAT diet, as it contained animal products that are rich in carnitine, while plant-based ingredients bring negligible dietary carnitine (Reuter and Evans, 2012). In humans, it has been demonstrated that although dietary carnitine intake was markedly lower in people consuming a plant-based diet, endogenous carnitine concentrations were minimally affected with urinary carnitine excretion was markedly lower (Lombard et al., 1989). These findings suggest carnitine levels were sustained by endogenous synthesis and upregulated renal conservation, which may explain the changes seen in the dogs in the current study as well. In one study, dogs with IBD were found to have elevated carnitine levels and total acylcarnitine levels, with a decrease in the ratio of short-chain acylcarnitines (butyrlcarnitine + isobutyrylcarnitine and methylmalonylcarnitine) to free carnitine, in comparison to healthy controls (Xu et al., 2016). In our study, free carnitine was also higher in the PLANT dogs, but total acylcarnitine concentration was comparable between the two groups and the ratios of short-chain acylcarnitines (C2, C3 and C4) to free carnitine did not differ between groups. In working sled dogs, it has been hypothesized that long-chain acylcarnitines, including C14:1, may be exported

by muscle to preserve glucose oxidation and insulin sensitivity, or exported by the liver as preferential energy substrates (Tosi et al., 2021). A link between long-chain acylcarnitines and insulin sensitivity has been demonstrated in humans as well, with higher medium and long-chain acylcarnitine concentrations, including C14:1, associated with reduced risk for gestational diabetes mellitus (Zhao et al., 2020). Considering only free carnitine and C14:1 were slightly increased in the dogs fed the PLANT diet compared to the MEAT-fed dogs, the clinical significance of this finding is unknown.

The findings from this study must be interpreted with consideration of the limitations, discussed in Chapter Six.

7.6 Conclusion

Although much focus is given to the protein and amino acid components of plant-based diets, serum metabolomic assessment showed minimal changes in protein and amino acid metabolism in the dogs fed the PLANT diet in comparison to the MEAT-fed dogs, and most could be explained by differences in diet composition. The association between eating a plant-based diet and changes in BCAA concentration in dogs warrants further investigation, as there was no explanation for the reduction in serum BCAA observed in this study. Furthermore, study of creatinine metabolism and potentially renal function in dogs fed plant-based diets should be considered. There are indications suggesting that plant-based diets could potentially be beneficial for renal health and may help conserve antioxidants such as SAME contribute to a less oxidative state. However, the effect on branched chain amino acid metabolism requires further investigation, as well as possible impairment of vitamin B12-mediated methylmalonyl

conversion. Many differences in FA profile and lipid metabolites were evident in the dogs fed the PLANT diet, yet again most were attributable to differences in the trial diets. Beneficial or detrimental outcomes associated with changes in phospholipids have not been well described in dogs, and at present the clinical significance of the alterations in PC and SM concentrations in the trial dogs are unknown. Further research investigating potential effects of plant-based diets of varying nutritional composition on canine metabolomics are warranted.

7.7 Footnotes:

- a. Petcurean Go! Solutions Skin + Coat Care Chicken Recipe
- b. MetaboAnalyst 5.0, 2021, www.metaboanalyst.ca
- c. Human Metabolome Database, 2021, www.hmdb.ca
- d. PubChem, 2021, www.pubchem.nlm.nih.gov

7.8 References

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7.9 Tables

Table 7.1: Significant results of univariate repeated measures mixed model of time and diet for each amino acid metabolite in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

Interaction	Amino acid metabolite	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	Arginine	PLANT	Exit	-7.94	-14.983- -0.901	0.011
	Glutamine	PLANT	Exit	54.16	8.786-99.537	0.019
	Methionine	PLANT	Exit	-4.32	-7.582- -1.063	0.005
	Proline	PLANT	Exit	-11.67	-20.887- -2.461	0.008
	Cysteine	PLANT	Exit	1.25	0.037-2.455	0.015
	Cystine	PLANT	Exit	-3.62	-5.891- -1.358	0.003
	Cystine	MEAT	Exit	-4.16	-6.461- -1.854	0.001
	Homocysteine	PLANT	Exit	0.04	0.017-0.064	0.001
Time*Group (referent = baseline timepoint)						
	Ornithine	PLANT	Exit	-3.17	-5.003- -1.336	<0.001
	Glycine	PLANT	Exit	-51.03	-68.324- -33.741	<0.001
	Isoleucine	PLANT	Exit	-3.67	-6.60- -0.752	<0.001
	Valine	PLANT	Exit	-14.40	-23.923- -4.883	0.001
	Acetylmethionine	PLANT	Exit	62.76	53.536-71.987	<0.001
	Methylhistidine	PLANT	Exit	-14.04	-18.204- -9.869	<0.001
	Creatinine	PLANT	Exit	-17.04	-22.862- -11.215	<0.001
	Trans-hydroxyproline	PLANT	Exit	-20.80	-26.386- -15.216	<0.001
	Alpha-aminoadipic acid	PLANT	Exit	0.22	0.081-0.354	<0.001
	dcSAM	PLANT	Exit	-0.004	-0.0083- -0.0003	0.008
Group*Time (referent = plant-based diet)						
	Ornithine	MEAT	Exit	3.67	1.814-5.532	<0.001
	Glycine	MEAT	Exit	47.90	30.314-65.489	<0.001
	Isoleucine	MEAT	Exit	6.02	3.026-9.024	<0.001
	Valine	MEAT	Exit	22.31	12.511-32.781	<0.001
	Acetylmethionine	MEAT	Exit	-66.06	-75.374- -56.753	<0.001
	Methylhistidine	MEAT	Exit	14.61	10.404-18.824	<0.001
	Trans-hydroxyproline	MEAT	Exit	19.85	14.155-25.549	<0.001
	Serotonin	MEAT	Exit	0.60	0.328-0.875	<0.001
	Creatinine	MEAT	Exit	17.81	11.939-23.683	<0.001
	Alpha-aminoadipic acid	MEAT	Exit	-0.29	-0.423- -0.147	<0.001
	dcSAM	MEAT	Exit	0.007	0.0031-0.0112	<0.001

dcSAM = S-adenosyl-methioninamine

Table 7.2: Significant results of univariate repeated measures mixed model of time and diet for each folate pathway metabolite in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

Interaction	Folate pathway metabolite	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	5-MTHF	PLANT	Exit	-0.002	-0.0031- -0.0002	0.006
	Cobalamin	PLANT	Exit	3.19e-06	1.33e-06-5.50e-06	<0.001
	Cobalamin	MEAT	Exit	2.87e-06	9.75e-07-4.76e-06	<0.001
	Cystine	PLANT	Exit	-3.62	-5.891- -1.358	0.003
	Cystine	MEAT	Exit	-4.16	-6.461- -1.854	0.001
	Homocysteine	PLANT	Exit	0.04	0.017-0.064	0.001
Time*Group (referent = baseline timepoint)						
	dcSAM	PLANT	Exit	-0.004	-0.0083- -0.0003	0.008
	Betaine	PLANT	Exit	12.51	6.0812-18.9354	<0.001
	MMA	PLANT	Exit	0.13	0.067-0.185	<0.001
Group*Time (referent = plant-based)						
	dcSAM	MEAT	Exit	0.007	0.0031-0.0112	<0.001
	Betaine	MEAT	Exit	-13.04	-19.5365- -6.5513	<0.001
	MMA	MEAT	Exit	-0.12	-0.180- -0.605	<0.001

5-MTHF = 5-methyltetrahydrofolate, dcSAM = S-adenosyl-methioninamine, MMA = methylmalonic acid

Table 7.3: Significant results of univariate repeated measures mixed model of time and diet for each carbohydrate metabolite in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

Interaction	Carbohydrate metabolite	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
	Alpha-Ketoglutaric acid	PLANT	Exit	3.05	1.298-4.804	0.001
	Alpha-Ketoglutaric acid	MEAT	Exit	3.20	1.420-4.984	<0.001
Time*Group (referent = baseline)						
	Citric acid	PLANT	Exit	26.97	12.092-41.843	<0.001

Table 7.4: Significant results of univariate repeated measures mixed model of time and diet for each fatty acid in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

Interaction	Fatty acid	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
Saturated						
	21:0	PLANT	Exit	1.27	0.570-1.964	0.001
Monounsaturated <i>trans</i>						
	18:1t12	PLANT	Exit	-0.30	-0.566- -0.034	0.010
Polyunsaturated						
	22:4n6	PLANT	Exit	-12.55	-23.350- -1.756	<0.001
	22:4n6	MEAT	Exit	14.44	3.075-25.814	<0.001
	22:5n6	PLANT	Exit	2.34	0.970-3.720	0.001
Time*Group (referent = baseline timepoint)						
	Total fatty acids	PLANT	Exit	-544.64	-827.824- -261.459	<0.001
Saturated						
	14:0	PLANT	Exit	-1.63	-2.587- -0.674	<0.001
	16:0	PLANT	Exit	-196.46	-242.305- -150.608	<0.001
	18:0	PLANT	Exit	-109.62	-155.288- -63.951	<0.001
	22:0	PLANT	Exit	5.58	3.898-7.255	<0.001
	23:0	PLANT	Exit	-1.81	-2.737- -0.891	<0.001
	24:0	PLANT	Exit	2.20	0.807-3.587	0.004
	Total saturated	PLANT	Exit	-296.43	-386.561- -206.307	<0.001
Monounsaturated <i>trans</i>						
	18:1t6-8	PLANT	Exit	1.74	1.149-2.336	<0.001
	18:1t16	PLANT	Exit	-0.55	-0.979- -0.114	<0.001
Monounsaturated <i>cis</i>						
	16:1c9	PLANT	Exit	-16.66	-21.895- -11.424	<0.001
	18:1c9	PLANT	Exit	276.66	224.194-329.126	<0.001
	18:1c11	PLANT	Exit	-19.72	-27.756- -11.685	<0.001
	20:1c11	PLANT	Exit	6.19	4.606-7.775	<0.001
Polyunsaturated						
	18:2n6	PLANT	Exit	-271.37	-360.226- -182.522	<0.001
	18:3n6	PLANT	Exit	-5.19	-6.964- -3.421	<0.001
	20:3n6	PLANT	Exit	-5.49	-9.459- -1.522	<0.001
	20:4n6	PLANT	Exit	-195.22	-243.268- -147.176	<0.001
	22:2n6	PLANT	Exit	-0.88	-1.636- -0.119	0.019
	24:1n9	PLANT	Exit	-7.36	-10.271- -4.454	<0.001
	22:6n3	PLANT	Exit	11.62	3.526-19.709	0.001
	n6:n3	PLANT	Exit	-3.13	-4.009- -2.253	<0.001
	n6:n3	MEAT	Exit	1.23	0.306-2.158	0.017
Group*Time (referent = plant-based)						
	Total fatty acids	MEAT	Exit	786.73	461.463-1112.003	<0.001
Saturated						
	14:0	MEAT	Exit	1.62	0.599-2.631	<0.001
	16:0	MEAT	Exit	231.04	180.594-281.482	<0.001
	18:0	MEAT	Exit	138.89	86.357-191.425	<0.001
	22:0	MEAT	Exit	-5.44	-7.347- -3.535	<0.001
	23:0	MEAT	Exit	2.09	1.101-3.072	<0.001
	24:0	MEAT	Exit	-2.63	-4.192- -1.075	0.001
	Total saturated	MEAT	Exit	360.74	257.337-464.141	<0.001

Monounsaturated <i>trans</i>						
	18:1t6-8	MEAT	Exit	-1.61	-2.241- -0.983	<0.001
	18:1t13&14	MEAT	Exit	2.56	1.179-3.946	<0.001
	18:1t16	MEAT	Exit	0.60	0.139-1.053	0.001
Monounsaturated <i>cis</i>						
	16:1c9	MEAT	Exit	23.41	17.901-28.924	<0.001
	18:1c9	MEAT	Exit	-229.40	-293.707- -165.095	<0.001
	18:1c11	MEAT	Exit	26.88	16.783-36.980	<0.001
	20:1c11	MEAT	Exit	-5.12	-6.866- -3.375	<0.001
Polyunsaturated						
	18:2n6	MEAT	Exit	324.41	224.419-424.393	<0.001
	18:3n6	MEAT	Exit	4.90	2.885-6.911	0.001
	20:3n6	MEAT	Exit	9.15	5.040-13.265	<0.001
	20:4n6	MEAT	Exit	247.77	167.957-327.578	<0.001
	24:1n9	MEAT	Exit	7.34	4.132-10.551	<0.001
	22:4n6	MEAT	Exit	20.25	8.997-31.498	<0.001
	22:6n3	MEAT	Exit	-11.63	-20.249- -3.005	0.010
	n6:n3	MEAT	Exit	3.65	2.551-4.747	<0.001

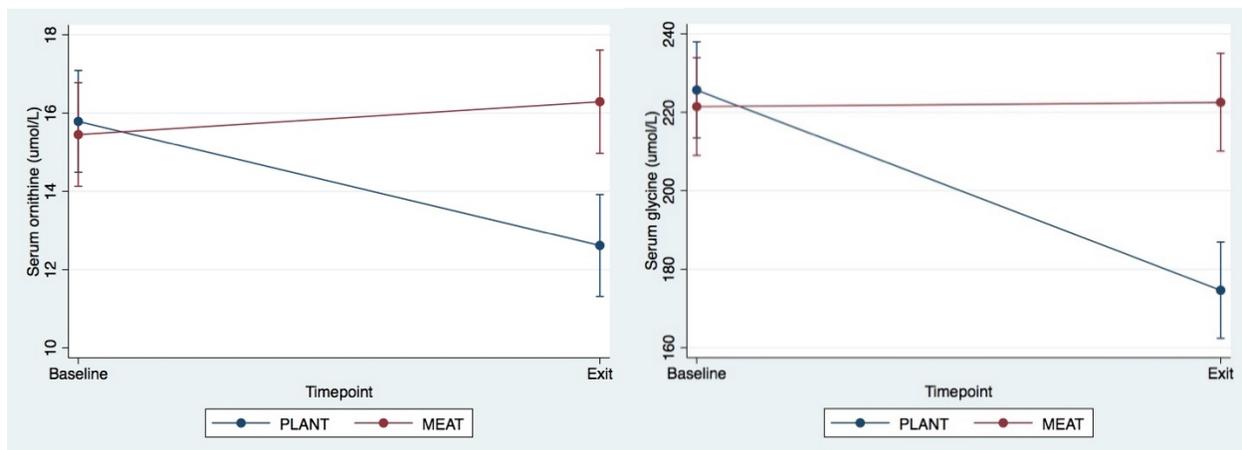
Table 7.5: Significant results of univariate repeated measures mixed model of time and diet for each lipid metabolite in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

Interaction	Lipid metabolite	Diet	Timepoint	Contrast	95% CI	P
Time (referent = baseline timepoint)						
Phosphatidylcholines						
	PC aa C38:0	PLANT	Exit	1.05	0.555-1.539	<0.001
	PC aa C38:0	MEAT	Exit	-0.54	-1.043- -0.043	0.015
	PC aa C40:6	PLANT	Exit	15.08	5.822-24.342	<0.001
	PC aa C40:6	MEAT	Exit	-11.78	-21.190- -2.364	0.008
Lysophosphatidylcholines						
	LPC a C18:0	PLANT	Exit	-5.00	-8.573- -1.426	0.002
	LPC a C18:2	PLANT	Exit	-4.02	-7.061- -0.980	0.009
	SMOH C14:1	MEAT	Exit	-0.81	-1.329- -0.287	0.002
	SMOH C16:1	PLANT	Exit	-0.65	-1.086- -0.208	0.004
	SMOH C16:1	MEAT	Exit	-0.66	-1.104- -0.212	0.004
	C5	PLANT	Exit	0.03	0.007-0.051	0.001
	C16	PLANT	Exit	-0.02	-0.033- -0.009	0.001
	C16:2 (OH)	PLANT	Exit	0.002	0.0063-0.0038	0.006
Time*Group (referent = baseline timepoint)						
Phosphatidylcholines						
	PC aa C32:2	PLANT	Exit	-0.79	-1.052- -0.531	<0.001
	PC aa C36:0	PLANT	Exit	3.07	1.570-4.564	<0.001
	PC aa C40:1	PLANT	Exit	0.12	0.048-0.195	0.001
	PC aa C40:2	PLANT	Exit	0.19	0.070-0.305	0.004
	PC ae 36:0	PLANT	Exit	-0.57	-0.832- -0.303	<0.001
	PC ae C40:6	PLANT	Exit	1.56	0.725-2.389	<0.001
Lysophosphatidylcholines						
	LPC a C16:0	PLANT	Exit	-18.29	-23.140- -13.440	<0.001
	LPC a C18:1	PLANT	Exit	10.72	8.013-13.430	<0.001
	LPC a C20:4	PLANT	Exit	-1.65	-2.536- -0.762	<0.001
	LPC a C24:0	PLANT	Exit	0.06	0.041-0.836	<0.001
Sphingomyelins						
	SM C16:0	PLANT	Exit	-37.13	-48.068- -26.195	<0.001
	SM C16:1	PLANT	Exit	-2.74	-3.714- -1.759	<0.001
	SM C18:0	PLANT	Exit	-9.61	-12.249- -6.970	<0.001
	SM C18:1	PLANT	Exit	-3.03	-3.400- -2.062	<0.001
	SM C20:2	PLANT	Exit	-0.15	-0.203- -0.105	<0.001
	SM (OH) C22:1	PLANT	Exit	-3.15	-4.483- -1.826	<0.001
	SM (OH) C22:2	PLANT	Exit	-2.55	-3.414- -1.691	<0.001
	SM (OH) C24:1	PLANT	Exit	-0.47	-0.680- -0.258	<0.001
Carnitines						
	C0	PLANT	Exit	6.99	3.481-10.498	0.001
	C2	PLANT	Exit	0.55	0.112-0.991	0.007
	C3	PLANT	Exit	0.04	0.011-0.622	0.004
	C4	PLANT	Exit	0.01	0.005-0.023	<0.001
	C14:1	PLANT	Exit	0.03	0.018-0.045	<0.001
	C16:1	PLANT	Exit	0.004	0.0007-0.0068	0.017
Group*Time (referent = plant-based diet)						
Phosphatidylcholines						
	PC aa C32:2	MEAT	Exit	0.81	0.548-1.077	<0.001

	PC aa C36:0	MEAT	Exit	-3.67	-5.188- -2.161	<0.001
	PC aa C38:0	MEAT	Exit	-1.59	-2.086- -1.095	<0.001
	PC aa C40:1	MEAT	Exit	-0.14	-0.218- -0.070	<0.001
	PC aa C40:2	MEAT	Exit	-0.19	-0.306- -0.069	0.002
	PC aa C40:6	MEAT	Exit	-25.74	-35.098- -16.382	0.006
	PC ae C36:0	MEAT	Exit	0.40	0.131-0.665	<0.001
	PC ae C40:6	MEAT	Exit	-2.33	-43.172- -1.493	0.001
Lysophosphatidylcholines						
	LPC a C16:0	MEAT	Exit	15.68	10.791-20.575	<0.001
	LPC a C17:0	MEAT	Exit	-0.26	-0.386- -0.130	<0.001
	LPC a C18:1	MEAT	Exit	-12.34	-15.074- -9.600	<0.001
	LPC a C20:4	MEAT	Exit	1.49	0.599-2.388	0.001
	LPC a C24:0	MEAT	Exit	-0.07	-0.087- -0.044	<0.001
Sphingomyelins						
	SM C16:0	MEAT	Exit	29.91	18.872-40.944	<0.001
	SM C16:1	MEAT	Exit	2.00	1.016-2.992	<0.001
	SM C18:0	MEAT	Exit	9.02	6.329-11.706	<0.001
	SM C18:1	MEAT	Exit	2.77	1.789-3.757	<0.001
	SM C20:2	MEAT	Exit	0.15	0.104-0.202	<0.001
	SM (OH) C22:1	MEAT	Exit	2.13	0.782-3.470	0.002
	SM (OH) C22:2	MEAT	Exit	1.97	1.098-2.840	<0.001
	SM (OH) C24:1	MEAT	Exit	0.37	0.156-0.583	0.001
	C0	MEAT	Exit	-7.05	-10.593- -3.509	<0.001
	C2	MEAT	Exit	-0.48	-0.931- -0.037	0.023
	C3	MEAT	Exit	-0.03	-0.058- -0.006	0.013
	C4	MEAT	Exit	-0.01	-0.019- -0.001	0.020
	C14:1	MEAT	Exit	-0.03	-0.046- -0.018	<0.001
	C16:1	MEAT	Exit	-0.005	-0.0083- -0.0020	<0.001

PC = Phosphatidylcholine, LPC = lysophosphatidylcholine, SM = sphingomyelin, C0 = carnitine, C2 = acetylcarnitine, C3 = propionylcarnitine, C4 = butyrylcarnitine, C5 = valerylcarnitine, C14:1 = tetradecenoyl carnitine, C16 = hexadecanoylcarnitine, C16:1 = dodecadenoylcarnitine, C16:2-OH = hydroxyhexadecadienylcarnitine

