APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY, FECAL CHARACTERISTICS, URINALYSIS, FECAL METABOLITES, SERUM CHEMISTRY, AND VOLUNTARY PHYSICAL ACTIVITY LEVELS OF HEALTHY ADULT DOGS FED EXTRUDED, MILDLY COOKED AND RAW FOODS

BY

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THESIS

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Abstract

The pet food market continues to be strong, with much of the growth coming from super-premium foods and those with novel formats or processing methods. In addition to canned and extruded diets, lightly cooked and raw diets are available today. Despite the increase in their popularity, little research has been performed on such diets. The objective of this study was to determine the apparent total tract macronutrient digestibility (ATTD), fecal characteristics and metabolites, serum chemistry metabolic profile, urinalysis, and voluntary physical activity levels of adult dogs fed the following commercial dog diets: 1) Purina Dog Chow (DC), as the control diet; 2) Freshpet Vital Balanced Complete Nutrition (CN); 3) Freshpet Roasted Meals (RM); 4) Freshpet Vital Raw (VR). All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to the study. Eight dogs (mean age = 3.6 yr ± 0.29; mean body weight (BW) = 13.0 kg ± 0.84) were used in a replicated 4x4 Latin square design. Each period consisted of 28 d, with a 14-d adaptation phase followed by a 7-d phase for measuring voluntary physical activity, a 1-d adaptation phase to metabolic cages, a 5-d phase for fecal and urine collection, and 1-d for blood collection. Fresh fecal samples were collected for pH, moisture, and metabolite measurements. Food was fed twice daily and at a rate to maintain BW. All data were analyzed statistically by mixed models of SAS. Dry fecal output and ATTD of dry matter (DM) and organic matter (OM) were not affected by treatment; however, ATTD of crude protein (CP) was greater ($P<0.05$) for dogs fed CN than dogs fed VR, with dogs fed RM being intermediate. Dogs fed CN or RM had greater ($P<0.05$) ATTD of CP than dogs fed DC. ATTD of fat was greater ($P<0.05$) by dogs fed VR than dogs fed RM, with dogs fed CN being intermediate. Dogs fed CN, VR, or RM had a greater ($P<0.05$) ATTD of fat than dogs fed DC.

Dogs fed CN had higher ($P<0.05$) fecal pH than dogs fed VR, with dogs fed RM and DC being intermediate. Dogs fed DC, CN or RM had higher ($P<0.05$) fecal DM% than dogs fed VR. Dogs fed VR had a higher ($P<0.05$) fecal acetate concentration than dogs fed RM, with dogs fed CN and DC being intermediate. Dogs fed RM had higher ($P<0.05$) fecal indole and total phenol and indole concentrations than dogs fed CN, VR and DC. Dogs fed VR had a higher ($P<0.05$) fecal ammonia concentration than dogs fed RM, CN and DC while dogs fed RM had a higher ($P<0.05$) ammonia concentration than dogs fed DC, with dogs fed CN being intermediate. All other fecal metabolites were not affected by treatment. Most serum metabolites and urinary measures were within reference ranges for dogs fed all dietary treatments and were not affected by diet ($P>0.05$). Serum triglycerides were within reference ranges, but greater ($P<0.05$) for dogs fed DC than dogs fed CN or VR, with those fed RM being intermediate. All diets were well tolerated and dogs remained healthy throughout the study. In conclusion, the lightly cooked and raw diets tested were highly palatable, highly digestible, reduced blood triglycerides, and maintained fecal quality and serum chemistry.

Dedication

This thesis is dedicated to my parents, Michael and Diane Algya, and my brother, Joshua. Thank you for the constant support over the years while I have been in school and always encouraging me to never give up.

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Chapter 1

Introduction

The relationship between humans and their pets has grown throughout the years. It started out as a symbiotic relationship and recently grew into companionship as owners started to view pets as family members. Because of this bond, the pet industry has been positively impacted by increased purchases of pet commodities such as food, supplies and medicine, veterinary care, live animals and pet services (e.g., grooming and boarding). In 2016, the pet product industry reached $66.8 billion in the U.S. market and a large portion of it came from pet food sales ($28.2 billion) (APPA, 2017).