7.10 Figures



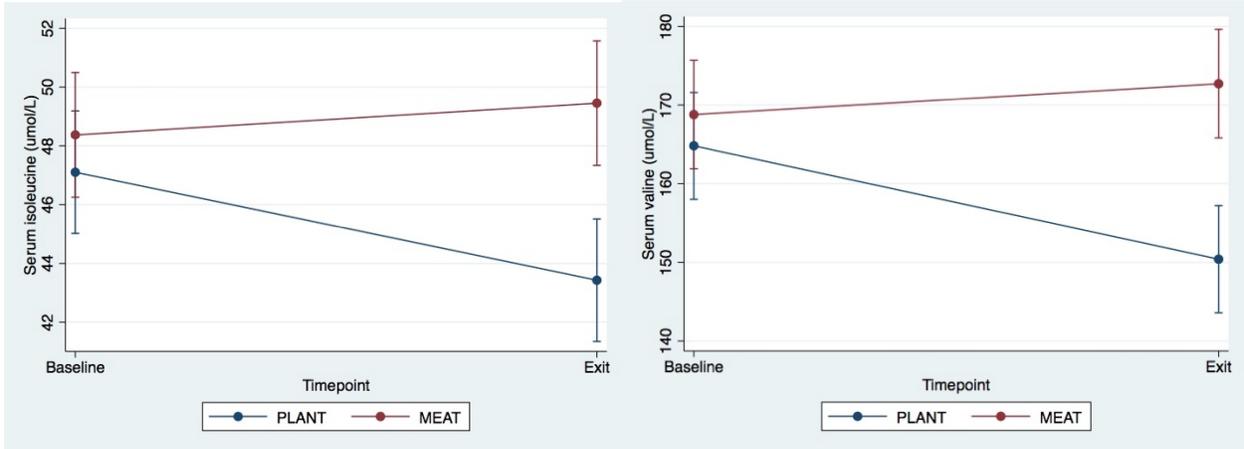
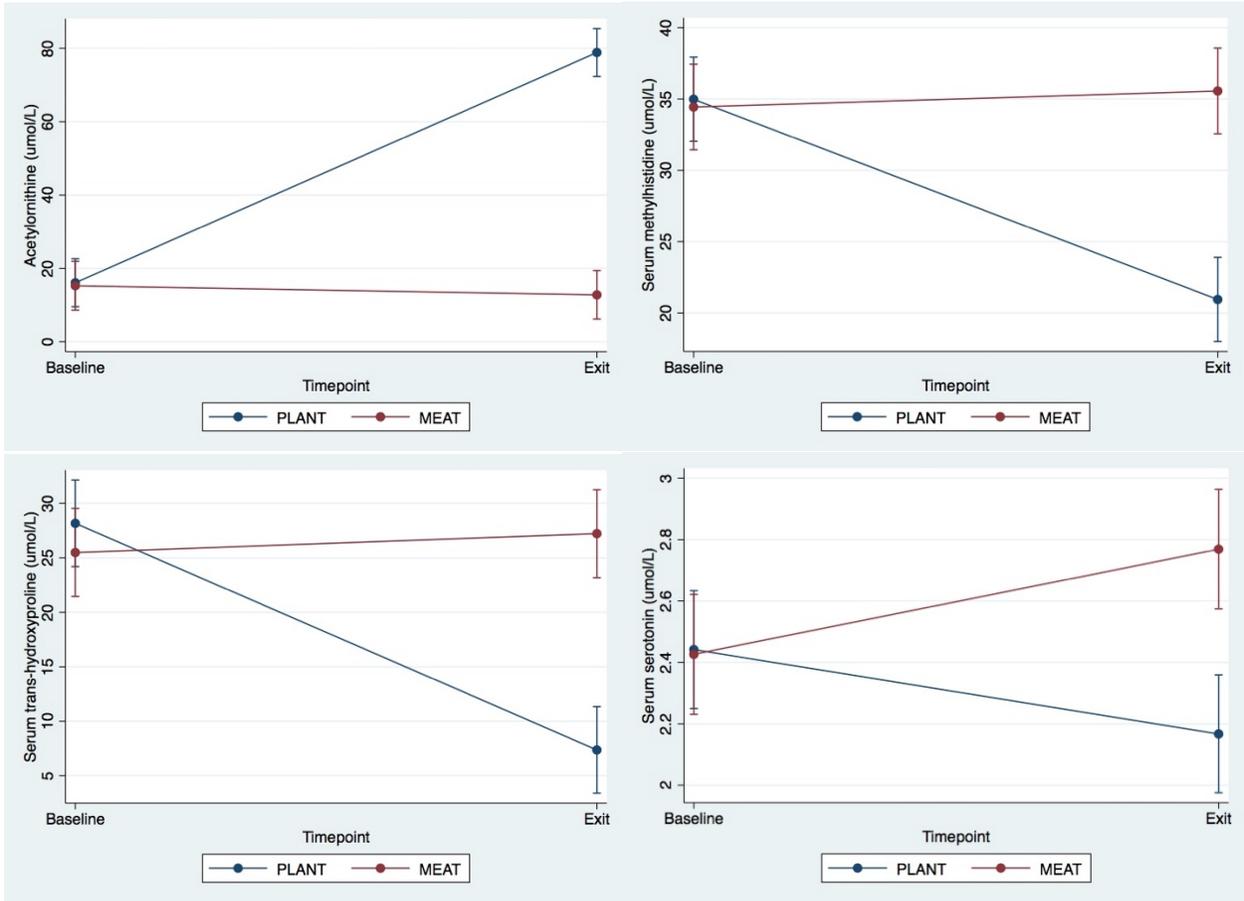


Figure 7.1. Amino acids with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.



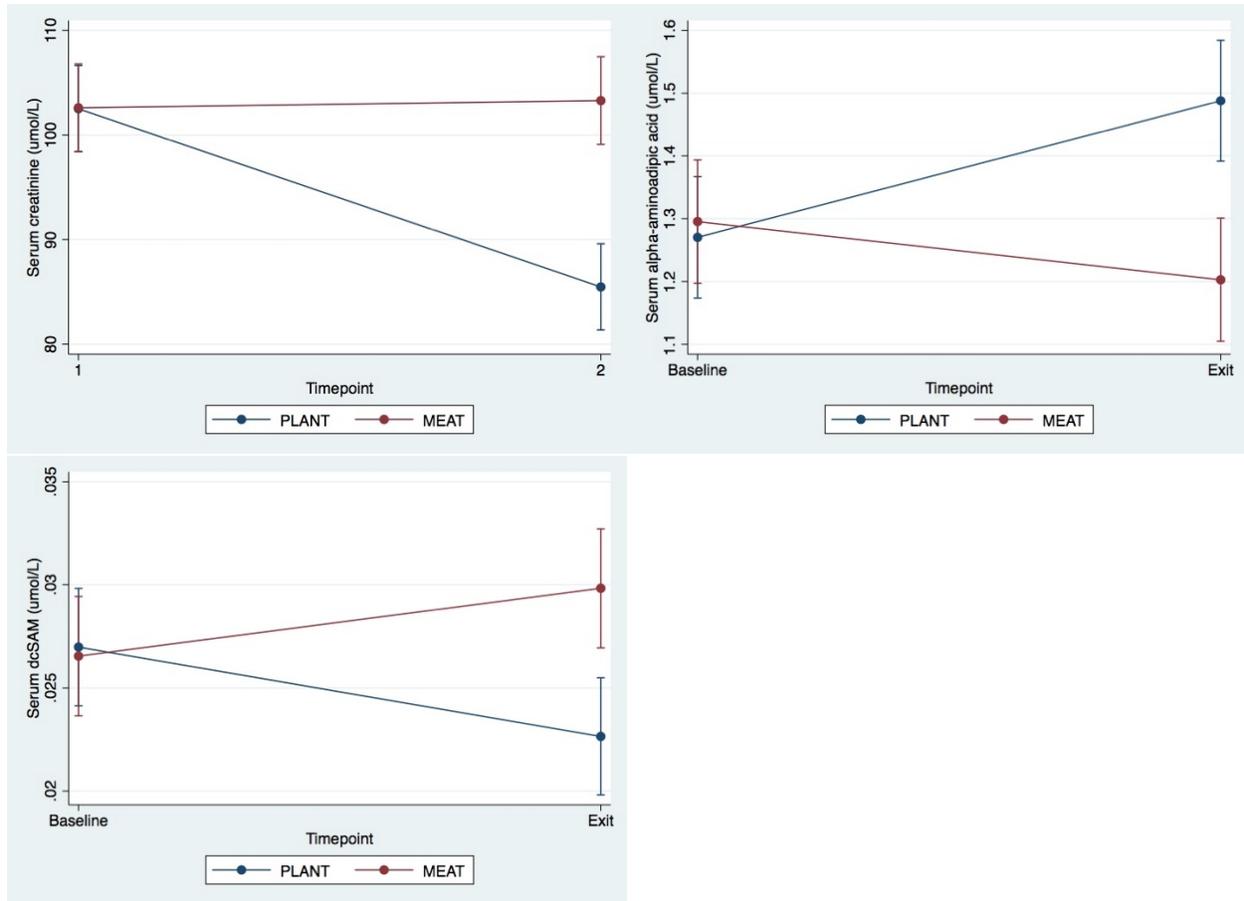


Figure 7.2. Amino acid derivatives with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

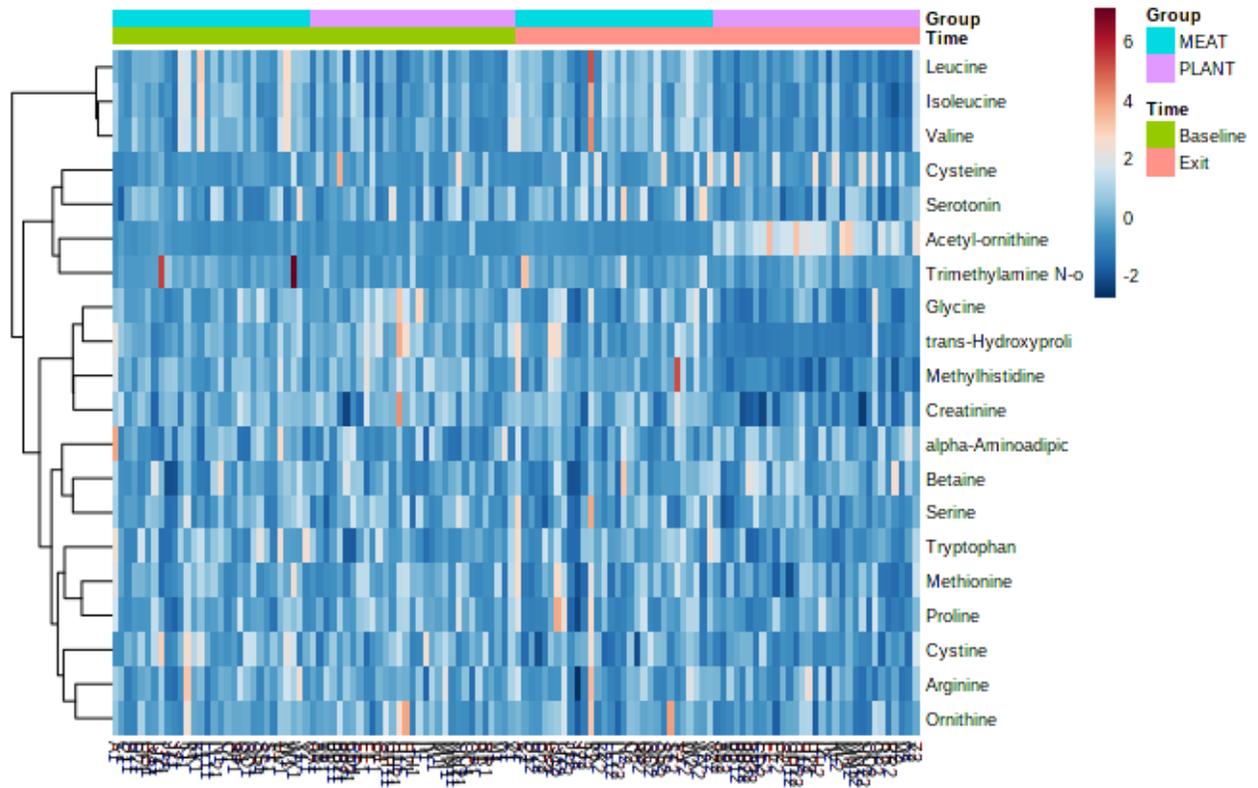


Figure 7.3. Two-way heatmap of significant protein and amino acid metabolites in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

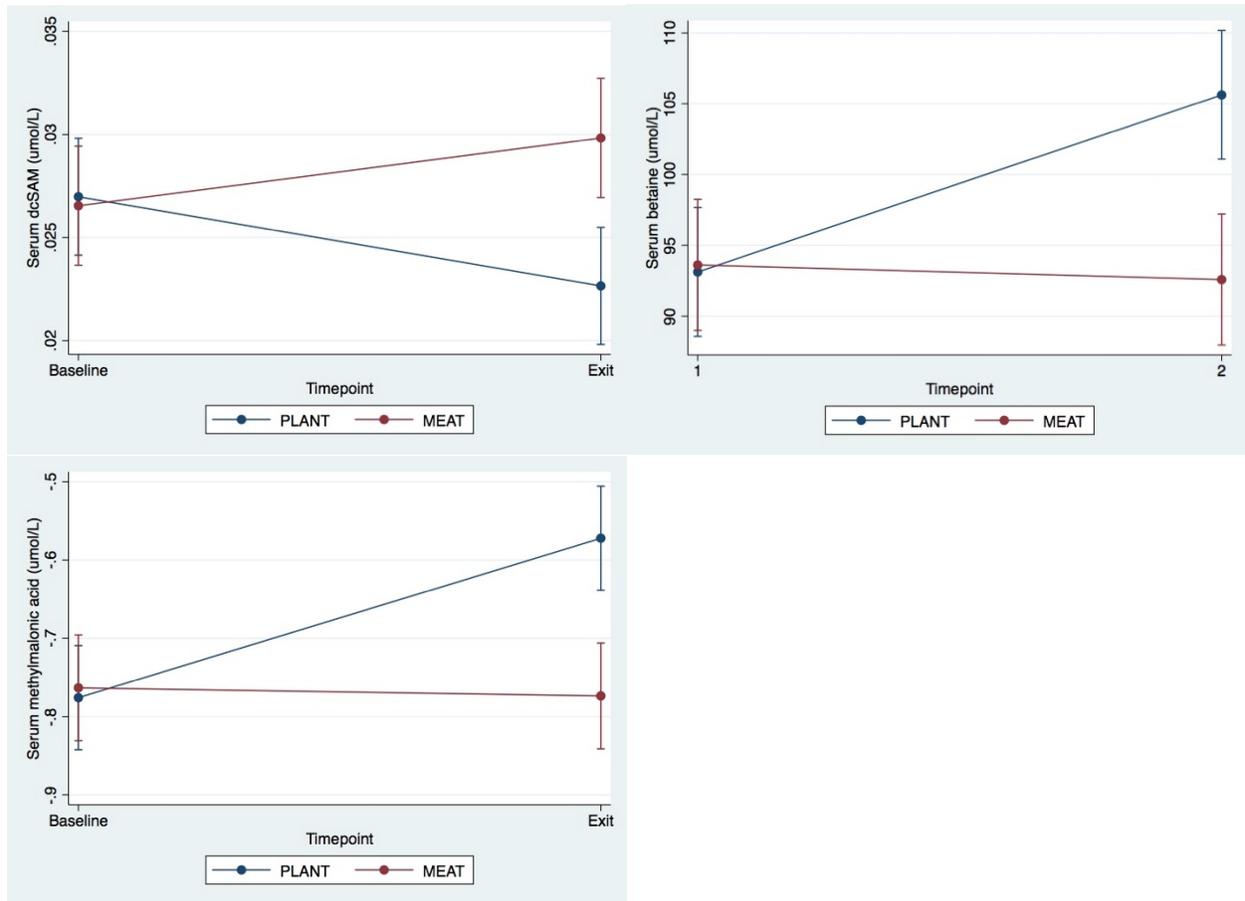


Figure 7.4. Folate pathway metabolites with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

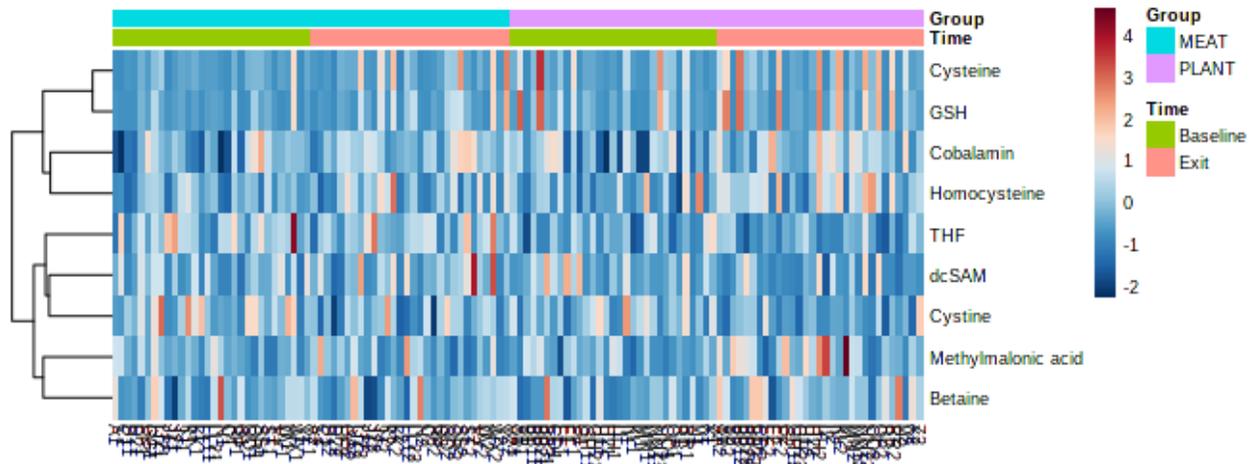
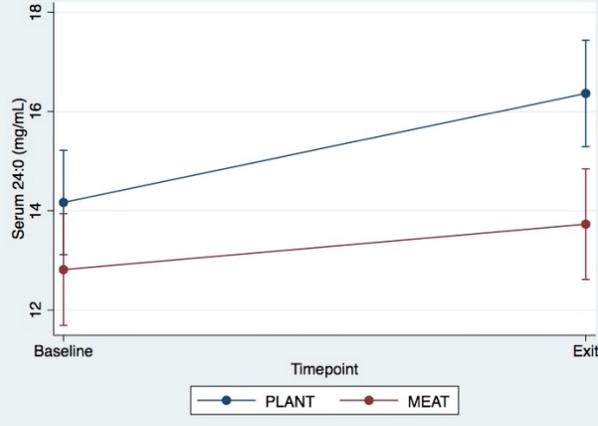
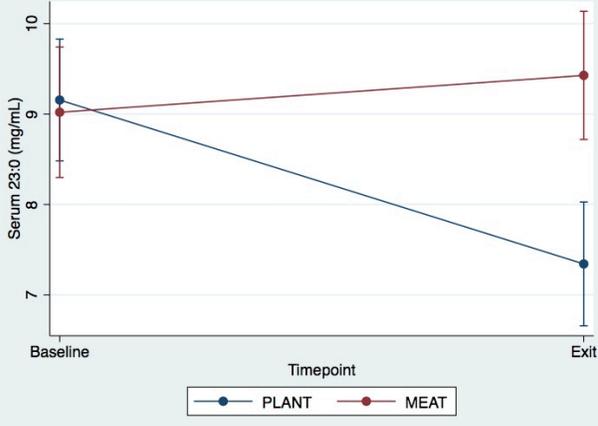
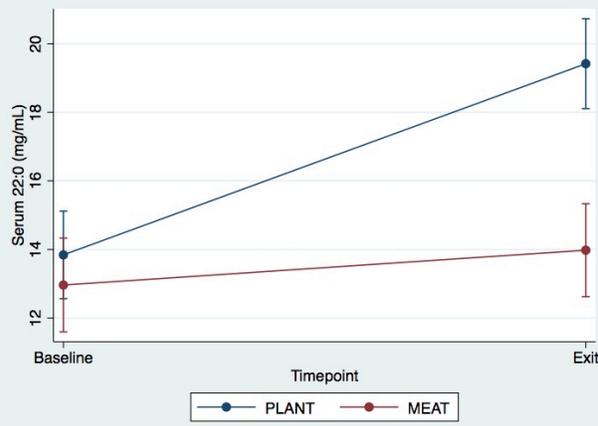
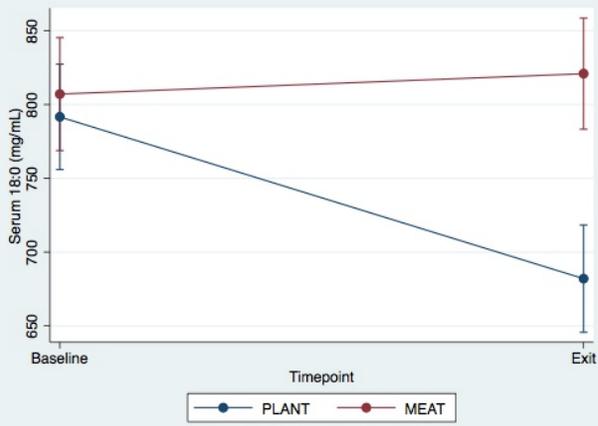
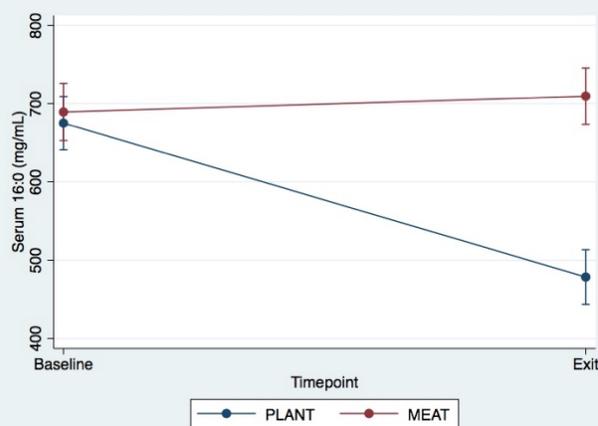
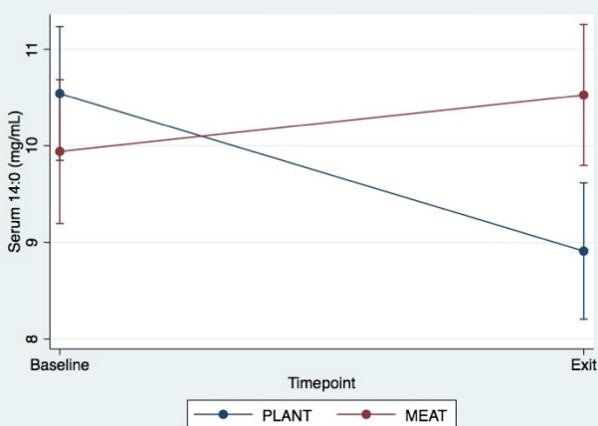