In 1866, James Spratt created the first commercial baked dog food in London and pet foods have come a long way since then. This phenomenal industry spread into the U.S. and expanded primarily due to scientific research pertaining to the metabolism and nutritional needs of pets, allowing the provision of all essential nutrients in a uniform format (Aldrich, 2006). An important part of this expansion occurred in 1954 when there was shift to low moisture extruded kibble diets. Drs. James Corbin and Joesph Vandepopuliere produced low moisture diets by extrusion, which is an industrial processing method using heat, moisture and pressure to cook the food. This cooking process improved the essential nutritive properties and increased protein and starch digestibility in pet foods (Singh et al., 2007). Extrusion, along with retorting, remain one of the most popular pet food formats today. Recently, diets have and continue to expand because of anthropomorphism or humanization views placed on pets. These views have strongly impacted the pet food industry over the past decade, with multiple pet food formats being developed that cater to consumer desires such as fresher looking and more convenient diets.

Some of the newer pet food formats include raw and dehydrated products. Unfortunately, little research has been performed on these novel pet food formats; therefore they need to be determined by their nutritional value and adequacy and physiological benefits to pet animals. With that in mind, the objective of this study was to determine ATTD, metabolizable energy, fecal characteristics and metabolites, serum chemistry, urinalysis, and voluntary physical activity levels of dogs fed extruded, mildly cooked, and raw diets. We hypothesized that the raw diets would have greater ATTD compared to the mildly cooked diets and the mildly cooked diets would have greater ATTD than the dry kibble diet control. We also hypothesized that there would be no negative effects on fecal characteristics and metabolites, urinalysis, or serum chemistry profile. Lastly, we hypothesized that the raw and mildly cooked diets would produce greater voluntary physical activity levels than the low moisture kibble diet. We came to this hypothesis because there was a study conducted by Deng et al. (2014) that observed cats having greater voluntary physical activity when they were being fed a high moisture diet (70% hydration) compared to a dry kibble diet control (8% moisture).

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Chapter 2:

Literature Review

Pet Food Market and Diet Formats

Throughout history, mankind has domesticated animals for numerous utilitarian purposes, including transportation, crop production, and as a source of food. Our relationship with animals has evolved over time, especially when it comes to cats and dogs. Dogs were domesticated by humans approximately 15,000 years ago (Savolainen et al., 2002; Wang et al., 2016). It is believed that dogs initially served man by guarding their family and livestock, warning them of intruders and hunting game (Serpell, 1996). Some dogs still serve those roles in today's society. In fact, the options for working dogs has actually expanded, with dogs serving as high performing members of the military and law enforcement, assistants for the disabled, a form of therapy for those with anxiety or depression, and more (Grandgeorge and Hausberger, 2011; Cobb et al., 2015). Similarly, cats have lived side by side with humans for thousands of years, initially for the purpose of disposal of vermin in and around homes, barns and other buildings. Although working roles are still present in both species, the majority of today's dogs and cats are kept for companionship. No matter what the role or relationship is, dogs and cats are primarily considered to be and treated as members of the family. Six out of ten (63.2%) households consider their pets to be family members and 35.8% consider them to be pets or companions, while only 1% consider their pets to be property (AVMA, 2012).

The relationship that humans have with their pets has impacted the pet product industry in many ways. First, the pet population is large and has steadily increased. The pet population is a bit difficult to estimate accurately; however, in 2011, 63.2% of households in the U.S. had a dog (36.5%; approximately 69 million) or a cat (30.4%; approximately 74 million) (AVMA,

2012). The percentage of households owning a pet has been fairly stable since the 1990's (AVMA, 2012.) In 2016, 68% of the U.S. households owned a pet, with the dog and cat populations estimated to be 89 and 94 million, respectively (APPA, 2017). Second, the annual number of veterinary visits and veterinary costs paid by consumers continues to grow and is related to the human-pet bond. Consumers that consider dogs a family member bring them in for vet checkups an average of 3 times per year, which is higher than if they consider them to be a pet or companion (2.1 times/year) or property (1.3 times/year) (AVMA, 2012). Third, these data can probably be translated to pet food products, with consumers spending more money on pet food depending on how they view their relationship with their pets. Total expenditures on pet products, which exceeded $66 billion in 2016, is expected to reach $69 billion in 2017 (APPA, 2017). The average annual household pet expenditures in the U.S. approach $500 and represent about 1% of total household expenses, a figure that remains relatively constant even during economic recessions (U.S. Bureau of Labor Statistics, 2013).