Figure 7.5. Two-way heatmap of significant folate pathway and one-carbon metabolites in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 month



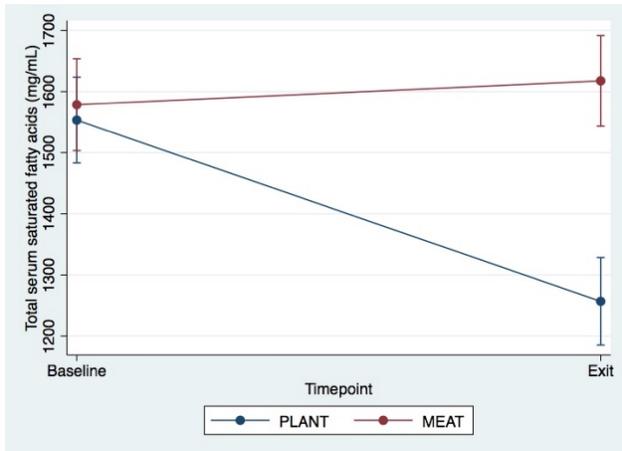


Figure 7.6. Saturated fatty acids with significant differences between diet groups at exit timepoint, as detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

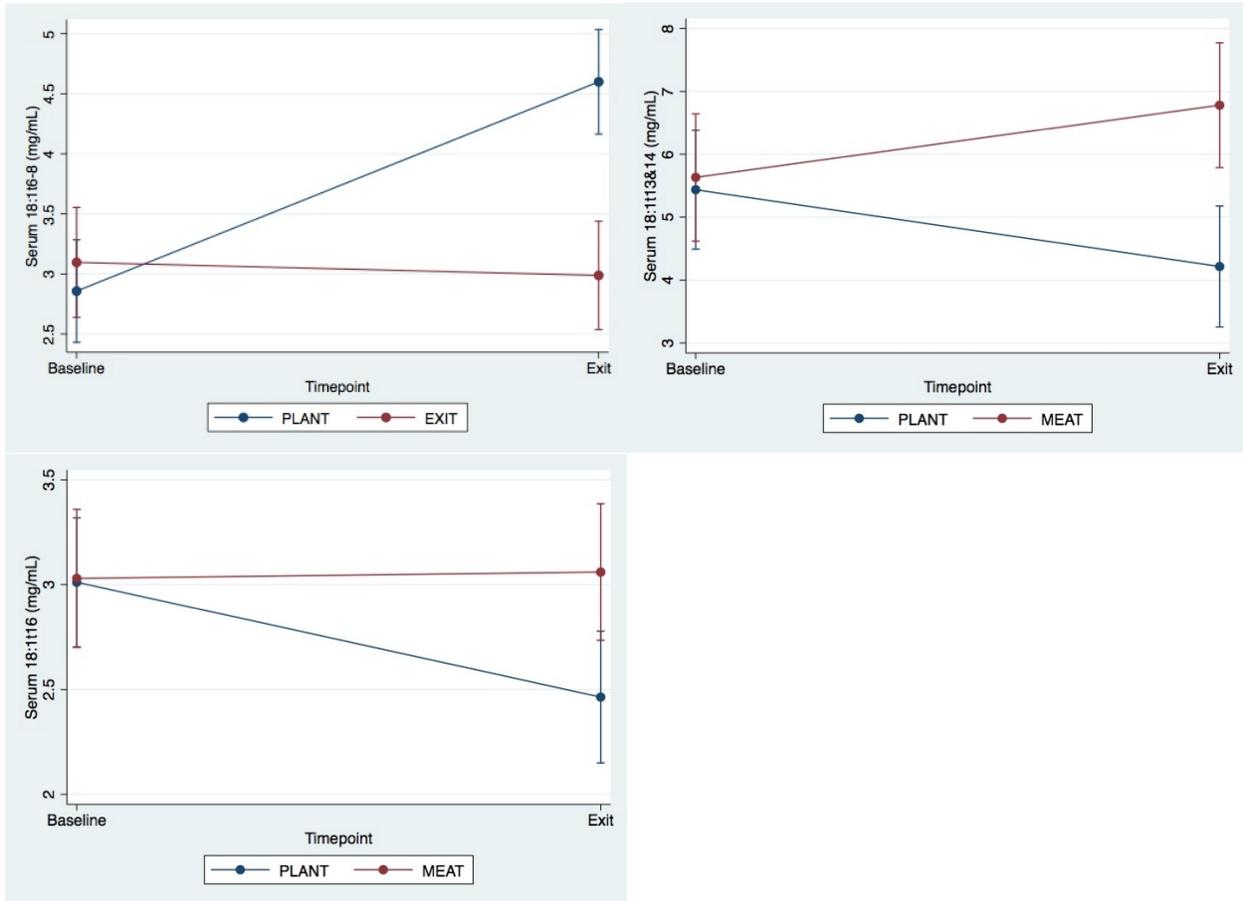


Figure 7.7. Mono-unsaturated *trans* fatty acids with significant differences between diet groups at exit timepoint, as detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

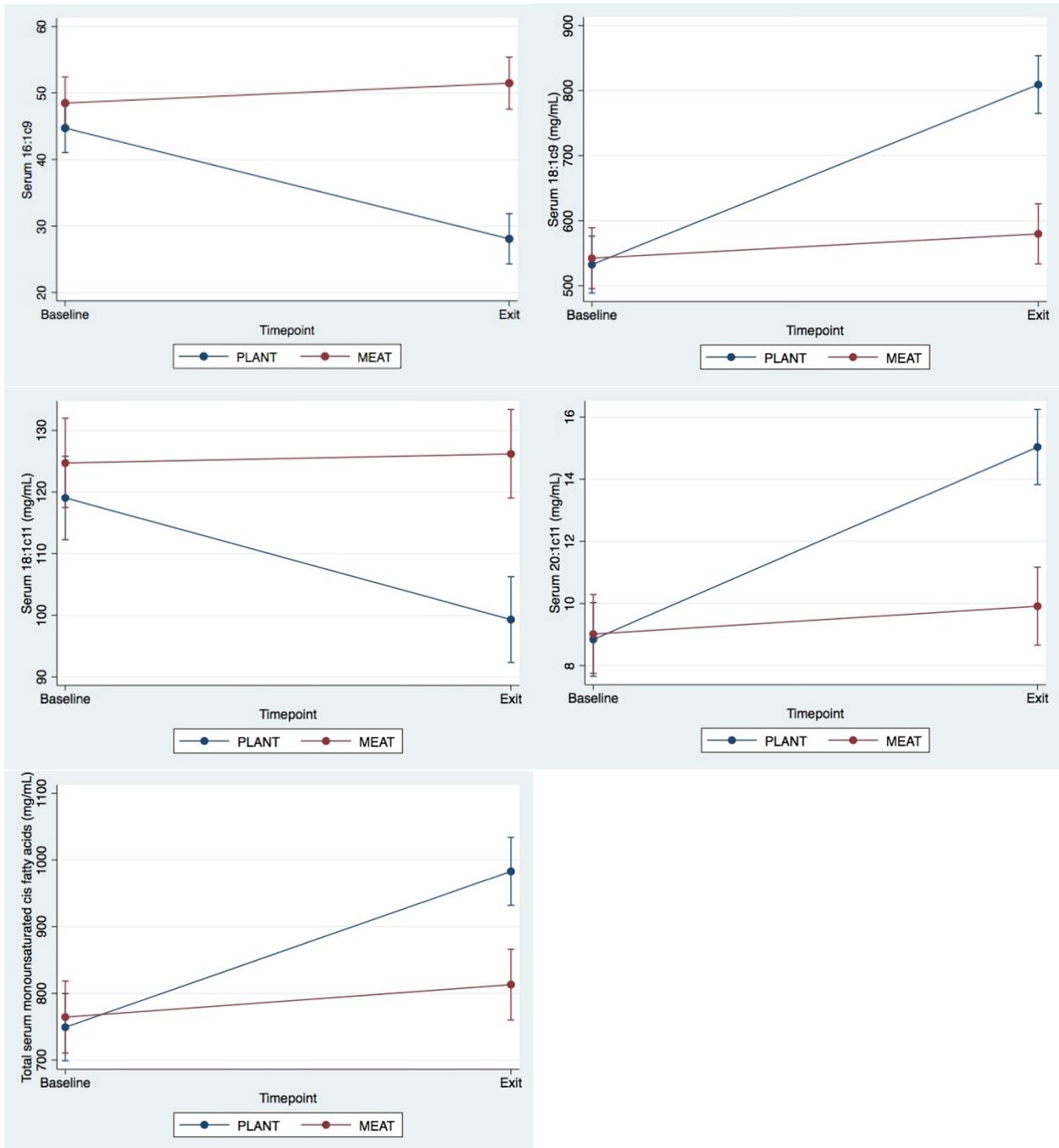
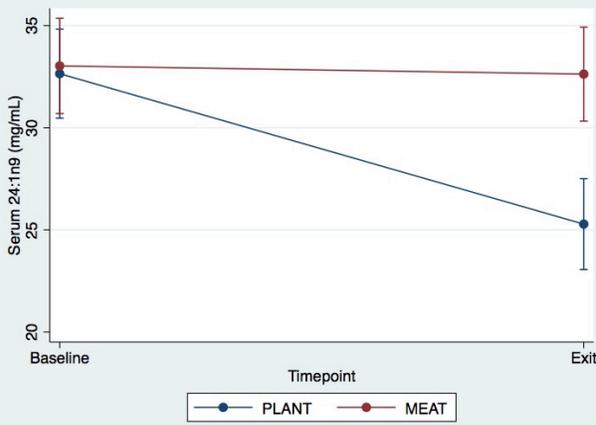
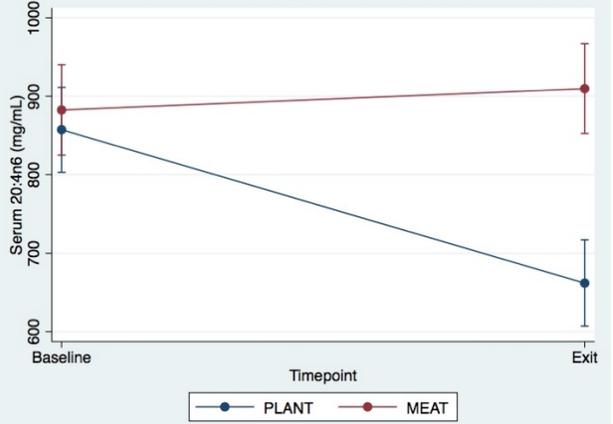
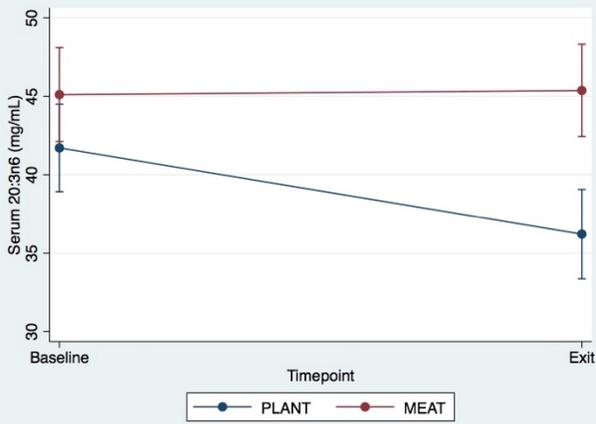
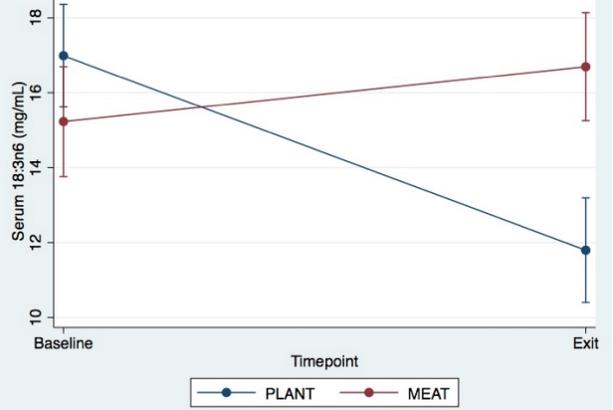
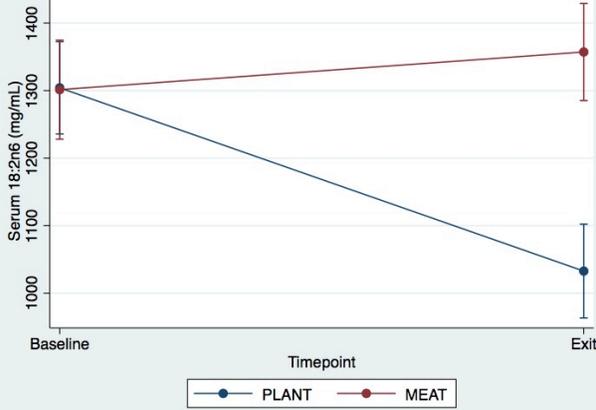


Figure 7.8. Mono-unsaturated *cis* fatty acids with significant differences between diet groups at exit timepoint, as detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.



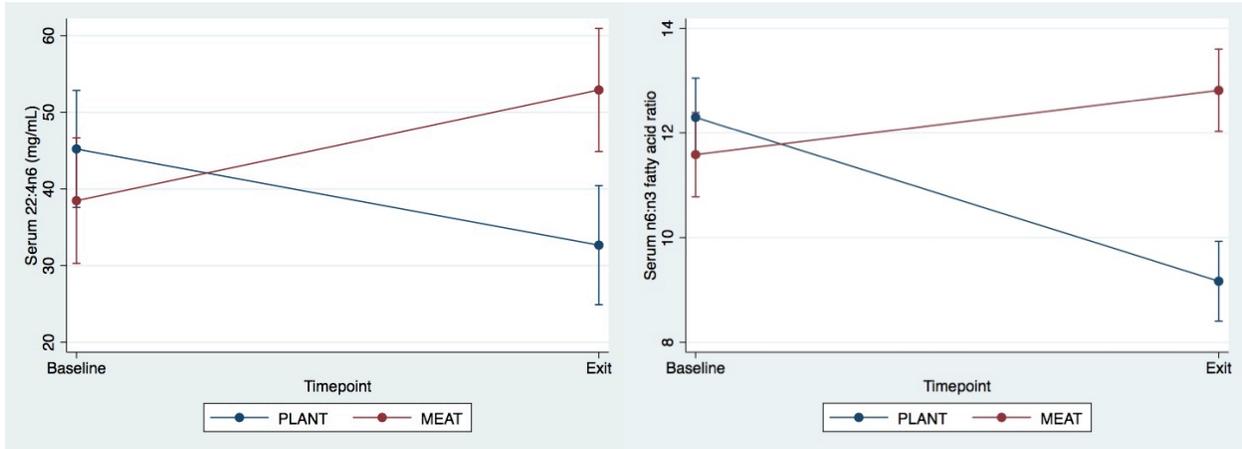


Figure 7.9. Polyunsaturated fatty acids with significant differences between diet groups at exit timepoint, as detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

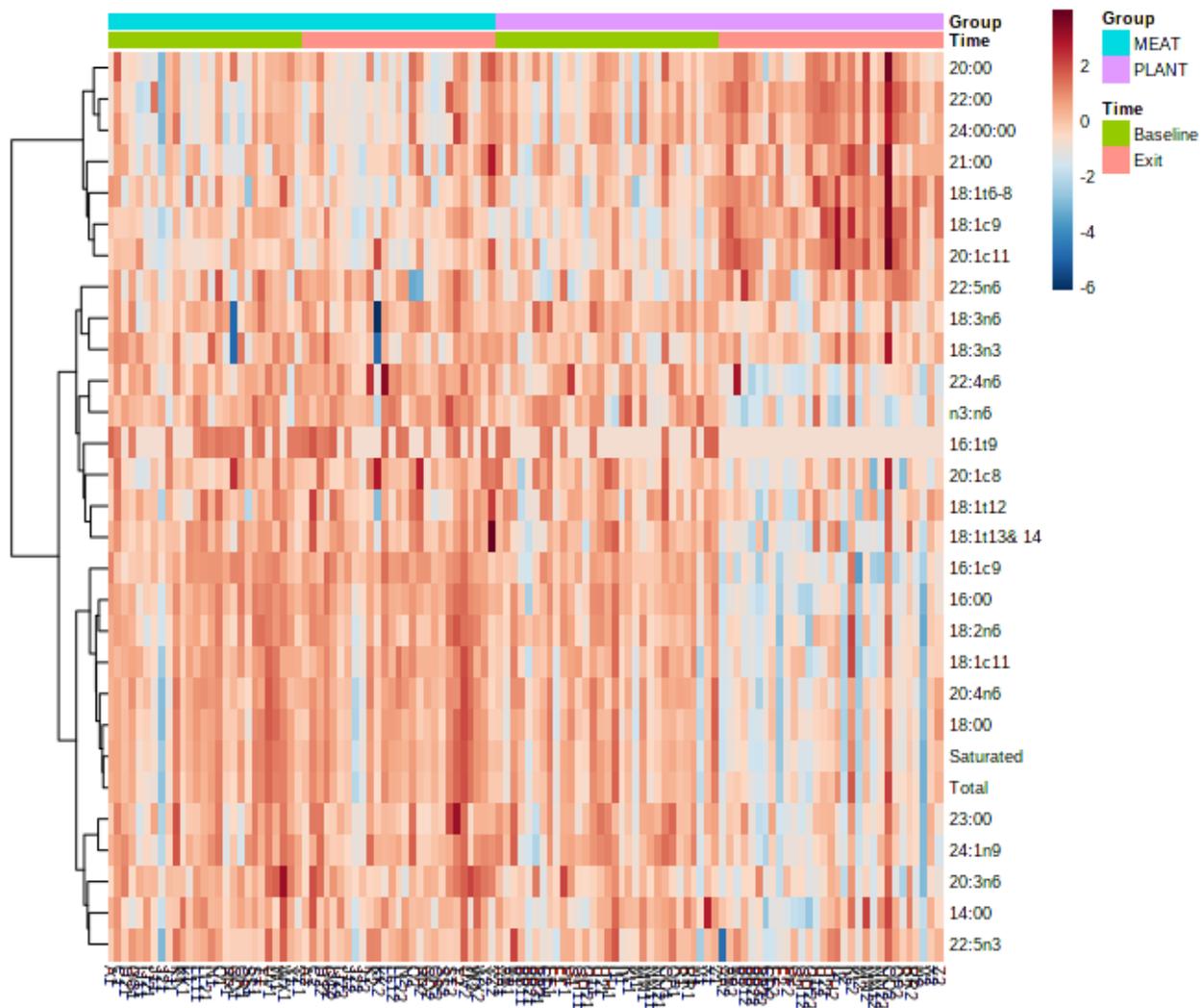
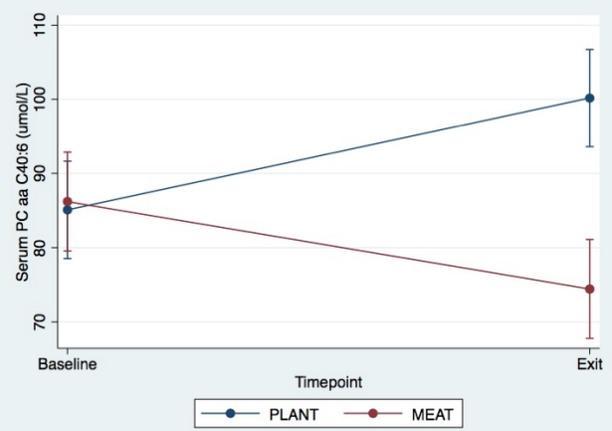
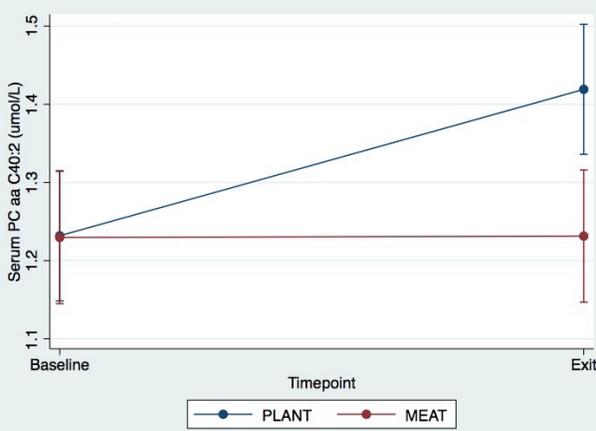
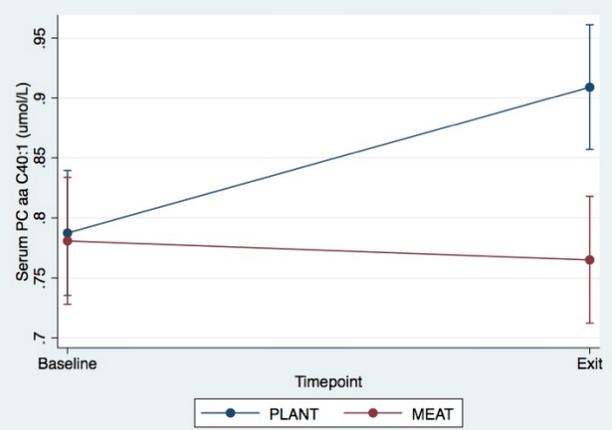
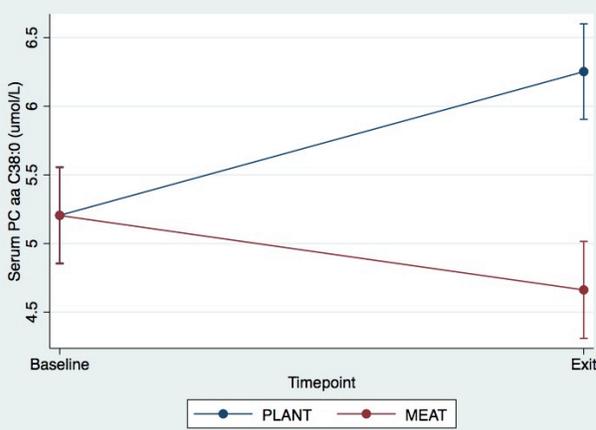
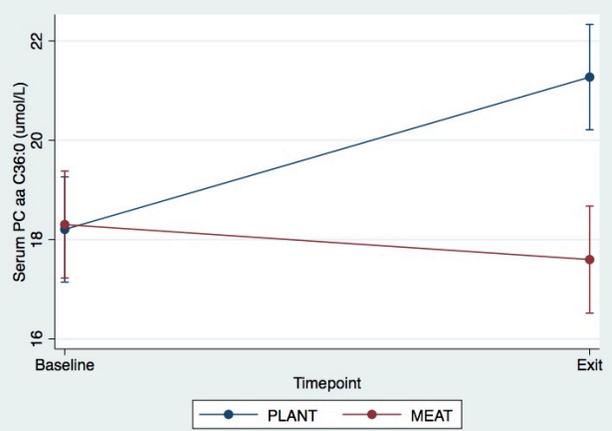
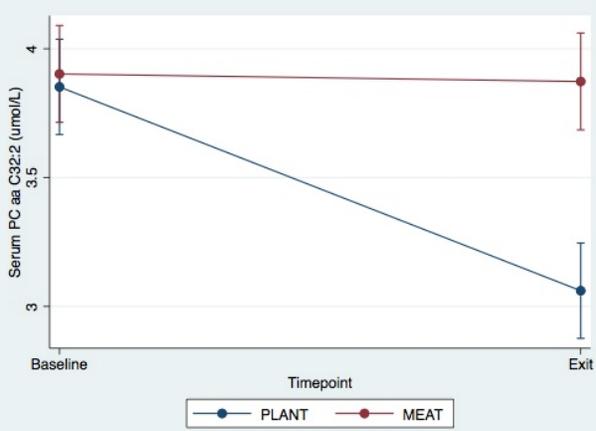


Figure 7.10. Two-way heatmap of significant fatty acids in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.



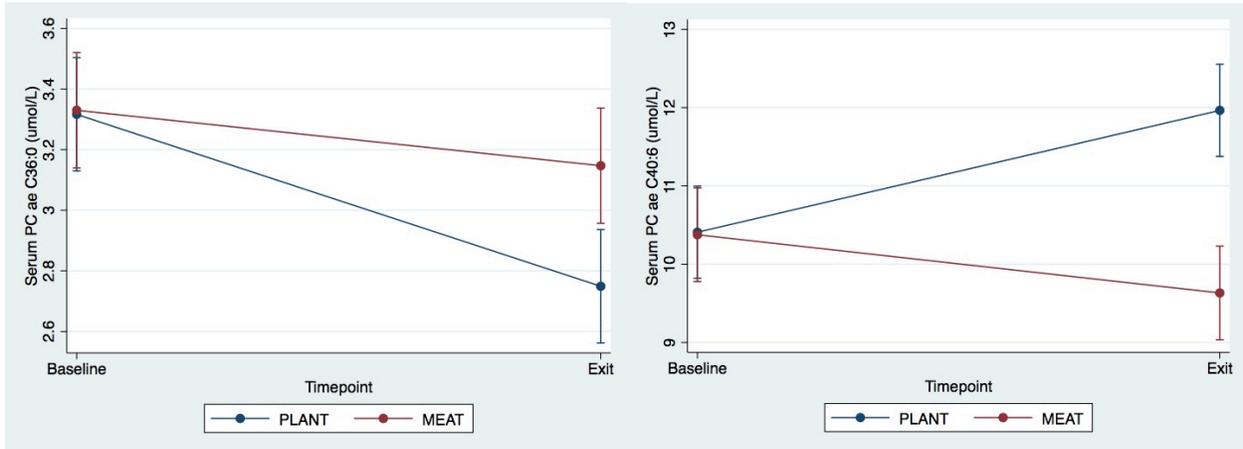


Figure 7.11. Phosphatidylcholines with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

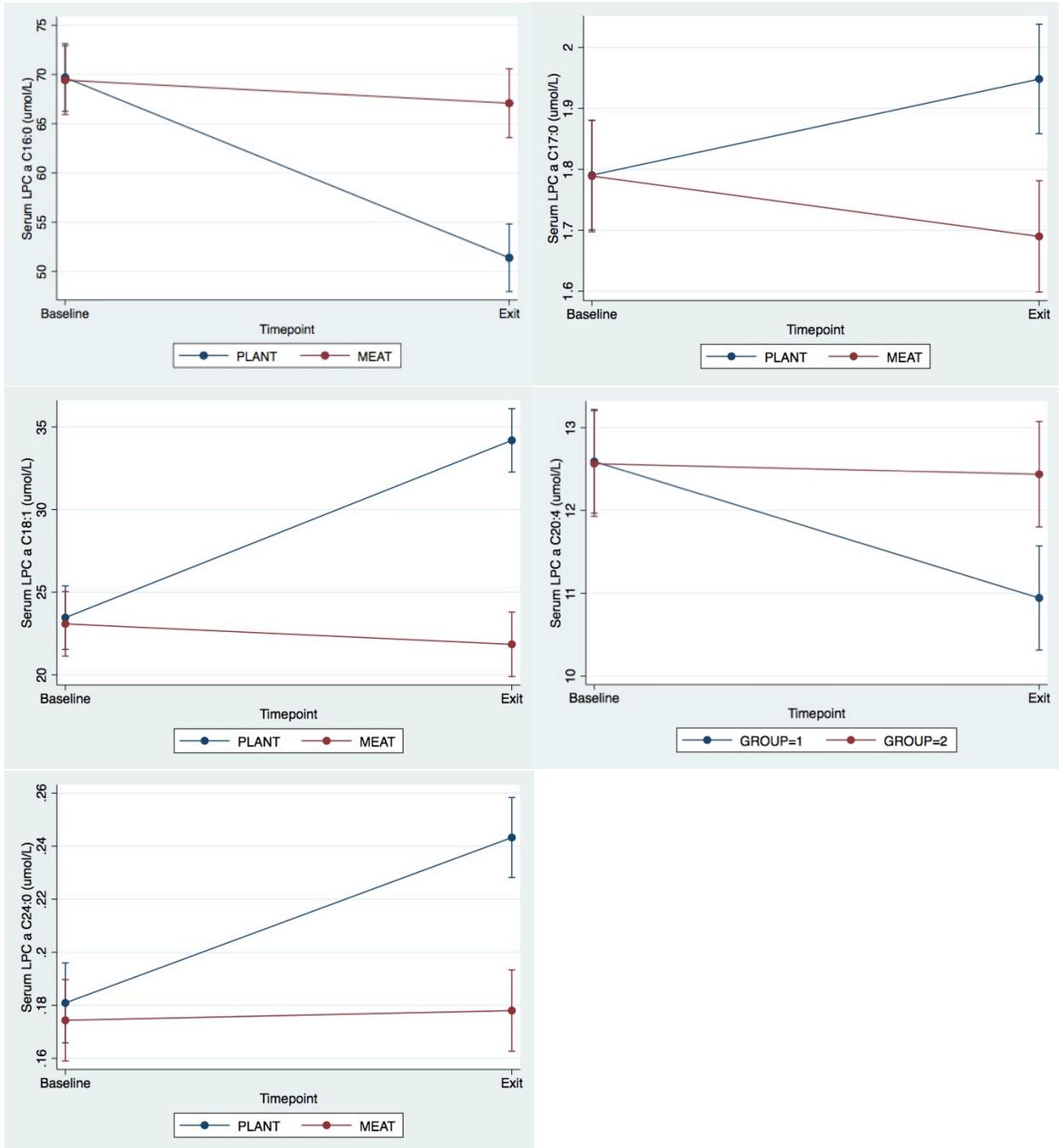
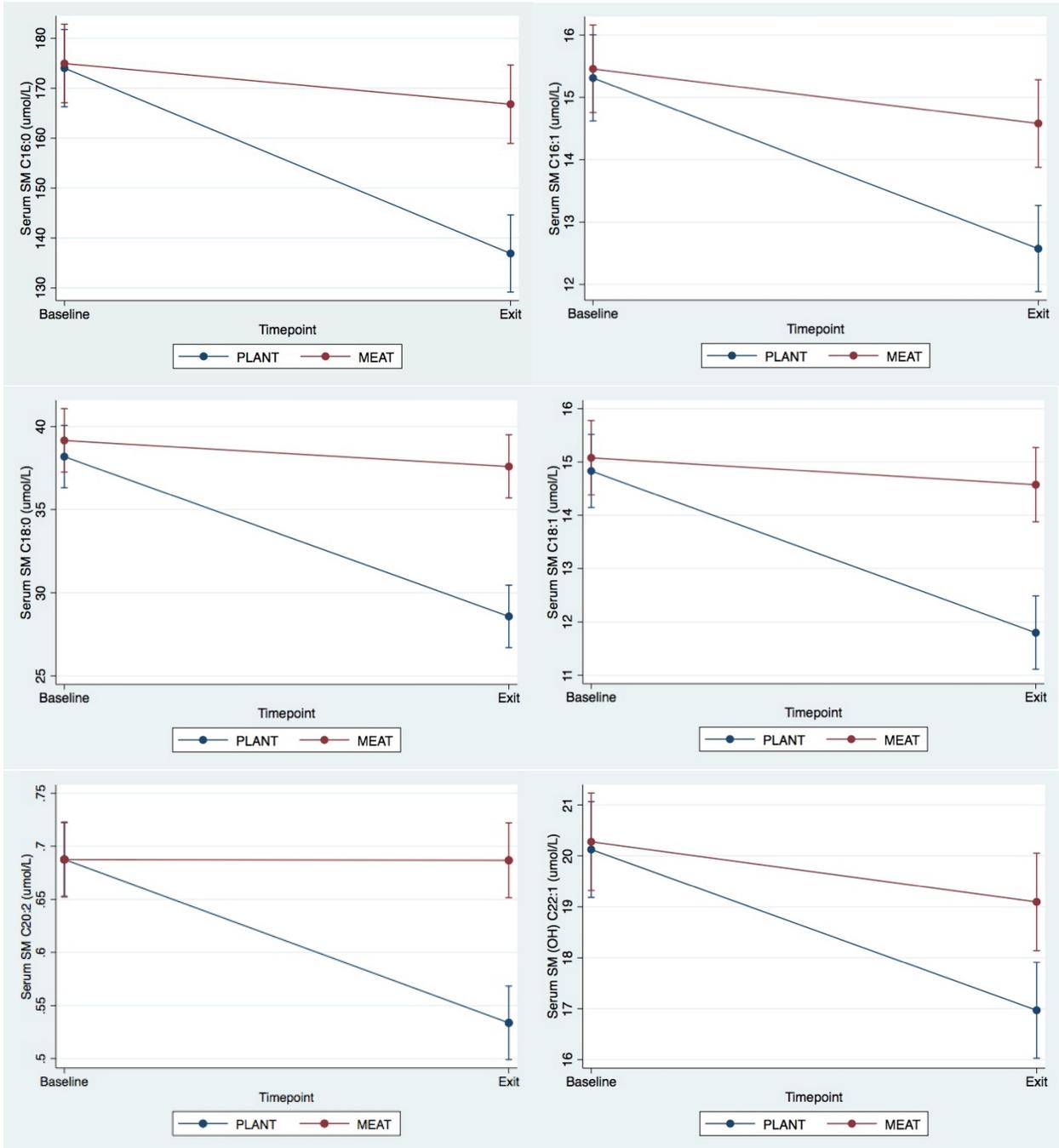


Figure 7.12. Lysophosphatidylcholines with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.



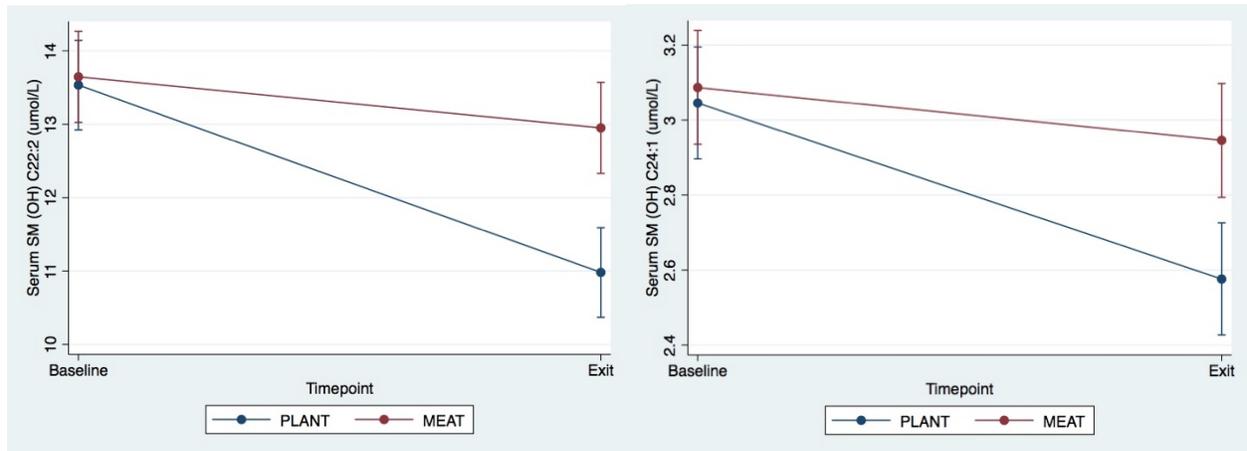


Figure 7.13. Sphingomyelins with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

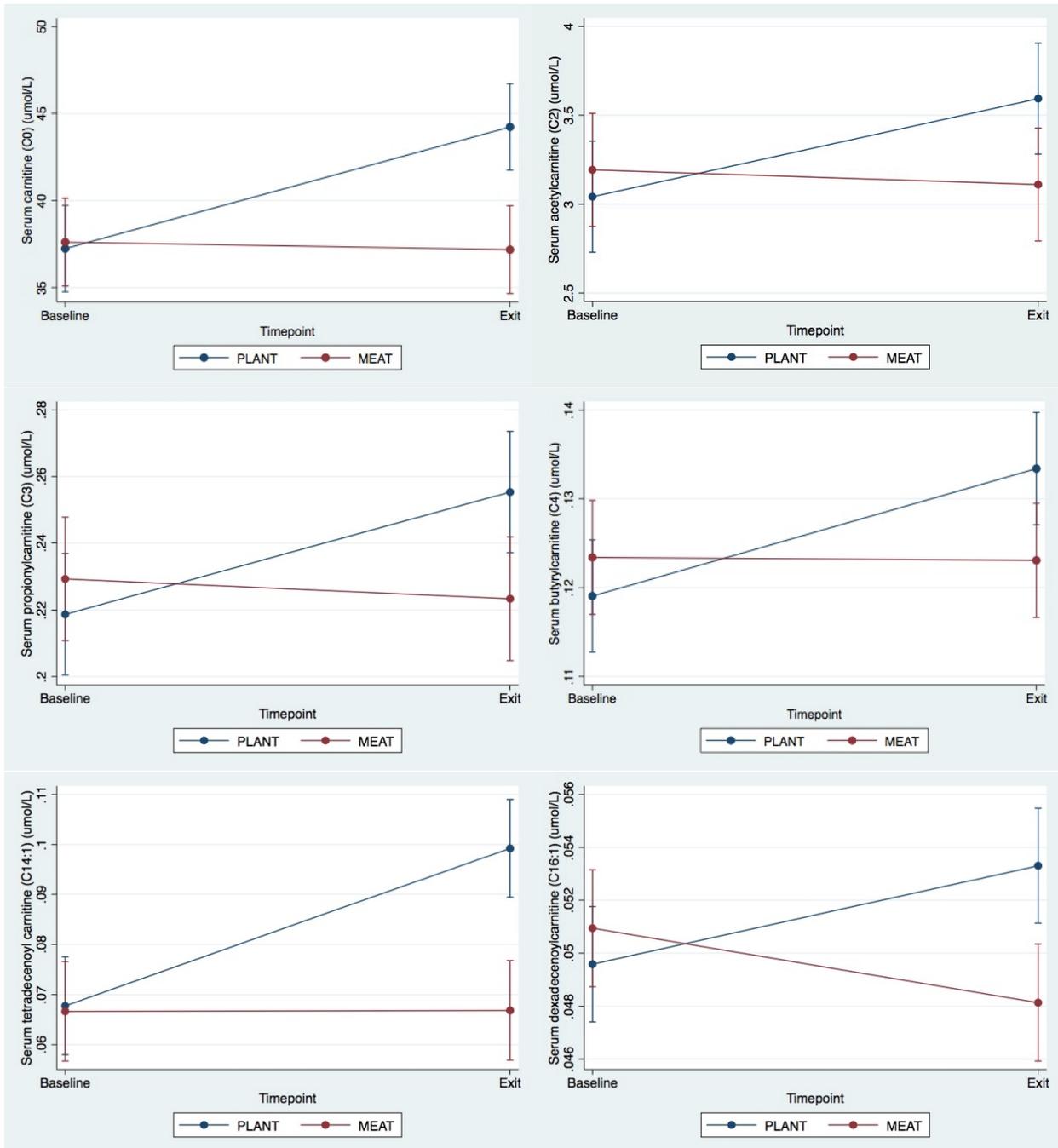


Figure 7.14. Carnitine and acylcarnitines with significant differences between diet groups at exit timepoint detected by repeated measures mixed modelling in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

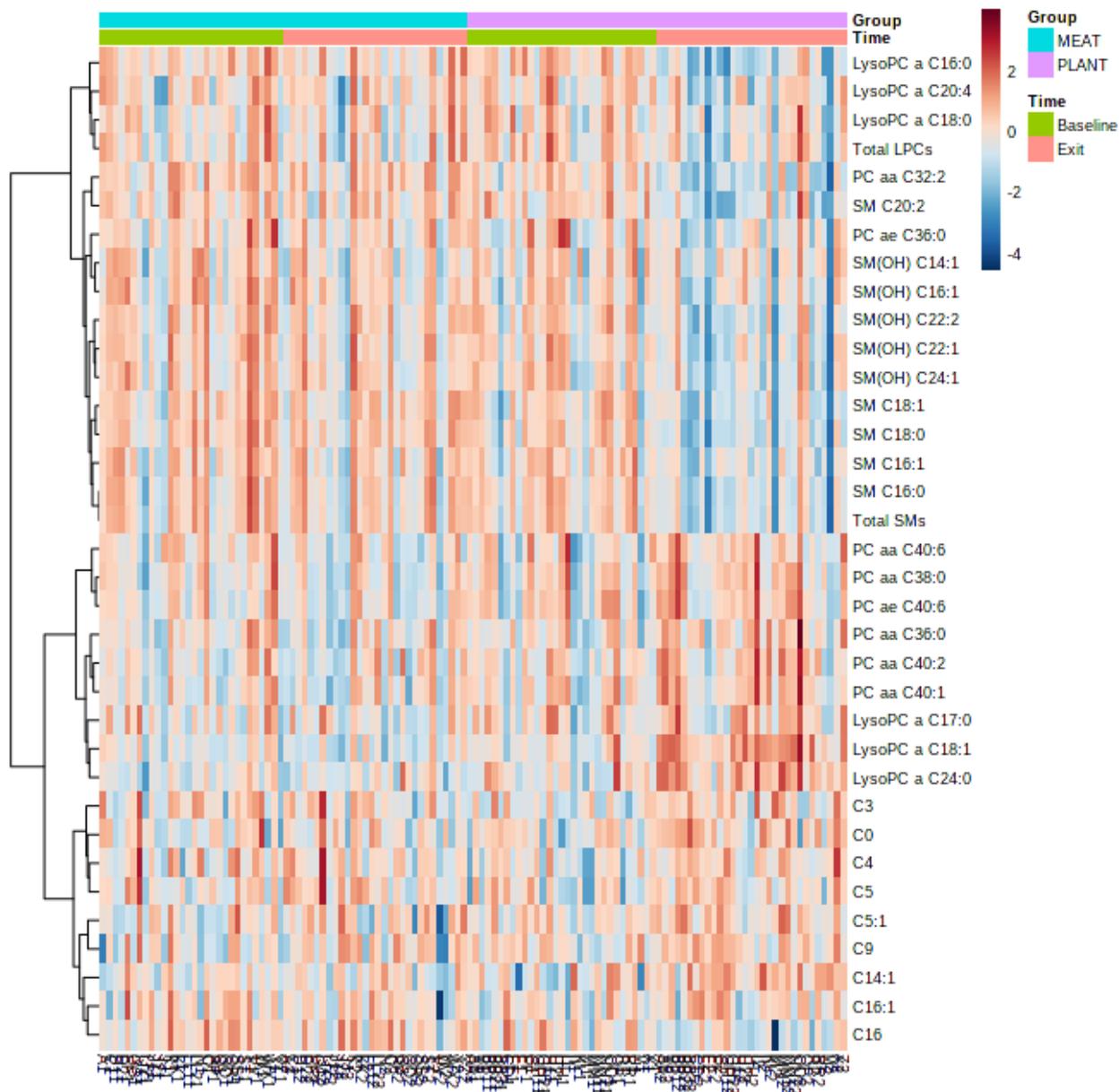


Figure 7.15. Two-way heatmap of significant lipid metabolites in 61 dogs fed an animal-based (MEAT) or plant-based (PLANT) diet for 3 months.

8 CHAPTER EIGHT: Discussion, future directions and conclusion

8.1 Overview of findings

The research presented in this thesis combined data from three separate but related studies: a survey of pet owner perception of pet health, nutrient analyses of plant-based pet foods, and a diet trial where dogs were fed a plant-based or conventional diet for 3 months.

There were nine key findings of this research:

1. The survey-based studies discussed in Chapters Three and Four did not identify health conditions common to cats and dogs fed plant-based diets (PBD). Instead, potential perceived positive health outcomes and a positive owner impression of health in dogs and cats fed PBD were reported.
2. Laboratory analyses of commercially-available plant-based pet food products revealed that, although all nutrients were able to be provided in PBD, most diets failed to meet the industry-recommended nutrient profile for the labelled species and life stage. Particularly, diets labelled for cats or for growth and development of puppies and kittens failed to meet recommendations (Chapter Five).
3. The diet trial described in Chapters Six demonstrated the efficacy of an experimental PBD (PLANT) to maintain normal health parameters, as typically measured during a veterinary wellness examination, as well as body condition and body composition.

4. Maintenance of serum vitamin D metabolite levels and skeletal mineralization in dogs fed plant-based vitamin D₂ compared to animal-derived vitamin D₃ provided, to the author's knowledge, the first empirical data supporting the inclusion of vitamin D₂ as a dietary vitamin D source for dogs (Chapter Six).
5. Differences in platelet morphology were detected between dogs fed the PLANT and MEAT diets, attributed to the reduction in serum arachidonic acid (AA) detected in dogs fed the PLANT diet (Chapters Six and Seven). In addition to lower serum AA, a reduction in serum cholesterol was also detected (Chapter Six).
6. The reduction in serum blood urea nitrogen (BUN) and creatinine detected in the plant-based trial dogs could be consistent with reports of lower kidney disease prevalence by the owners feeding PBD in the dog survey study (Chapters Six and Seven).
7. Though no differences in muscle metabolism or body composition were detected, trial dogs fed the PLANT diet demonstrated a decrease in serum branched-chain amino acids (BCAA), despite the PLANT diet containing a greater proportion of BCAA than the conventional meat-based (MEAT) diet (Chapters Six and Seven).
8. Remarkably few significant differences were detected between the metabolomic profile of dogs fed PLANT or MEAT diets. Most were attributable to differences in the nutrient profiles of the diets (Chapter Seven).

9. No differences in sulfur amino acid metabolism were detected between dogs fed PLANT or MEAT diets. This does not support a hypothesis that PBD may contribute to dilated cardiomyopathy secondary to induced taurine deficiency (Chapter Seven).

8.2 Discussion and implications of key findings

8.2.1 Key finding 1: Owner perception of good health in dogs and cats fed plant-based diets

Contrary to expectations, as described in Chapters Three and Four, neither dog nor cat owners reported a higher prevalence of urinary tract disorders in pets fed plant-based diets, providing the first tentative evidence against the widely-referenced hypothesis proposed by Knight and Leitsberger (2016). Also unexpectedly, dogs and cats fed a PBD had fewer reported health disorders, particularly with respect to gastrointestinal (GI) and hepatic disorders. These were novel findings, the significance of which warrants further investigation. Due to the cross-sectional nature of the study, no causality could be inferred and no association could be determined regarding PBD and improved health. However, there are plausible explanations that could potentially support these findings. Dogs and cats with dietary hypersensitivities may demonstrate GI signs, such as vomiting and diarrhoea, which may be ameliorated by transition from an animal-based to a PBD, as the most common dietary allergens in dogs are predominantly animal-based. As such, elimination of the offending ingredients would be expected to improve GI health (Bethlehem et al., 2012, Martín et al., 2004, Ricci et al., 2012). Another potential explanation, if pets did not have dietary hypersensitivity, but had fibre-responsive diarrhoea, would be that transition to a PBD, possibly higher in fibre than most animal-based diets by virtue of the fibre-rich nature of plant-based ingredients, may improve faecal consistency (Rossi et al.,

2020, Lecoindre and Gaschen, 2011, Alves et al., 2021, Frieche et al., 2011, Mandigers and German, 2010). Similarly, a fibre-rich PBD may have ameliorated constipation if pets were previously passing dry, hard faeces. Alternatively, it is possible that the reason fewer pets fed PBD were reported to have GI disorders could be attributable to a diet change of affected dogs *away from* a PBD. It is common practice in veterinary medicine to empirically ‘treat’ acute GI disorders with a highly-digestible therapeutic diet (Lawrence and Lidbury, 2015). Thus, the lower proportion of animals with reported GI conditions may have been not due to better GI health when fed a PBD, but due to a diet shift to a therapeutic diet, which are almost exclusively meat-based. Similarly, plant-proteins may be beneficial for liver metabolism in dogs and cats with hepatic disease and may thus have ameliorated or prevented clinical signs in pets fed PBD, or potentially transition to a therapeutic hepatic diet may have reduced prevalence (Lidbury et al., 2016, Proot et al., 2009). Though the numbers were too low to detect statistical significance (only one cat and no dogs fed a PBD were reported to have impaired renal health), there may be an association between diet and kidney conditions. In humans, PBD and plant proteins have been suggested to improve renal health, thus the lack of plant-based pets reported to have kidney disease may have been a real, though unexpected, finding (Kim et al., 2019a). As with GI and hepatic conditions, it may also be the case that pets diagnosed with chronic kidney disease were transitioned to a renal therapeutic diet, again, eliminating pets with renal disease from the PBD group (McGrotty, 2008). These findings present areas for future research to investigate possible positive health outcomes that may be associated with feeding PBD.

8.2.1.1 Implications

The implications of the results presented in Chapters Three and Four suggest further research is warranted to investigate whether there could be possible health benefits of feeding PBD to cats and dogs under certain conditions. If the cats and dogs fed PBD did truly have fewer health disorders and improved health compared to those fed non-PBD, there could be two possible explanations: it is possible that there are beneficial components provided from plant-based ingredients and/or damaging components provided from animal-derived ingredients. Plant-based ingredients may bring health promoting phytochemicals along with dietary nutrients (Okarter and Liu, 2010, Fragua et al., 2017, Sagols and Priymenko, 2011). It must be remembered, however, that dogs and cats are adapted to a more carnivorous diet than humans, and that dietary components from plants with health benefits in humans may not provide the same health benefits for dogs or cats. Furthermore, plant-based ingredients can also bring anti-nutritional factors and impair nutrient absorption and bioavailability (Mansilla et al., 2019). For example, phytates in plants can bind essential minerals, rendering them inaccessible to the animal unless properly processed; similarly, trypsin inhibitors in undercooked legumes can impair protein digestion (Cargo-Froom et al., 2019, Félix et al., 2013, Yamka et al., 2005). Due to the presence of lectins, tannins, trypsin inhibitors and oligosaccharides, high levels of soybean meal, a common plant-based protein source for pet foods, has been demonstrated to impair digestibility even after heat processing (Yamka et al., 2003). In humans, there have been concerns raised about possible negative health impacts from excessive consumption of some animal-derived foods, particularly processed muscle tissues (meats) (Soladoye et al., 2015, Thøgersen and Bertram, 2021, Rohrmann and Linseisen, 2016). It is possible that some of the

same factors suspected to potentially contribute to adverse health outcomes in humans, such as presence of haeme iron or products of protein and amino acid degradation from meat processing, may impact health of dogs and cats, though there have been few investigations regarding these possibilities (Gu et al., 2012, Knize et al., 2003). Furthermore, dogs and cats are more carnivorous than humans and may be better adapted to consuming diets rich in animal tissues without adverse consequences, and it is the opinion of some veterinarians and nutritionists that cats and dogs require animal products in their diet to thrive and maintain their best health status (Zoran, 2002, Fox, 2005, Loeb, 2020). However, current dietary recommendations are based upon essentiality of nutrients, not ingredients (NRC, 2006, AAFCO, 2020, FEDIAF, 2020). Plant-based diets have been demonstrated to be at risk of nutrient insufficiencies, which may have negative impacts on the health of cats and dogs to which they are fed, although nutritional deficiencies may take years to manifest and consequences may not be recognized as being attributable to an imbalanced diet (Kanakubo et al., 2015, Gray et al., 2004, Zafalon et al., 2020). The greatest implications of this research is that the results suggest that PBD may provide some health benefits in certain circumstances and further research is warranted to investigate the risks and benefits of PBD for dogs and cats.

8.2.2 Key finding 2: Nutrient insufficiencies in plant-based pet foods products

Within Canada, pet food products have been demonstrated to have poor compliance with guaranteed analyses and labelling regulations (Burdett et al., 2018). However, no comparable studies on PBD sold in Ontario, Canada have been performed. In previous studies of PBD, performed in Brazil and the USA, relatively consistent nutritional insufficiencies have been documented in plant-based pet foods (Zafalon et al., 2020, Gray et al., 2004, Kanakubo et al.,

2015). In products for dogs, methionine (Met), Met+Cysteine (Met+Cys), tryptophan (Trp), and calcium (Ca) most commonly failed to meet recommendations (either European (FEDIAF), American (AAFCO), or both). In products for cats, leucine (Leu), lysine (Lys), Met, Met+Cys, Trp, taurine (Tau), arachidonic acid (AA), and Ca were most often found in insufficient concentrations. Thus, it was unknown if the PBD fed to the dogs and cats reported in the survey study (Chapters Three and Four) were nutritionally complete and balanced.

These findings were mostly repeated in our study investigating PBD available in Ontario, Canada (Chapter Five), though, potentially owing to the larger samples size and/or comprehensive analysis including multiple nutrients (amino acids, fatty acids, minerals, and vitamins A, B12 and D) more insufficiencies were detected. In addition to Met, Met+Cys and Trp, canine maintenance diets were also found with insufficiencies in Lys. Previous studies had not measured fatty acids in canine diets labelled for growth, but our study demonstrated insufficiency of eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) in all growth diets. Calcium, Ca to phosphorus (P) ratio, and vitamin D were also found to be insufficient in multiple products. Contrary to expectations, feline diets performed better in terms of amino acid profile, though Tau was found insufficient in some extruded and all canned feline diets. As with previous findings, AA was below recommendation in most feline products, and some had low Ca and/or an inverse Ca:P ratio. Concerningly, amino acid provision in kitten diets was poorer than in adult maintenance diets, with multiple products containing insufficient Met, Met+Cys and/or Tau.

While these findings mostly reinforce those previously reported, in contrast to previous studies, the results presented here demonstrated that it is *possible* to formulate and manufacture diets containing all essential nutrients for both dogs and cats for growth or maintenance, as there were no nutrients that were consistently non-existent in all diets for a given species or life stage. This suggests that complete and balanced plant-based foods for dogs, cats, puppies and kittens are possible. However, the only nutrient recommendations that were met by any product in this study were those for adult dogs.

8.2.2.1 Implications

The implications of the study presented in Chapter Five highlight the necessity to advocate for the use of skilled, experienced formulators and for post-production testing of commercial products, especially niche products with limited ingredient lists. The findings of this study are particularly concerning, as deficiencies and imbalances of Ca, P, and vitamin D can lead to nutritional secondary hyperparathyroidism with severe consequences. In adult dogs, this may take months to years to manifest, but in growing puppies signs can develop within weeks (Dodd et al., 2019a, Tal et al., 2018, Verbrugghe et al., 2011). As with dogs, adult cats can likely accommodate for minor nutrient deficiencies for longer periods of time than growing kittens. There are fewer reports of dramatic skeletal abnormalities in cats fed diets with inadequate Ca, improper Ca to P ratio, and/or insufficient vitamin D. Possibly, this is due to the comparatively lower body weight and leaner bodies in kittens than in puppies, thus pathological fractures and significant skeletal deformities may be less likely. However, numerous cases have still been reported, highlighting the risk of imbalanced diets (Tomsa et al., 1999).

Documentation of the nutrients commonly insufficient in commercial plant-based products will provide a resource from which veterinarians can educate their clients regarding potential risks of PBD and supplementation required to improve the nutrient profile and balance the diet. For dogs and cats fed commercial PBD, supplementation with Tau and vitamin B12 may be safe and prudent, although best practice would be to analyze the at-risk nutrients in the diet of each presenting animal and tailor nutritional recommendations to the individual, as other nutrients, such as Ca and vitamin D, may not be as safe to supplement without knowing the typical dietary provision. For example if a diet contains sufficient Ca and a balanced Ca:P ratio, chronic administration of additional Ca or vitamin D could predispose to hypercalcaemia (Cline, 2012, Mellanby et al., 2005). Furthermore, due to rapid development, increased metabolic rate and deposition of lean tissue and bone in growing animals, dietary manipulation should be performed under the guidance of a veterinary nutritionist only.

8.2.3 Key finding 3: Comparable wellness and body composition in dogs fed plant- or meat-based diets

Over the course of the 3-month diet trial, dogs fed the plant-based trial diet (PLANT) maintained all health parameters within published reference ranges and had no changes detected on veterinary physical examination (Chapter Six). Notably, in conjunction with the lack of differences in reported urinary tract disorders between dogs fed a plant-based or non-PBD detailed in Chapter Three, the lack of differences in urinary parameters in the dogs fed the plant-based trial diet may cast further doubt on the widely-referenced hypothesis that PBD may predispose dogs to urinary alkalinisation and subsequent urinary tract disorders (Knight and Leitsberger, 2016, Dodd et al., Submitted).

Previously, it has been demonstrated that muscle wasting and fat accumulation in dogs fed a low-protein diet was worsened when the protein source was plant-based (Wakshlag et al., 2003). It was suggested that the poorer amino acid profile of the plant-based protein contributed to the worse muscle wasting when protein was restricted. The present study demonstrates that when fed in excess of minimum requirements, the amino acids present in plant-based protein can be sufficient to maintain a relatively comparable serum amino acid profile and support normal lean soft tissue mass and proportion (Chapters Six and Seven). Furthermore, there were no increases in fat mass or fat proportion in the plant-based dogs (Chapter Six). Dogs in both groups maintained body weight, body condition, and all measures of body composition as determined by dual-energy x-ray absorptiometry (Chapter Six).

8.2.3.1 Implications

The results of the diet trial contribute greatly to the limited body of knowledge surrounding the suitability of PBD for dogs. This was the first study of its kind to demonstrate this and is a benchmark for future research.

Apparently healthy, adult dogs fed a complete and balanced PBD for 3 months maintained all measures of health and wellness and maintained their body condition and composition. This suggests that, at least over a relatively short period (3 months), the nutrition provided by the PLANT diet formulated to exceed recommendations for canine adult maintenance was comparable to that of the conventional MEAT diet (AAFCO, 2020). This supports the concept that nutrients are essential, not the ingredients from which they are delivered. As such, a possible interpretation is that a complete and balanced PBD, replete in all

essential nutrients, is suitable for adult canine maintenance for at least 3 months, supporting the findings reported by pet owners in Chapter Three of perceived good health in dogs fed PBD (Dodd et al., Submitted).

8.2.4 Key finding 4: Dogs fed plant-based maintained serum vitamin D concentrations and skeletal bone mineralization

The findings of this study, presented in Chapter Six, were the first to demonstrate efficacy of vitamin D₂ in maintaining serum vitamin D concentrations (hydroxyvitamin D, ionized calcium, parathyroid hormone) and supporting normal skeletal bone metabolism. In some species, vitamin D₂ has been demonstrated to have reduced efficacy compared to vitamin D₃ as a dietary source of vitamin D (Morris, 2002a, Trang et al., 1998). Industry recommendations are published only for ‘vitamin D’, referencing studies in which dogs were fed varying levels of vitamin D₃ (AAFCO, 2020, FEDIAF, 2020, NRC, 2006). There were no published studies demonstrating efficacy of vitamin D₂ in dogs, yet it has been used as a source of dietary vitamin D with little understanding of its suitability. The lack of published cases of dogs with hypovitaminosis D from consuming a diet supplemented with D₂ suggests that this practice has been acceptable, but no empirical evidence had been demonstrated to support it.

In Chapter Six, it was demonstrated that dogs fed a diet supplemented with D₂ experienced a replacement of all vitamin D metabolites with the D₂ form. Hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D (1,25(OH)₂D) and 24,25-dihydroxyvitamin D (24,25(OH)₂D) were all found present in the D₂ form, whereas in the dogs fed the conventional animal-based diet, D₂ forms were negligible and all vitamin D metabolites were in the D₃ form. Nevertheless, total serum levels of 25(OH)D, the main marker of vitamin D in the body, and

1,25(OH)₂D, the most active metabolite, were comparable between groups. Of note, however, there was a reduction in total 24,25(OH)₂D, considered a catabolic metabolite of vitamin D, generated for excretion (Yoo et al., 2021, Makris et al., 2021), in the plant-based group. 24,25(OH)₂D has been considered a catabolic metabolite of vitamin D, generated for excretion (Yoo et al., 2021, Makris et al., 2021). However, this metabolite appears to have a biological role in its own right. Although the activity of 24,25(OH)₂D is less potent than calcitriol, it is considered to exert some influence on skeletal metabolism, primarily affecting cartilage and bone (Boyan et al., 2001, van Leeuwen et al., 2001). Considering serum levels of 25(OH)D, 1,25(OH)₂D, Ca, iCa and P were unchanged, as were bone mineral content and bone mineral density, and without normal reference ranges for serum 24,25(OH)₂D, it is hard to interpret the reduction demonstrated by the PBD dogs. Further investigation into the fate of this vitamin D metabolite in dogs fed PBD is warranted.

8.2.4.1 Implications

Most notably, the documented changes in vitamin D metabolites and demonstration of maintenance of stable vitamin D concentration and skeletal mineralization in dogs fed vitamin D₂ compared to D₃ are the first of their kind. This work represents a significant contribution to the understanding of vitamin D metabolism in dogs and may serve to guide industry recommendations regarding provision of vitamin D₂ as a source of dietary vitamin D in dog foods. Previously, industry vitamin D recommendations were based on studies of vitamin D₃ metabolism in dogs, as data for vitamin D₂ were lacking. Nevertheless, recommendations for vitamin D allowed for supplementation of diets with D₂ instead of D₃, despite a lack of empirical evidence supporting the practice. While the findings presented in Chapter Six support a potential

safe inclusion rate for vitamin D₂ in diets formulated for canine maintenance, they also highlight areas for new research. A dose-response relationship was not established for vitamin D₂, and trials with growing puppies are indicated to determine if D₂ is also sufficient to meet the more metabolically demanding requirements of developing skeletons.

8.2.5 Key finding 5: Changes in fatty acids, lipid metabolites, and potential consequences for dogs fed plant-based

As explored in Chapter Seven, the most striking differences in metabolomics were found in fatty acids and lipid metabolites, though these were mostly explainable by the difference in the fatty acid compositions of the diets.

As with amino acids, changes in serum fatty acids corresponding with differences in dietary provision were expected. Dietary AA provision was lower in the PLANT diet, with concurrent reduction in serum AA in PLANT-fed dogs. The fatty acid composition of platelets is known to affect platelet function. In animals receiving diets with low AA content, a reduction in platelet AA has been demonstrated, associated with altered platelet function (Turner et al., 2015). In this case, dogs fed the plant-based diet demonstrated lower serum arachidonic acid, which may be consistent with lower platelet arachidonic acid, as, although platelet fatty acid composition was not measured in this study, it has been demonstrated in humans that dietary fatty acid composition influences fatty acid composition of platelet membranes (Lankinen et al., 2018). Indeed, in this study it was found that platelet count decreased slightly, in conjunction with a slight increase in platelet size. Both parameters were within the normal reference range, but it is unclear if they were continuing to change by the exit point of the study. In a previous study in dogs, platelet function was demonstrated to be affected by the fatty acid profile of

platelet lipids, but platelet count and morphology were not (Westgarth et al., 2017). In our study, only count and morphology were measured, function was not, and so the clinical significance of this finding is not yet known.

In addition to the alterations in fatty acid concentrations, a reduction of cholesterol was demonstrated in the PLANT-fed dogs. Consistent with the current findings, a previous study in which dogs were fed a mostly plant-based product (containing all plant ingredients, with added meat and bone meal) containing no animal fat, demonstrated a reduction in serum cholesterol (Swanson et al., 2004). Cholesterol is synthesized only by animals, not plants, thus the contribution of diet to cholesterol levels is negligible in animals fed a PBD.

8.2.5.1 Implications

The fatty acid profiles of plant lipids vary from those of animal-derived lipids, and in this study the serum fatty acid profile of the dogs varied based on their dietary fatty acid profiles. This presents an opportunity to tailor the dietary fatty acids to produce a desired outcome. For example, the lipid profiles of the dogs fed the PBD shared some similarities with dogs fed a cardioprotective diet (Li et al., 2020). This could represent a potential benefit to heart health, though further investigation is necessary. However, this also highlights that some fatty acids that would usually be provided in conventional ingredients, such as AA, require consideration in PBD. Though platelet fatty acid composition was not measured in the study, it is possible that platelet morphology was affected by the change in fatty acid availability – specifically the reduction in AA. No reports of altered haemostasis in dogs fed PBD have been published, suggesting that possibly these alterations were either specifically as a result of the formulation of

the PLANT diet used in the trial, or that changes in platelet count and morphology remain minimal even when a PBD is fed long term, with no adverse effects. However, this warrants further investigation as to possible implications for both the health of dogs fed plant-based diets and future formulation of plant-based diets

With respect to the decrease in serum cholesterol, this is likely attributable to the lack of dietary cholesterol, with all circulating cholesterol being endogenously synthesized – since mammals are capable of *de novo* cholesterol synthesis, dietary cholesterol is not required. As such, there is no recognized adverse effects of a decreased dietary intake. Indeed, there are canine health conditions in which reduction of serum cholesterol is beneficial, such as hypercholesterolaemia, hyperlipidemia, diabetes mellitus, cholestatic disease and pancreatitis (Johnson, 2005, Xenoulis and Steiner, 2010). This presents a potential opportunity for a therapeutic effect of PBD for reducing serum cholesterol.

8.2.6 Key finding 6: Reduced serum BUN and creatinine in dogs fed plant-based

A novel finding from this study was a reduction in blood urea nitrogen (BUN) and serum creatinine (Chapters Six and Seven). Both metabolites are indicators of protein and muscle metabolism. BUN is recognized to be elevated in dogs fed high protein diets or absorbing digested blood from bleeding lesions within their gut, but does not vary greatly with body size or composition (Prause and Grauer, 1998). Creatinine, on the other hand, is directly associated with muscle mass, and is lower in small and lean dogs and higher in larger, muscular dogs (Freeman III et al., 2003, Médaille et al., 2004). However, despite reductions in serum BUN and creatinine,

dietary protein was sufficient and was not significantly lower in the dogs fed PLANT, thus decreased protein intake cannot explain the reductions. Many of the serum amino acids measured by HPLC in Chapter Six were included in The Metabolomics Innovation Centre PRIME DI/LC-MS/MS assay reported in Chapter Seven, with comparable results, including the reduction in serum creatine in PLANT-fed dogs. There were few differences in amino acid metabolism between the PLANT and MEAT-fed dogs, specifically, there were no indicators of alterations in protein catabolism or muscular metabolism to support the reductions in BUN and creatinine. Similarly, the dogs maintained muscle condition and lean soft tissue mass, so loss of body muscle cannot likely explain the reduction in serum creatinine. Body composition was comparable in both groups of dogs, those fed the PLANT diet and those fed the conventional, meat-based diet (MEAT), demonstrating the efficacy of PLANT to maintain lean soft tissue, bone and fat in the sample population of adult dogs over a 3-month period.

Another mechanism that could explain the decreases could be regarding renal filtration. BUN and creatinine are markers of kidney function, with concurrent increases in BUN and creatinine, termed azotaemia, recognized as indicators of kidney damage (Médaille et al., 2004). It is possible that the dogs fed PLANT experienced improved renal filtration, reducing BUN and creatinine from baseline levels (Finco et al., 1995). Considering the lack of renal disease in the dogs fed PBD reported by owners in Chapter Three, these findings may suggest a therapeutic benefit of PBD for kidney health (Dodd et al., Submitted). Indeed, this has been postulated in humans as well (Kim et al., 2019a).

8.2.6.1 Implications

These biochemical findings warrant further investigation, as they may imply health benefits. Considering that PLANT-fed dogs showed little alteration in protein metabolism, with no change in creatine, the precursor of creatinine, or muscle mass, the reductions in serum BUN and creatinine suggest improved clearance as opposed to reduced production. There were no other findings from this study that could explain these findings or indicate a mechanism for this, thus these results point to further research to investigate potential effects of PBD on renal parameters in dogs. With respect to the nutrients known to have the most effect on kidney health and function (protein, phosphorus, potassium and EPA+DHA), the diets provided very similar quantities. Furthermore, in healthy dogs, these nutrients are not recognized to impact kidney health or function; they are only of concern in dogs with impaired kidney function. Thus, it would appear that, in this case, the sources (ingredients) providing the nutrients may have some relevance to the impact of the diet, either through additional provision of beneficial non-essential nutrients or non-nutrient compounds, or through reduction of deleterious non-essential nutrients or non-nutrient compounds. Degenerative kidney disease is a common condition of aging dogs, thus if it is found that either a specific plant-based component (for example, plant-based protein) or a wholly PBD are beneficial for kidney health and/or function, this could have a large impact on formulation of therapeutic diets to promote better health for dogs.

8.2.7 Key finding 7: Alterations in serum amino acids in dogs fed plant-based

No consistent reference ranges for serum amino acids are published for dogs. This research thus utilized dogs fed the conventional animal-based diet as controls and considered their serum amino acid profiles to be ‘normal’ or ‘healthy’, from which comparison could be

made with the PLANT dogs. The only changes not attributable to dietary composition demonstrated by the PLANT dogs were a decrease in BCAAs (isoleucine, leucine and valine) and an increase in glutamine (Chapter Six and Seven). Glutamine content in the diets was not measured, thus the origin of the increase could not be determined and it is possible that differences in glutamine concentration in the diets may explain the difference in serum glutamine. The BCAAs, however, were provided in a higher concentration in the PLANT diet, yet serum BCAAs decreased. Descriptions of reduced serum BCAAs are scarce amongst any species, and the significance of this finding is unknown. The BCAA are a key component of skeletal muscle, which provides the main pool of BCAA in the body. The reduction in serum BCAA in the PLANT dogs did not correspond with any detectable changes to muscle quantity, as the PLANT dogs maintained lean soft tissue mass and proportion throughout the study. In both groups, ratio of Ile:Leu:Val was 1.0:2.5:3.5 at both baseline and exit timepoints, thus the reduction in serum BCAA in the PLANT-fed dogs were proportional. Serum amino acids represent only a single component of protein and amino acid flux, thus further investigation of BCAA metabolism in dogs fed a PBD are indicated (Elwyn *et al.*, 1968).

8.2.7.1 Implications

Possible health impacts of the changes in serum amino acids were more challenging to predict. Changes in serum amino acids corresponding with differences in dietary provision were expected, and some were identified during the diet trial. However, an unexpected finding was the decrease in BCAA detected in the serum of the dogs fed the PBD, despite a slightly higher dietary provision. This indicates a potential alteration in BCAA metabolism, though no changes in serum total protein, albumin, muscle condition or lean soft tissue were detected to suggest a

difference in total body protein (Chapter Six). Furthermore, neither products of BCAA catabolism, alpha-ketoglutarate and glutamate, nor carnitines associated with BCAA oxidation, propionylcarnitine and valerylcarnitine, were increased in the dogs fed the PLANT diet (Chapter Seven).

There are few studies discussing BCAA metabolism in dogs, while in other species the focus has been on the prognostic value of elevated, not reduced, serum BCAA (Li et al., 2019, Merz et al., 2018, Zhang et al., 2020). Similarly, though lower serum glutamine has been reported to be associated with gastrointestinal disease in humans and experimental intestinal injury in dogs, few studies have described clinical significance of elevated glutamine levels (Xin et al., 2015, Gouttebel et al., 1992, Puurunen et al., 2018). Further research into amino acid metabolism in dogs fed PBD is warranted to elucidate the alterations in glutamine and BCAA demonstrated here.

8.2.8 Key finding 8: few metabolomic differences between dogs fed a plant-based or conventional diet

Measurement of serum metabolites includes effects of dietary composition, gut microbiome and the genetic expression of the host animal themselves. Thus, metabolomics can offer a wide picture of the metabolic activity of an animal, including effects of diet, microflora and individual variability. Inclusion of serum metabolomics in the diet trial allowed for detection of diet effects within each animal.

Concentrations of essential nutrients in complete and balanced commercial pet food products lie within ranges recommended by industry guidelines (AAFCO, 2020, FEDIAF, 2020). However, non-essential nutrients and biologically active compounds such as polyphenols and amides may vary based on dietary composition. The metabolomic profiles were consistent with other findings from the trial. Many of the serum amino acids measured by HPLC in Chapter Six were included in The Metabolomics Innovation Centre PRIME DI/LC-MS/MS assay reported in Chapter Seven, with comparable results. Similarly, creatinine as measured in the PRIME assay was reduced, consistent with the reducing in creatinine detected on serum biochemistry reported in Chapter Six.

Changes noted in amino acids were consistent with those reported in serum amino acid profile detailed in Chapter Six. Namely, amino acid concentrations reflected dietary composition, with one notable exception. As described in Chapter Six and in section 9.2.7 above, BCAAs were all reduced, despite being provided in higher dietary concentrations. This has been discussed in Key Finding 3. Furthermore, no other metabolomic changes could explain this finding – there were no corresponding alterations in metabolites associated with BCAA catabolism, such as the BCAA-derived ketoacids or tricarboxylic acid cycle intermediates. Furthermore, considering muscle is the main pool of BCAA in the body, other changes in amino acid metabolites were noted, including decreases in methylhistidine and trans-hydroxyproline, that would be inconsistent with an increase in muscle catabolism or protein turnover. Whether this was a unique finding of the dogs fed this particular PLANT diet and potential causes for reduction in serum BCAA in dogs fed PBD thus requires further investigation.

8.2.8.1 Implications

Few differences were detected in the serum metabolome of dogs fed the PLANT diet as compared to dogs fed the conventional MEAT diet, supporting the concept introduced in Key Finding 1 (Chapters Three and Four): dogs have an essential requirement for nutrients, independent of the ingredients used to provide those nutrients. By and large, nutrient metabolism in dogs fed PLANT was the same as dogs fed MEAT, or else attributable to differences in nutrient provision in the PLANT and MEAT diets with a few exceptions. The most marked alterations were in fatty acid and lipid metabolites, but these were also largely attributable to the differences in fatty acid composition of the PLANT and MEAT diets. Notable exceptions included reduced BCAA and creatinine.

As mentioned previously in Key Finding 5, the cause of the decrease in BCAA in dogs fed the PLANT diet could not be determined and warrants further investigation. The reduction in creatinine without any change in its precursor, creatine, suggests that the decrease was not associated with changes in muscle mass or muscle protein metabolism. Instead, considering the concurrent reduction in serum BUN, it is possible that increased renal filtration may be the cause of the reduction. This also warrants further study.

The implication of the finding that the changes that were seen in conjunction with differences in the nutritional provision in the diet were that changes in dietary nutrient provision directly influenced metabolic pathways in dogs within 3 months. This was most marked in the fatty acids and lipid metabolites, where the differences in animal-derived and plant-derived fats were clearly seen. On the other hand, when dietary nutrient provision was comparable, the

ingredient source, either animal- or plant-based, did not impact nutrient metabolism, with the possible exception of BCAA. This supports the concept that a PBD formulated to meet target nutrient profiles can provide nutrients available and utilisable by the animal.

8.2.9 Key finding 9: No evidence of alterations in sulfur amino acid metabolism in dogs fed plant-based

The findings of the study reported in Chapter Seven demonstrated comparable SAA metabolism. Considering the role that SAA play as methyl donors, and the activity of folate and folate-pathway intermediates in regeneration of methionine, and the lack of differences in these pathways detected between PLANT and MEAT dogs, it was determined that SAA metabolism was comparable between the two groups. Serum taurine levels in the dogs fed the PBD decreased over the course of the trial and were lower in the PLANT dogs, though within the normal range, attributable to lower dietary provision of Tau in the PLANT diet. There were no notable differences in SAA, one-carbon or folate pathways to suggest dysregulation or impaired Tau synthesis. However, it is possible that Tau excretion was increased, as neither urinary nor fecal Tau were measured in this study.

8.2.9.1 Implications

Considering recent concerns regarding SAA metabolism in dogs fed diets with plant-based protein, it is worth noting that there were no differences detected in serum SAA, one-carbon or folate pathway metabolism, with the exception of a reduction in serum dcSAM and Tau. The decrease in Tau is likely explainable by a lower Tau content in PLANT than MEAT. This is reinforced by no corresponding changes in serum SAA or SAA metabolites in the taurine biosynthetic pathway. This suggests that provision of SAA was sufficient in PLANT to maintain

normal SAA metabolism and Tau synthesis in healthy adult dogs. As serum Tau was maintained within the normal reference range, these findings did not support the hypothesis that PBD necessarily induce Tau deficiency.

8.3 Limitations

8.3.1 Survey study

Questionnaire-based surveys have limitations inherent in their methodology. Participants are expected to accurately recall information, which introduces a potential for incorrect data reporting. Bias may also be introduced if participants believe there is a ‘correct’ answer or wish for their answers to be perceived in a certain manner. Bias mitigation can be implemented, through careful phrasing of questions, utilising previously-validated questions, and pre-testing of surveys prior to data collection, but ultimately it must be assumed that participants answer the questions honestly and factually. Nevertheless, there are limitations to the interpretation of survey-based data. In the case of the studies presented in Chapters Three and Four, the surveys were anonymous and answers were recorded in a written format. As such, there was no ability to clarify responses and ambiguous or confusing answers could not be interpreted. Selection bias may also have been introduced, as a non-random sampling strategy was employed. Pet owners were convenience sampled by advertising the survey at pet food retailers and in pet-related social media groups. This form of sampling method was utilized to facilitate large sample sizes across Canada and the USA. However, there was risk of selection bias since results from owners electing to participate in a pet health related survey may not be generalizable to the wider pet-owning population. Furthermore, incentive, in the form of gift cards to pet food retailers, was

used to encourage participation, potentially introducing bias. Nevertheless, this is a common sampling strategy, and demographic variables of the survey respondents were comparable to other published survey-based pet nutrition studies. Lastly, due to the cross-sectional study design, causality cannot be inferred for any of the associations reported, resulting in different potential implications of the results, as discussed in Chapters Three and Four.

The studies presented in Chapters Three and Four measured self-reported perceptions of pet health and wellbeing by pet owners and compared the results between pets fed plant-based or non-PBD. No objective measures of disease presence or absence, such as veterinary records, were obtained, and animals were not examined, so confirmation of owner perception of their pet's health could not be verified. Accuracy of owner estimation of body condition, health status, and behavioural indicators of health and wellness may vary based on owner relationship with their animal, their knowledge of animal health, and the animal's lifestyle (such as indoor/outdoor cats who may not be observed often by their owner). Thus, the scope of the study was reporting owner *perceptions* of their pets' health, and cannot be inferred as accurate representations of pet health in general. Nevertheless, perception of health is important, particularly in consideration the application of the Health Belief Model to veterinary medicine (Kamleh, 2019).

8.3.2 Nutrient analyses study

Nutrient analyses performed in this study were based on single samples of diets obtained from a single batch and may not be representative of the typical analysis of the product at any given time. Variability from batch to batch and even intra-batch is inherent when mass producing products, and so single point analyses must be interpreted with caution. Although samples were

collected and submitted for analysis in a timely manner, with additional samples collected, vacuum-sealed and frozen at -20°C, storage time and conditions prior to acquisition by the research team are unknown. Some nutrients are temperature, moisture, and/or light sensitive, and degradation may have occurred prior to testing, reducing the apparent content in the diet.

Nutrient-nutrient interactions are also possible.

To reduce the risk of inaccuracies in nutrient measurements, samples were analyzed by laboratories following AAFCO guidelines for nutrient detection and AOAC methodologies were utilised, where feasible. In-house analyses were performed under the guidance of experienced technical staff, following published protocols. Nevertheless, there is also accepted variance in nutrient analyses between labs, and within labs between protocols. Often, analyses were performed in duplicate and an average result reported.

In addition to the limitations associated with analytical methodology, limitations regarding interpretation of the results must be highlighted. Detection of a nutrient within a food matrix does not infer digestibility or bioavailability in the animal to which it is intended to be fed. Demonstration of adequacy of nutrient provision in the diet is an indicator of nutritional sufficiency, but further testing, such as *in vitro* or *in vivo* digestibility and/or diet trials provide more information about the performance of the diet.

8.3.3 Diet trial

One of the biggest limitations of the diet trial was the use of client-owned animals and the resultant reliance on owner compliance. This could impact the diet trial in numerous ways:

owners may or may not consistently measure or feed the recommended amount of the trial diet; they may or may not feed or allow their dog to be fed foods outside the scope of the trial; they may or may not accurately record data; their dog may or may not scavenge food from external sources (ie: “counter-surfing”, garbage, edible items in their yard or when out on walks), etc. Within this trial in particular, specific concerns were the provision of foods containing animal products and/or vitamin D₃, which could interfere with the evaluations performed. Participants were required to complete daily records of the food their dogs were offered, their consumption, other foods acquired (treats, snacks, scraps, etc), fecal output, exercise and activities and any notable events in order for the research team to determine adherence to the study protocol. This required the assumption that owners would complete the records and complete them honestly and with factual accuracy, and provide the records to the research team at the requested interval (once weekly). Furthermore, owner participation affected completion of the trial, with some study dogs being lost to follow-up, particularly during the COVID-19 lockdowns. For the remaining dogs in the study, an indicator of good compliance was the lack of D₃ metabolites detected in the PLANT group. This suggests that participants complied with the dietary recommendations and refrained from feeding animal products to their dogs for the duration of the study.

The heterogeneity of the sample population may introduce additional confounding factors which complicate data analysis and interpretation – although this also may improve external validity to the general dog population. In this case, dogs could vary in age from 2-10 years, sex could be sterilized female or male, there were no limitations on breed, though a minimum size of 5kg was required for safety of administration of sedation and collection of the necessary blood

samples. Due to the randomization of assignment of dogs to each diet group, there was a risk of groups being imbalanced with respect to the aforementioned characteristics. Distribution of these independent variables was tested and found to be equal between groups, reducing possible confounding effects on the outcomes of interest.

A limiting factor with respect to the data collected and variables evaluated as outcomes from the diet trial was the frequency of sampling. Apart from a screening wellness examination, data were collected from dogs only at the beginning and end of the trial. Thus, with any variables with significant time effects, the pattern of change was indeterminable. It could not be determined if variables were still changing at the exit timepoint, or if they had changed and then stabilised, or reversed, between the start and the end of the trial. This reduces the ability to interpret the significance of some of the changes. For example, dogs in the plant-based group had lower platelet counts with higher platelet volume at the end of the trial, but both parameters were within the confines of the normal reference range, making interpretation of the clinical relevance of this finding challenging. As it is unclear if platelet count and volume would continue to change if the diet trial were longer, it could not be determined if these parameters would eventually exit the reference ranges and potentially impact platelet function and haemostatic measures. More frequent measurements to document the temporal trends and patterns in variables would have been beneficial. A longer trial would also be beneficial to determine longer-term effects of the PBD on participating dogs, but due to concerns with owner compliance over longer time periods the trial length was set at three months. This was determined, based on previous diet trials in canines and other species, to be an adequate time for

which to detect differences in the variables of interest, without exceeding the compliance of participating owners.

Extrapolation of findings from this trial to the greater population of dogs fed PBD should be made with caution. The PLANT diet used in the trial was specifically formulated to exceed minimum nutrient recommendations and was tested to ensure nutrient sufficiency (AAFCO, 2020). As stated in Key Finding 2 and demonstrated in Chapter 3, commercial PBD may not meet all nutrient recommendations for canine adult maintenance. Furthermore, the specific nutrient profile of PLANT may not be representative of other complete and balanced PBD. In particular, the metabolic differences detected in dogs fed PLANT, including reduced serum BCAA and alterations in fatty acid and lipid metabolites, may only be applicable to dogs fed PLANT, as opposed to any other PBD. Further investigations to determine the effects of PBD, per se, as opposed to the effects of this specific diet would be required in order to determine if the results were attributable to the diet being plant-based as opposed to simply attributable to the specific PLANT nutrient profile.

Lastly, the impact of the COVID-19 lockdowns introduced some variability into the duration of the diet trial. Initially, dogs were scheduled at fixed intervals, with a screening appointment followed four weeks later by the baseline evaluations, followed twelve weeks later by exit evaluations. However, dogs were in variable stages of the study (post-screening or post-baseline) when data collection was forcibly paused during the global COVID-19 pandemic. This resulted in some dogs within the study consuming the control diet for longer than 4 weeks prior to baseline evaluations, and some dogs consuming either the plant-based or animal-based diet for

longer than 12 weeks between baseline and exit evaluations. The length of each dog's participation in each phase of the study was recorded, and there did not appear to be an impact of length of participation on measured outcomes, and these dogs were not found to be outliers in statistical analyses.

8.4 Future research

The studies presented in this thesis provide novel insights to guide further investigations into the suitability of PBD for dogs and cats. They serve as a starting point for the understanding of potential risks and benefits, setting up future work with the following focuses:

- **Association between plant-based pet foods and GI, hepatic, renal and cardiac disorders:** Owners of both dogs and cats fed plant-based diets reported lower prevalence of these health disorders than owners feeding non-PBD. However, this was a cross-sectional study relying upon owner-reporting and requires further investigation. A plausible next step would be to partner with veterinary clinics to examine veterinary records and collect data regarding diet, dietary changes, and diagnoses in dogs and cats. This would be more objective and risk of bias would be lower than in the owner-reported survey.
- **Renal function testing:** Further investigation into the effects of PBD on renal function is warranted given that owners perceive lower prevalence of renal disease in dogs and cats fed PBD along with the observed reduction in blood urea nitrogen and creatinine detected in the dogs fed a PBD while maintaining lean soft tissue, muscle condition, and serum proteins. It is possible that these changes indicated improved glomerular filtration

(Finco et al., 1995). Urine samples that were collected and frozen during data collection could be used to measure urine protein to creatinine ratio as an indicator of renal filtration (Borsook and Dubnoff, 1947, Braun et al., 2003). Future diet trials could also include direct measurement of glomerular filtration rate (Von Hendy-Willson and Pressler, 2011).

- **Cardiac health testing:** Although serum taurine levels decreased in dogs fed the PBD, the change was numerically small and not clinically significant. However, considering the trial documented taurine at two timepoints only, the rate of the change was unknown, and it cannot be determined if serum taurine was continuing to trend downwards or not. Since reduced taurine is a risk factor for dilated cardiomyopathy in dogs, investigation of cardiac health is warranted. Frozen sera collected during this study could be tested for markers of cardiac health, such as B-type natriuretic peptide and cardiac troponins (Baisan et al., 2016).
- **Branched-chain amino acid investigation:** Dogs fed the PBD, which was higher in BCAAs than the conventional animal-based diet, had reductions in serum isoleucine, leucine and valine. Muscle condition, lean soft tissue mass and proportion were all maintained, suggesting that skeletal muscle metabolism was not impacted by the changes in BCAA metabolism, but muscle was not directly investigated in this study. In a previous study, biopsies of skeletal muscle revealed markers of proteolysis in dogs with evidence of muscle wasting fed low protein diets (Wakshlag et al., 2003). Future studies may incorporate muscle biopsies to investigate if changes in serum amino acids and skeletal muscle protein and amino acid stores are related.

- **Platelet function:** Although platelet count and size were maintained within the normal laboratory reference ranges, count decreased and volume increased over time in the PLANT dogs. To determine if there are any effects on platelet function in dogs fed a PBD diet, serial blood sampling with haematology and platelet function testing, as well as determination of platelet membrane fatty acid composition, is indicated.
- **24,25-dihydroxyvitamin D excretion:** Most vitamin D metabolite concentrations were maintained in the dogs fed the PBD, though one difference was detected between those dogs and the dogs fed the animal-based diet: serum 24,25-dihydroxyvitamin D was lower. To investigate vitamin D catabolism further, measurement of vitamin D metabolites in frozen urine samples collected during the trial would be of interest.
- **Vitamin D₂ dose-response:** Although vitamin D concentration and skeletal mineralization was comparable between the dogs fed the plant-based or animal-based diet, provision of vitamin D₂ in the plant based diet was one third higher than vitamin D₃ in the animal-based diet. Thus, it could not be concluded that vitamin D₂ could be a one-to-one replacement for vitamin D₃ in canine diets. In other animals, a reduced efficiency of vitamin D₂ as compared to D₃ has been reported (Morris, 2002a). This study demonstrated that vitamin D₂ activity was equivalent at a rate of 130% of vitamin D₃. Future studies investigating different D₂ doses are warranted to further guide industry recommendations.
- **Vitamin D₂ as a vitamin D source for growing puppies:** The present study only examined the efficacy of vitamin D₂ in adult dogs so as to obtain data in this population

prior to studying growing puppies at risk of skeletal mineralization defects as a result of insufficient vitamin D. The findings of this study suggest that, at least at the inclusion rate used here, vitamin D₂ was sufficient to maintain vitamin D concentration and skeletal metabolism in adult dogs. Now, investigation in growing puppies is warranted to determine appropriate inclusion rates in diets formulated for growth or all life stages.

- **Feline diet trial:** No diet trials have been published in which cats were maintained on a PBD. Building from these findings in dogs, a diet trial with cats is indicated, in order to investigate the effect of PBD on feline health parameters, body composition, vitamin D concentration, bone mineralisation, and renal and cardiac health.

8.5 Concluding remarks

This thesis presents a comprehensive, multi-faceted investigation into plant-based foods and contributes to the small but growing body of knowledge pertaining to this niche field. Though at present, PBD are fed to a small proportion of dogs and cats, if the popularity of PBD continues to grow as predicted there will be an even greater requirement for further understanding of how these diets may impact the health and wellbeing of the animals to which they are fed (Dodd et al., 2019b). In Chapters Three and Four, it was reported how owners of cats and dogs fed PBD have a perception that their pet's health is equal to or greater than the health perceived by owners feeding meat-based diets (Dodd et al., 2021a, Dodd et al., Submitted). This is despite the reality that many commercial PBD fail to meet the nutrient profiles recommended by American and European pet food industry associations, as demonstrated in Chapter Five (Dodd et al., 2021b). These findings provide insight for

veterinarians to monitor pet health and educate clients regarding potential dietary risks and benefits. Furthermore, the findings suggest changes required to improve the nutritional quality of commercial plant-based diets. Additional research is indicated to objectively measure health outcomes in dogs and cats fed plant-based diets to determine how these are associated with reported owner perceptions.

It was demonstrated in the feeding trial (Chapters Six and Seven) that feeding a nutritionally complete and balanced PBD formulated to meet the recommended nutrient profile for canine adult maintenance was sufficient to maintain wellness parameters and most serum metabolites in a heterogenous population of healthy, adult dogs for at least three months. Importantly, to the author's knowledge this study presented the first evidence for the use of vitamin D₂ as a source of dietary vitamin D for dogs (Chapter Six). These findings suggest that, at the inclusion utilised in PLANT, which was 137% of the vitamin D₃ inclusion, plant-based vitamin D₂ was an effective dietary source of vitamin D for healthy, adult dogs.

Additionally, the findings from this research support the concept that essential nutrients can be adequately provided in pet foods without the use of animal ingredients, and that, at least in dogs, these nutrients are metabolised similarly to those from animal-derived ingredients. It thus falls to the formulation of the diet to ensure that it includes necessary nutrients in appropriate quantities and in available forms. Within the industry, formulation, manufacturing, and post-production analyses should be performed by skilled, knowledgeable and experienced professionals to improve the compliance of PBD with meeting recommended nutrient profiles for dogs, cats, puppies and kittens. Given the current situation, pet parents should be aware of the

risks of nutritional insufficiencies in commercial PBD, and, if feeding a PBD to their pet, could consider testing their food products for the content of key essential nutrients demonstrated to be at greatest risk of imbalance, or evaluating and monitoring nutritional status in the animal themselves.

Possible health implications of PBD were identified, including potential risks for platelet production and thus potentially haemostasis, and possible benefits for reducing serum cholesterol, BUN and creatinine. However, these require further investigation before the veterinary community may be able to make recommendations for or against a PBD for specific health conditions.

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10 Appendices

10.1 Appendix I

Survey instrument for Chapters three and Four.

Survey of Dog and Dog Health and Wellbeing

Consent

Thank you for considering being a participant in this study. On the next page you will find the consent form. Please read it thoroughly and indicate whether you would like to consent to participate by clicking Yes or No. Once you have reached the end of the survey, you will have the option to be entered into a draw to win one of eight \$25 gift cards to a pet supply retailer of your choice.

DOG AND DOG HEALTH AND WELLNESS SURVEY PARTICIPANT CONSENT FORM

What we will ask you to do if you agree to participate:

If you live with a dog and/or dog and are their primary caregiver, you are invited to participate in research to understand factors relating to dog and cat health and wellness as reported by their keepers. If you agree to participate, you will be asked to fill out an eSurvey, which will take approximately 15-20 minutes. The eSurvey will ask you general questions about your companion animal and will inquire into their health history.

Are there any potential risks to participation?

You do not have to respond to any questions or provide information you do not feel comfortable providing. There is no consequence to you if you do not want to complete the survey. The survey will be anonymized, which means that we will not be able to connect you to the answers after the data collection period. Please note that while every effort is made to securely store data once they have been received, confidentiality cannot be guaranteed while data are in transit over the internet. Data provided by you may NOT be withdrawn from the research project once the completed survey is submitted. You may withdraw during the survey by closing your browser. Non-identifying information may be used in publishing materials and presentations.

Are there any potential benefits to participation?

Your input will benefit the area of study, and a summary of the results will be available, once the study has been completed, at: <http://bulletin.ovc.uoguelph.ca/>

Are there any financial incentives?

If you complete the survey, you can choose to enter your email into a draw for one of five \$50 gift certificates to a pet supply distributor of your choice. If you enter this draw, you will have to enter your email in order to be contacted in the event that you win. No other identifying information will be requested.

Please remember that your PARTICIPATION IS VOLUNTARY and you may decide to skip or not participate at any time.

Please print or screenshot this page for your records.

Researcher information:

Dr. Adronie Verbrugghe DVM, PhD, DECVCN, Assistant Professor

Dr. Doge Dewey DVM, PhD, Professor

Dr. Sarah Abood DVM, PhD, Assistant Professor

Dr. Sarah Dodd BVSc, MSc, PhD Student

AGREEMENT TO PARTICIPATE

Please choose Yes or No (below) indicating your consent to participation.

- a) Yes
- b) No

Introduction

1. Do you live with dogs or dogs?
 - a. Cat(s)
 - b. Dog(s)
 - c. Both cat(s) and dog(s)

Cats

General

2. How many cats do you have?
 - a. 1
 - b. More than 1
 - i. If more than 1, please specify the numbers of cats {Open Text}
3. How old is your cat?
 - a. {Open Text}
4. What is the sex of your cat?
 - a. Male
 - b. Female
5. Is your cat neutered (castrated / spayed)?
 - a. Yes
 - b. No
 - c. I don't know
6. What breed is your cat?
 - a. {Open Text}
7. How long has your cat lived with you?
 - a. Their whole life
 - b. Other

i. {Open Text}

8. Does your cat live indoors or outdoors? If not exclusively indoors or outdoors, please describe.

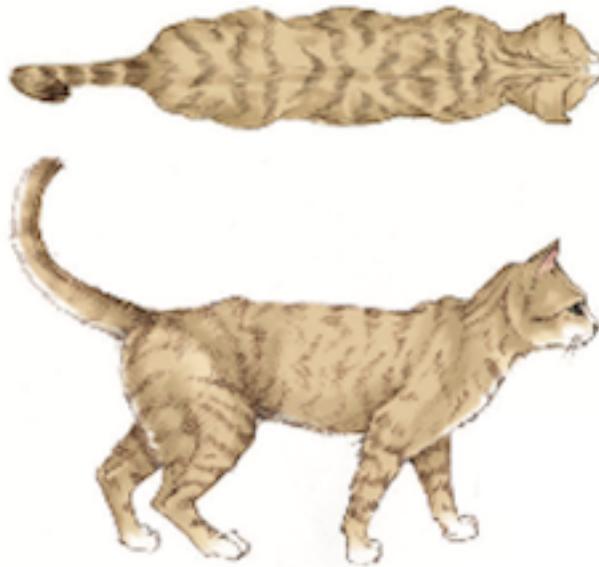
- a. Indoors only
- b. Outdoors only
- c. Mostly indoors {Open Text}
- d. Mostly outdoors {Open Text}
- e. Half indoors and half outdoors {Open Text}

9. If not indoors only:

Is your cat able to hunt for prey? Do you ever see your cat catch prey?

- a. Yes my cat could hunt prey, but I have never seen them catch prey
- b. Yes, my cat hunts prey and I have seen them catch prey
- c. No, my cat could not hunt prey, they are not loose outside

10. Please select the image which best corresponds to your cat's body condition:



a.



b.



c.



d.

Health

11. Please list any veterinary conditions your cat has experienced over the past year.
 - a. {Open Text}

12. Please rate your cat's current general health:
 - a. Excellent
 - b. Very good
 - c. Good
 - d. Fair
 - e. Poor

13. What do you use as a source of information about cat health?
 - a. Books
 - b. Breeder
 - c. Discussion/support groups
 - d. Friends/family
 - e. Internet articles written by veterinarians
 - f. Internet articles written by pet owners
 - g. Pet health magazines
 - h. Pet health websites
 - i. Pet stores

- j. Veterinarian
- k. Other
 - i. {Open text}

14. How many hours per week do you spend learning about pet health related topics?
- a. < 1
 - b. 1 – 10
 - c. 10 – 20
 - d. 20 – 30

Please answer the following questions about your cat's health during the past 4 weeks:

15. Has your cat been ill and vomited?
- a. Not at all
 - b. A little
 - c. Quite a bit

16. Has your cat been inactive or had low energy?
- a. Not at all
 - b. A little
 - c. Quite a bit

17. Please select the image which correlates with your cat's stool consistency.





b.



c.



d.



e.



f.



g.

18. Has the quality of your cat's hair coat changed?

- a. Quite worse
- b. A little worse
- c. No change
- d. A little better
- e. Quite better

19. Has your cat been grooming themselves (licking, scratching) as much as usual?

- a. A lot less

- b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
20. Has your cat been jumping (e.g. onto cat tower, furniture, counter tops) as much as usual?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
21. Has your cat been drinking as much as usual?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
22. Has your cat had bowel movements (passed faeces) with usual frequency?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
23. Has your cat urinated with usual frequency?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more

Wellbeing

Please answer the following questions about your cat's behaviour and wellness during the past 4 weeks:

24. Has your cat appeared happy?
- a. Not at all

- b. A little
 - c. A moderate amount
 - d. A great deal
25. Has your cat yowled or hissed in distress?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
26. Has your cat moved away when you attempt to touch them?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
27. Has your cat been affectionate towards you?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
28. Has your cat been curious and shown an interest in their surroundings?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
29. Has your cat been kneading (e.g. pawing laps, cushions or blankets) as usual?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
30. Has your cat slept more than normal?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more

- e. A lot more

Nutrition

Please answer the following questions about your cat during the past 4 weeks:

31. How much food has your cat eaten compared to normal?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
32. Has your cat's weight changed?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
33. What do you use as a source of information about cat nutrition?
- a. Book
 - b. Breeder
 - c. Discussion/support group
 - d. Friends/family
 - e. Internet
 - f. Pet Store
 - g. Veterinarian
 - h. Other
 - i. {Open text}
34. What factors do you consider important when selecting food for your cat? (select all that apply)
- a. Convenience to feed
 - b. Convenience to purchase
 - c. Hair ball treatment

- d. Homemade
- e. Human-grade ingredients
- f. Specific ingredients
 - 1. Presence of:
 - 2. Lack of:
- g. Natural/organic/holistic
- h. Palatability
- i. Plant-based/vegan
- j. Price/value
- k. Raw meat-based
- l. Skin/coat health
- m. Stool odor
- n. Stool quality
- o. Veterinary therapeutic diet prescribed for specific health condition
- p. Other
 - i. {Open text}

35. Please rank the following factors in order of importance, with 1 being most important

- a. __ (Choices carried over from previous question)

36. Please completely describe your cat's diet. Include treats, snacks, table scraps or other 'human food', supplements, and any other sources of nutrition (e.g. Brand "X" kibble free choice and Brand "Y" canned for breakfast and dinner, plus fish oil supplement and dental treats daily)

- a. {Open text}

37. Has your cat been fed that type of diet for as long as you've had them?

- a. Yes
- b. No
 - i. How long have they been fed that type of diet?
 - 1. {Open text}
 - ii. Why did you change the type of diet?
 - 1. {Open text}
 - iii. Please describe any changes you have noticed in the health or wellbeing of your cat since changing to that type of diet?
 - 1. {Open text}

38. Have you had previous cats, and would you be willing to answer a brief series of questions regarding them?

- a. If yes:
 - i. Please indicate the age(s) your previous cat(s) lived to:
 - 1. {open text}
 - ii. What diet was/were your previous cat(s) fed?
 - 1. {open text}

39. Would you like to submit answers for your dog as well?
- a. Yes
 - b. No

Dogs

General

40. How many dogs do you have?
- a. 1
 - b. More than 1
 - i. If more than 1, please specify the numbers of dogs {Open Text}
41. How old is your dog?
- a. {Open Text}
42. What is the sex of your dog?
- a. Male
 - b. Female
43. Is your dog neutered (castrated / spayed)?
- a. Yes
 - b. No
 - c. I don't know
44. What breed is your dog?
- a. {Open Text}
45. How long has your dog lived with you?
- a. Their whole life
 - b. Other

i. {Open Text}

46. Does your dog live indoors or outdoors? If not exclusively indoors or outdoors, please describe.

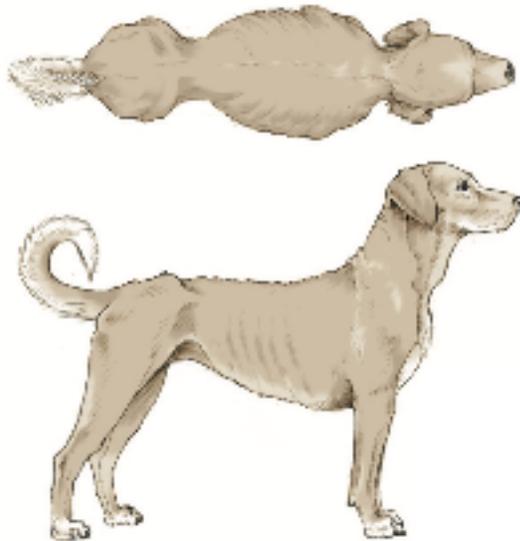
- a. Indoors only
- b. Outdoors only
- c. Mostly indoors {Open Text}
- d. Mostly outdoors {Open Text}
- e. Half indoors and half outdoors {Open Text}

47. If not indoors only:

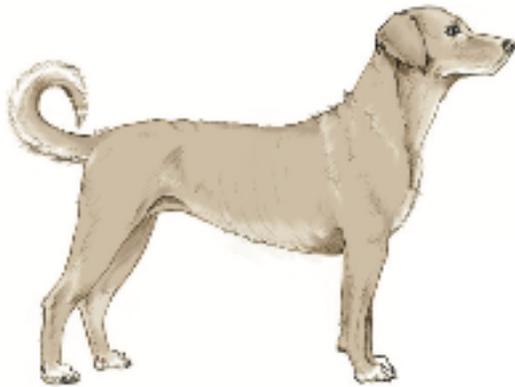
Is your dog able to hunt for prey? Do you ever see your dog catch prey?

- a. Yes my dog could hunt prey, but I have never seen them catch prey
- b. Yes, my dog hunts prey and I have seen them catch prey
- c. No, my dog could not hunt prey, they are not loose outside

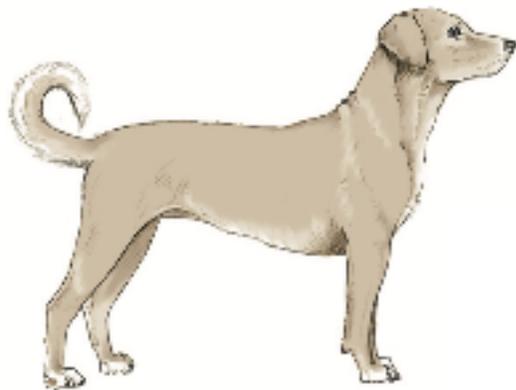
48. Please select the image which best corresponds to your dog's body condition:



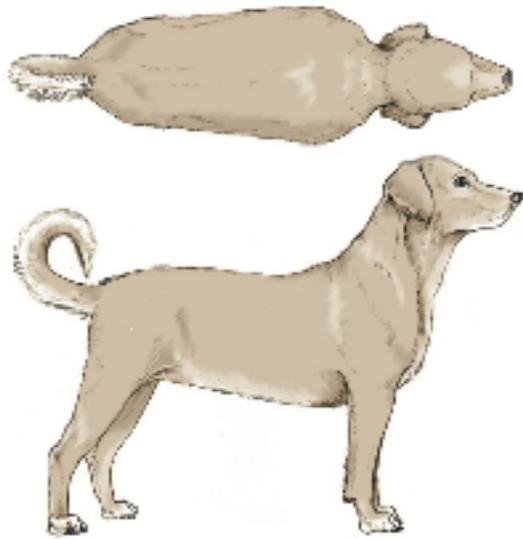
a.



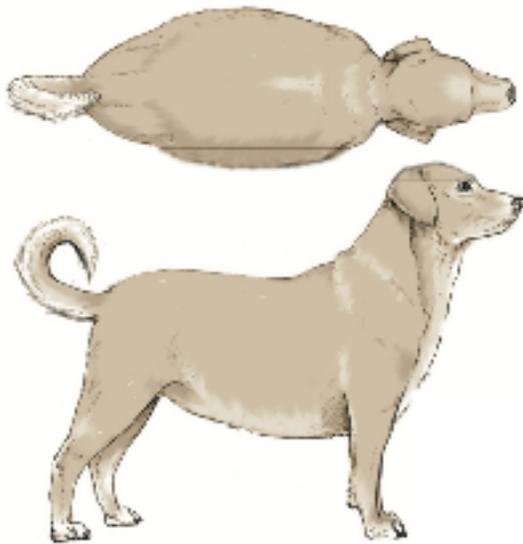
b.



c.



d.



e.

Health

49. Please list any veterinary conditions your dog has experienced over the past year.

a. {Open Text}

50. Please rate your dog's current general health:

a. Excellent

- b. Very good
- c. Good
- d. Fair
- e. Poor

51. What do you use as a source of information about dog health?

- a. Books
- b. Breeder
- c. Discussion/support groups
- d. Friends/family
- e. Internet articles written by veterinarians
- f. Internet articles written by pet owners
- g. Pet health magazines
- h. Pet health websites
- i. Pet stores
- j. Veterinarian
- k. Other
 - i. {Open text}

Please answer the following questions about your dog's health during the past 4 weeks:

52. Has your dog been ill and vomited?

- a. Not at all
- b. A little
- c. Quite a bit

53. Has your dog been inactive or had low energy?

- a. Not at all
- b. A little
- c. Quite a bit

54. Please select the image which correlates with your dog's stool consistency.



a.



b.



c.



d.



e.



f.



g.

55. Has the quality of your dog's hair coat changed?

- a. Quite worse
- b. A little worse
- c. No change
- d. A little better
- e. Quite better

56. Has your dog been slower to move around or get up after lying down?

- a. Not at all
- b. A little less
- c. Quite a bit

57. Has your dog been grooming (licking, chewing, scratching) themselves as much as usual?

- a. A lot less
- b. A little less
- c. The same
- d. A little more
- e. A lot more

58. Has your dog been easily going up or down stairs and/or jumping (such as onto a couch or into/out of a vehicle) as much as usual?

- a. A lot less
- b. A little less
- c. The same

- d. A little more
- e. A lot more

59. Has your dog been drinking as much as usual?

- a. A lot less
- b. A little less
- c. The same
- d. A little more
- e. A lot more

60. Has your dog had bowel movements (passed faeces) with usual frequency?

- a. A lot less
- b. A little less
- c. The same
- d. A little more
- e. A lot more

61. Has your dog urinated as usual?

- a. A lot less
- b. A little less
- c. The same
- d. A little more
- e. A lot more

Wellbeing

Please answer the following questions about your dog's behaviour and wellness during the past 4 weeks:

62. Has your dog appeared happy?

- a. Not at all
- b. A little
- c. A moderate amount
- d. A great deal

63. Has your dog vocalized in distress (eg: howl, bark, yelp, whine)?

- a. Not at all
- b. A little

- c. A moderate amount
 - d. A great deal
64. Has your dog moved away when you attempt to touch them?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
65. Has your dog been affectionate towards you?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
66. Has your dog been curious and shown an interest in their surroundings?
- a. Not at all
 - b. A little
 - c. A moderate amount
 - d. A great deal
67. Has your dog been playing (e.g. chewing on toys, fetching a ball, playing with other dogs) as usual?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
68. Has your dog slept as much as usual?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more

Nutrition

Please answer the following questions about your dog during the past 4 weeks:

69. What has your dog's appetite been like?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
70. Has your dog's weight changed?
- a. A lot less
 - b. A little less
 - c. The same
 - d. A little more
 - e. A lot more
71. What do you use as a source of information about dog nutrition?
- a. Book
 - b. Breeder
 - c. Discussion/support group
 - d. Friends/family
 - e. Internet
 - f. Pet Store
 - g. Veterinarian
 - h. Other
 - i. {Open text}
72. What factors do you consider important when selecting food for your dog? (select all that apply)
- a. Convenience to feed
 - b. Convenience to purchase
 - c. Hair ball treatment
 - d. Homemade
 - e. Human-grade ingredients
 - f. Specific ingredients
 - 1. Presence of:
 - 2. Lack of:
 - g. Natural/organic/holistic

- h. Palatability
- i. Plant-based/vegan
- j. Price/value
- k. Raw meat-based
- l. Skin/coat health
- m. Stool odor
- n. Stool quality
- o. Veterinary therapeutic diet prescribed for specific health condition
- p. Other
 - i. {Open text}

73. Please rank the following factors in order of importance, with 1 being most important

- a. __ (Choices carried over from previous question)

74. Please completely describe your dog’s diet. Include treats, snacks, table scraps or other ‘human food’, supplements, and any other sources of nutrition (e.g. Brand “X” kibble free choice and Brand “Y” canned for breakfast and dinner, plus fish oil supplement and dental treats daily)

- a. {Open text}

75. Has your dog been fed that type of diet for as long as you’ve had them?

- a. Yes
- b. No
 - i. How long have they been fed that type of diet?
 - 1. {Open text}
 - ii. Why did you change the type of diet?
 - 1. {Open text}
 - iii. Please describe any changes you have noticed in the health or wellbeing of your dog since changing to that type of diet?
 - 1. {Open text}

76. Have you had previous dogs, and would you be willing to answer a brief series of questions regarding them?

- a. If yes:
 - i. Please indicate the age(s) your previous dog(s) lived to:
 - 1. {open text}
 - ii. What diet was/were your previous dog(s) fed?
 - 1. {open text}

10.2 Appendix II

Characteristics of the dogs enrolled to participate in the diet trial, with notes regarding drop-outs and losses.

Dog	Breed	Sex	Age (years)	Weight (kg)	BCS (1-9)	Group	Screening date	Baseline date	Exit date	Note
1	Shih tzu	F	5	6.9	6	Excluded	July 2 2019	DNF	DNF	Elevated liver enzymes
2	Shih tzu	F	2	4.9	5	Dropped	July 2 2019	DNF	DNF	Did not like food
3	Labrador x	M	6	36.5	7	2	July 2 2019	Aug 1 2019	Oct 22 2019	
4	Boxer x	F	7	26.2	4	1	July 2 2019	July 30 2019	Oct 22 2019	
5	Labrador retriever	F	7	28.3	6	2	July 4 2019	July 30 2019	Oct 22 2019	
6	Golden retriever	F	4	28.3	6	2	July 4 2019	Aug 1 2019	Oct 24 2019	
7	Golden retriever	M	5	33	6	2	July 4 2019	Aug 1 2019	Oct 24 2019	
8	Australian shepherd	M	3	21.8	5	1	July 4 2019	July 30 2019	DNF	Lost to follow-up
9	St Bernard x	F	7	50.0	6	Dropped	July 9 2019	DNF	DNF	Gained excessive weight
10	German shepherd x collie	F	7	25.5	4	1	July 9 2019	Aug 15 2019	Nov 12 2019	
11	German shepherd x collie	F	4	25.0	4	Excluded	July 9 2019	DNF	DNF	Hypocalcaemia
12	Labrador retriever	F	6	32.3	7	1	July 11 2019	Aug 8 2019	Nov 7 2019	
13	Labrador retriever	M	4	32.5	5	1	July 11 2019	Aug 8 2019	Nov 5 2019	
14	Labrador retriever	M	3	27.2	5	1	July 11 2019	Aug 8 2019	Nov 5 2019	

15	Akbasha	M	7	45.3	6	1	July 23 2019	Aug 20 2019	Nov 21 2019	
16	Collie x	F	3	16.5	6	2	July 23 2019	Aug 20 2019	DNF	Lost to follow-up
17	Klee kai	M	3	8	6	2	Feb 10 2020	July 7 2020	Sept 29 2019	Delay due to COVID
18	Klee kai	M	5	6	5	2	Feb 10 2020	July 7 2020	Sept 29 2019	Delay due to COVID
19	Irish wolfhound	F	4	48	5	2	July 23 2019	Aug 15 2019	Nov 12 2019	
20	Pug x Dachshund	F	6	10	7	2	July 23 2019	Aug 20 2019	Nov 14 2019	
21	Australian shepherd	M	9	17.2	4	1	July 25 2019	Aug 14 2019	Nov 20 2019	
22	Poodle	F	3	17.4	4	2	July 25 2019	Sept 5 2019	Jan 9 2020	
23	Australian shepherd	F	6	23	6	2	July 25 2019	Aug 15 2019	Nov 12 2019	
24	Corgi x	F	3	11.5	7	1	Nov 13 2019	Dec 18 2019	DNF	Developed UTI
25	Husky x labrador retriever	F	5.5	27	5	DNF	March 5 2020	DNF	DNF	Dropped out due to COVID
26	Boxer x	M	2.5	35	5	2	Sept 10 2019	Oct 10 2019	Mar 18 2020	Delay due to COVID
27	Labrador x poodle	F	2	28	7	1	Oct 1 2019	Oct 29 2019	Feb 11 2020	
28	Labrador x	M	2.5	31.5	6	2	Nov 13 2019	Dec 19 2019	DNF	Dropped out due to COVID
29	Bichon x	M	3.5	7.6	7	2	Sept 12 2019	Nov 14 2019	Feb 13 2020	
30	Golden retriever	M	5	25	5	1	Oct 3 2019	Oct 31 2019	Feb 13 2020	
31	Potcake	F	3	14	5	1	Feb 12 2010	July 6 2020	Sept 28 2020	
32	Cattledog x collie	M	8	28	6	2	Oct 3 2019	Nov 7 2019	Feb 13 2020	

33	Cocker spaniel x miniature poodle	M	2	8.2	7	1	Oct 3 2019	Oct 31 2019	July 9 2020	Delay due to COVID
34	Soft-coated wheaten terrier	M	2	21.5	6	2	Nov 19 2019	Dec 18 2019	Mar 17 2020	
35	Cattledog x collie	M	9	40	7	1	Nov 19 2019	Jan 9 2020	Mar 18 2020	
36	French bulldog	M	7	17.2	6	DNF	Nov 20 2019	DNF	DNF	Dropped out due to COVID
37	German shepherd x	F	7	22	7	1	July 9 2020	Aug 4 2020	Oct 27 2020	
38	Coton du Tulier x miniature poodle	F	4	7.5	7	1	Nov 21 2019	Dec 18 2020	Mar 19 2020	
39	Labrador retriever	F	4.5	33	7	2	Nov 21 2019	Jan 9 2020	Mar 19 2020	
40	Labrador x	F	5	33.5	7	1	Nov 26 2019	March 17 2020	July 8 2020	Delay due to COVID
41	Potcake	M	3	18.5	4	1	Nov 26 2019	March 17 2020	July 8 2020	Delay due to COVID
42	German shorthair pointer	M	6	34	4	DNF	Feb 14 2020	DNF	DNF	Dropped due to anal gland issues
43	German shorthair pointer	M	2	27	4	DNF	Feb 14 2020	DNF	DNF	Dropped due to anal gland issues
44	German shorthair pointer	F	2	17	4	2	July 13 2020	August 10 2020	Nov 27 2020	
45	German shorthair pointer	F	8	30.5	6	2	July 13 2020	August 10 2020	Nov 27 2020	

46	Labrador x	F	2	20	7	1	Feb 12 2020	July 14 2020	Oct 9 2020	Delay due to COVID
47	Border collie	M	3	24	7	1	Feb 12 2020	July 8 2020	Sept 30 2020	Delay due to COVID
48	Springer spaniel	M	2	17.7	5	DNF	Feb 14 2020	DNF	DNF	Dropped out due to COVID
49	Mastiff x	M	8	36.5	5	1	June 30 2020	Aug 3 2020	Oct 26 2020	
50	Yorkshire terrier	F	5	5.2	5	2	Feb 14 2020	July 10 2020	Oct 29 2020	Delay due to COVID
51	Border collie	F	5	17.5	5	2	June 30 2020	July 30 2020	Oct 23 2020	
52	Golden retriever x poodle	F	8	28.5	7	2	June 29 2020	July 31 2020	Oct 26 2020	
53	Labrador x australian shepherd x cattledog	M	2	30.8	6	2	July 10 2020	Aug 5 2020	Nov 5 2020	
54	Labrador	F	4	24	6	DNF	June 30 2020	DNF	DNF	Dropped out due to COVID
55	Australian shepherd	F	2	16.5	5	1	June 30 2020	July 28 2020	Oct 21 2020	
56	Mix	F	8	20	5	1	July 13 2020	Aug 27 2020	Nov 20 2020	
57	Boxer x	F	9	20	5	1	July 13 2020	Aug 27 2020	Nov 20 2020	
58	Miniature fox terrier	M	7	5.6	5	1	July 13 2020	Aug 26 2020	Nov 29 2020	
59	Anatolian shepherd	M	2.5	40	5	2	Feb 10 2020	July 6 2020	Sept 28 2020	Delay due to COVID
60	Jack Russel terrier	M	5	9.7	7	1	July 10 2020	Aug 7 2020	Nov 6 2020	
61	German shepherd dog	F	3	28	4	1	July 13 2020	Aug 3 2020	Nov 3 2020	
62	Shepherd x	M	5	35.5	6	2	June 29 2020	July 29 2020	Oct 23 2020	

63	Golden retriever	F	3	27.4	6	1	June 29 2020	July 31 2020	Oct 30 2020	
64	Mix	M	4	24.5	5	1	June 29 2020	July 31 2020	Oct 23 2020	
65	Springer spaniel	F	2	13.3	5	2	June 29 2020	July 27 2020	Oct 19 2020	
66	Australian shepherd	M	9.5	19	6	2	June 30 2020	July 27 2020	Oct 19 2020	
67	Australian shepherd	F	9	20.3	7	2	June 30 2020	July 30 2020	Oct 22 2020	
68	Nova Scotia Duck Tolling Retriever	F	9	25	7	2	June 30 2020	July 30 2020	Oct 22 2020	
69	Irish wolfhound	F	5.5	62	7	1	June 30 2020	July 28 2020	DNF	GI ulcers after NSAID administration
70	Hound x	M	2	25.7	5	1	June 30 2020	Aug 4 2020	Oct 26 2020	
71	German shepherd	F	2	27.5	4	1	June 30 2020	Aug 4 2020	Oct 26 2020	
72	Cattledog x collie	F	3	33.5	7	1	June 30 2020	Aug 6 2020	Oct 28 2020	
73	Miniature schnauzer	F	6	7.3	5	2	June 29 2020	July 27 2020	Oct 20 2020	
74	Boxer	M	3	33.8	6	2	July 10 2020	Aug 7 2020	Nov 3 2020	
75	Shepherd x	F	2.5	18	5	1	June 29 2020	Aug 5 2020	Oct 30 2020	
76	German shepherd	F	5	25.5	5	2	July 9 2020	Aug 3 2020	Oct 28 2020	

F = female spayed, M = male castrated, DNF = did not finish, x = cross breed, COVID = global Coronavirus pandemic, NSAID = non-steroidal anti-inflammatory

10.3 Appendix III

Nutrient profile and ingredient list of the commercial animal-based diet (MEAT) and the experimental plant-based diet (PLANT) fed to client-owned dogs in the diet trial, compared to industry recommendations at the time of manufacture.

Nutrient (/100kcal)	PLANT	MEAT	Adult Maintenance (AAFCO, 2018)(AAFCO, 2018)
Moisture (g)	1.62	1.44	No recommendation
**Metabolizable energy (kcal/100g)	419	410	
Ash (g)	1.69	1.97	
Crude fibre (g)	0.93	0.83	
*Nitrogen-free extract (g)	11.36	11.00	
Crude protein (g)	5.65	6.76	4.5
Crude fat (g)	3.56	3.22	1.38
Alanine (g)	0.27	0.42	No recommendation
Arginine (g)	0.43	0.48	0.128
Aspartic acid (g)	0.64	0.58	No recommendation
Cystine (g)	0.10	0.19	No recommendation
Glutamate (g)	0.80	0.77	No recommendation
Glycine (g)	0.30	0.60	No recommendation
Histidine (g)	0.15	0.15	0.048
Isoleucine (g)	0.27	0.23	0.095
Leucine (g)	0.50	0.47	0.170
Lysine (g)	0.35	0.38	0.158
Methionine (g)	0.14	0.18	0.083
Methionine + cystine (g)	0.24	0.37	0.163
Phenylalanine (g)	0.36	0.29	0.113
Phenylalanine + tyrosine (g)	0.49	0.43	0.185
Proline (g)	0.32	0.43	No recommendation
Serine	0.33	0.31	No recommendation
Taurine (g)	0.02	0.06	No recommendation
Threonine (g)	0.26	0.26	0.120
Tryptophan (g)	0.03	0.03	0.040
Tyrosine (g)	0.13	0.14	No recommendation
Valine (g)	0.33	0.30	0.123
Linoleic acid (g)	1.5	1.9	0.28
Alpha-linolenic acid (g)	0.39	0.21	No recommendation
Dihomo-gamma-linolenic acid (g)	0.00	0.01	No recommendation
Arachidonic acid (g)	0.00	0.05	No recommendation
Eicosapentaenoic acid (g)	0.00	0.01	No recommendation
Docosapentaenoic acid (g)	0.00	0.01	No recommendation
Docosahexaenoic acid(g)	0.03	0.02	No recommendation

Total omega-6 fatty acids	0.46	0.71	No recommendation
Total omega-3 fatty acids	0.13	0.09	No recommendation
Omega-6:omega-3 fatty acids	3.54	7.89	< 30:1
Calcium (g)	0.33	0.39	0.125 – 0.625
Phosphorus (g)	0.20	0.27	0.100
Ca:P	1.69	1.44	1-2:1
Potassium (g)	0.19	0.19	0.15
Sodium (g)	0.06	0.10	0.020
Chloride (g)	0.19	0.37	0.030
Magnesium (g)	0.03	0.03	0.015
Iron (mg)	9.31	4.88	1.0
Copper (mg)	0.43	0.54	0.183
Manganese (mg)	0.88	0.98	0.125
Zinc (mg)	3.58	4.63	2.0
Iodine (mg)	0.05	0.10	0.025 – 0.275
Vitamin A (IU)	189	291	125.0 - 6,250.0
Vitamin D2 (IU)	22.44	0	No recommendation
Vitamin D3 (IU)	0	16.34	No recommendation
Vitamin D (IU)	22.44	16.34	12.5 - 75.0
Vitamin E (IU)	2.12	3.07	1.25
Thiamin (mg)	0.25	0.34	0.056
Riboflavin (mg)	0.33	0.44	0.13
Pantothenic acid (mg)	1.14	1.73	0.30
Niacin (mg)	0.63	1.03	0.34
Pyridoxine (mg)	0.10	0.15	0.038
Folate (mg)	0.04	0.02	0.0054
Cobalamin (mg)	0.001	0.002	0.0007
Choline (mg)	52.3	43.9	34.0
PLANT ingredients			
Peas, barley, oats, potato protein, sunflower oil (preserved with mixed tocopherols), pea protein, lentils, quinoa, calcium carbonate, dicalcium phosphate, primary dried yeast, flaxseed, natural vegetable flavouring, salt, dried marine algae, choline chloride, vitamins (vitamin A supplement, vitamin D2 supplement, vitamin E supplement, niacin, L-ascorbyl-2-polyphosphate (a source of vitamin C), d-calcium pantothenate, thiamine mononitrate, riboflavin, pyridoxine hydrochloride, folic acid, biotin, vitamin B12 supplement), minerals (zinc proteinate, iron proteinate, copper proteinate, zinc oxide, manganese proteinate, copper sulphate, ferrous sulphate, calcium iodate, manganous oxide, selenium yeast), DL-methionine, potassium chloride, L-lysine, taurine, L-carnitine, dried rosemary			
MEAT ingredients			
Chicken meal, de-boned chicken, whole brown rice, white rice, oatmeal, chicken fat (preserved with mixed tocopherols), potatoes, salmon meal, natural chicken flavour, whole dried egg, flaxseed, pea fibre, alfalfa, apples, carrots, cranberries, sodium chloride, potassium chloride, dried chicory root, dried Lactobacillus acidophilus fermentation product, dried Enterococcus faecium fermentation product, vitamins (vitamin A supplement, vitamin D3 supplement, vitamin E supplement, niacin, L-ascorbyl-2-polyphosphate (a source of vitamin			

C), d-calcium pantothenate, thiamine mononitrate, beta-carotene, riboflavin, pyridoxine hydrochloride, folic acid, biotin, vitamin B12 supplement), minerals (zinc proteinate, iron proteinate, copper proteinate, zinc oxide, manganous proteinate, copper sulphate, ferrous sulphate, calcium iodate, manganous oxide, selenium yeast), DL-methionine, L-lysine, taurine, yucca schidigera extract, dried rosemary.

*Nitrogen free extract is an approximation of carbohydrate content, calculated by subtraction of the sum of crude protein, crude fat, crude fibre, moisture, and ash from a total of 100 (AAFCO, 2018).*Nitrogen free extract is an approximation of carbohydrate content, calculated by subtraction of the sum of crude protein, crude fat, crude fibre, moisture, and ash from a total of 100 (AAFCO, 2018).

Metabolizable energy (kcal/kg) is calculated as: $10[(3.5 \times \text{crude protein}) + (8.5 \times \text{crude fat}) + (3.5 \times \text{nitrogen-free extract})]$ (AAFCO, 2018).Metabolizable energy (kcal/kg) is calculated as: $10[(3.5 \times \text{crude protein}) + (8.5 \times \text{crude fat}) + (3.5 \times \text{nitrogen-free extract})]$ (AAFCO, 2018).

Fatty acid of the commercial animal-based diet (MEAT) and the experimental plant-based diet (PLANT) fed to client-owned dogs in the diet trial.

Fatty acid (g/100kcal)	PLANT	MEAT
Saturated FA		
14:0	0.015	0.056
16:0	0.712	1.933
18:0	0.409	0.494
20:0	0.041	0.013
21:0	0.002	0.003
22:0	0.090	0.007
23:0	0.006	0.002
24:0	0.035	0.007
Total saturated fat	1.310	2.515
Monounsaturated <i>cis</i> FA		
16:1c9	0.047	0.444
18:1c-9	7.844	3.262
18:1c11	0.096	0.171
18:1c12	0.001	0.001
20:1c8	0.003	0.009
20:1c11	0.049	0.037
24:1n-9	0.006	0.007
Monounsaturated <i>trans</i> FA		
16:1t9	0.000	0.002
18:1t6-8	0.001	0.002
18:1t12	0.004	0.002
18:1t13&14	0.002	0.001
Omega 6 polyunsaturated FA		
18:2n-6	1.501	1.851

18:3n-6	0.001	0.013
20:2n-6	0.008	0.017
20:3n-6	0.002	0.014
20:4n-6	0.005	0.049
22:4n-6	0.001	0.010
22:5n-6	0.012	0.004
Omega-3 polyunsaturated FA		
22:6n-3	0.032	0.017
Omega-6 : omega-3 ratio	3.54	7.89

10.4 Appendix IIV

Distribution of independent variables between groups in the diet trial.

	PLANT (n = 34)		MEAT (n = 32)	
	Median	Range	Median	Range
Age (years)	4.0	2 – 9.5	4.8	2 – 9.5
Weight (kg)	24.9	5.4 – 59.1	26.6	4.8 – 45.7
BCS (1-9)	5	4 - 7	6	4 – 7
	Frequency	Proportion	Frequency	Proportion
Female	18	43%	18	56%