Before owner preferences drove the pet food industry like they often do today, expansion of pet food diets in the 1980's and 1990's was due primarily to the scientific research pertaining to the metabolism and nutritional needs of pets. At that time, U.S. pet foods expanded to include different life stages, use of a greater variety of ingredients, diets designed to prevent or treat clinical diseases, and expanded portfolios to include different diet package sizes and formats (Aldrich, 2006). The commercial pet diets at that time, primarily extruded dry and retorted canned diets, provided all of the essential nutrients [e.g., crude protein (CP) and amino acids (AA), crude fat and fatty acids, vitamins and minerals] in a uniform format.

The committed and growing relationships with pets are complicated by the anthropomorphism or humanization placed on them. These views have strongly impacted the pet

food industry over the past decade, with multiple pet food formats being developed that cater to consumer's view of pets. In the early 2000's, pet foods began to expand in a way that was based not on scientific research, but on consumer beliefs. The result has been a variety of foods that have replicated many human food trends. While ingredient functionality, packaging, shelf-life and food format have been important qualities for many years (Aldrich, 2006), recent trends have accommodated consumer desires for inclusion of natural, organic or fresh ingredients; convenient packaging sizes (e.g., single serve pouches); and diet formats that are more similar to human foods (The Nielson Company, 2016). An example of the humanization of pets and their diets may be demonstrated by the use of vegetables like peas and carrots. In the past, such ingredients were added only in meal form and were secondary products of the human food industry. Today, these ingredients often are not processed secondary products, but are included in the diet as whole pieces that are of human grade quality.

The variety of pet diet formats also has expanded and now includes kibble, semi-moist, canned, baked, raw, refrigerated, frozen and dehydrated foods. All of these diet formats undergo specific handling and/or processing methods that influence the overall functionality of the diet and possibly the performance of the pet. The performance of raw ingredients and those undergoing traditional processes techniques (e.g., extrusion and retort) have been tested over the past few decades. Based on the published literature, the newer processes have not be been well tested. Therefore, further research needs to be done on the nutrition and health of pets as regards different pet food processing techniques and formats.

Macronutrient Metabolism and Recommendations for Dogs

In today's pet foods, most micronutrients are provided in premixes. A wide variety of ingredients that vary by source, cost, nutrient quality and balance, and digestibility provide the macronutrients (e.g., proteins, fats, and carbohydrates). Each macronutrient class plays specific roles and functions in the body and may serve as an energy source to dogs. Proteins are organic compounds composed of carbon, hydrogen, oxygen and nitrogen. Amino acids are the building blocks and are essential for numerous functions associated with proper maintenance and growth of tissues, including the regulation of metabolic pathways (e.g., AA synthesis and catabolism, and protein turnover) that influence growth, development, lactation, and reproduction (Wu et al., 2014). Amino acids may be divided into those that are non-essential and those that are essential in the diet. In general, non-essential AA are synthesized by the body in sufficient amounts to maintain the physiological state in question, while essential AA are not, requiring provision in the diet. Whether or not the AA are essential depends on the species pathological state, environmental factors (e.g., pH), age and physiological factors (e.g., gene expression and protein synthesis) (Wu et al., 2010; Wu et al., 2014). The 10 essential AA for dogs include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (AAFCO, 2016). If insufficient amounts of essential AA are present in the diet, protein synthesis will be compromised and homeostasis will decline. The AA that is the first to become unavailable is designated to be the limiting AA. When AA are deficient, health issues such as a decrease in muscle mass, a decline in immune response, poor skin conditions, and reduced growth may result (van Rooijen, 2015). In ingredients, AA concentrations can vary. For example, in ingredients such as cereal grains, lysine often is considered the most limiting AA and if not, then the second in commercial pet foods. However, different ingredient sources can be

deficient in other essential AA, being deficient in methionine, cysteine, and tryptophan.(Boisen et al., 2000; Kerr et al., 2014).

Dogs are monogastric animals and they have a relatively large stomach with respect to their short gastrointestinal tract and a very small non-functional cecum. Dietary proteins are digested by a variety of proteases in the stomach and small intestine and this process is initiated by pepsin. Pepsin breaks down long chain peptides (e.g., oligopeptides). Other proteolytic enzymes (i.e., endopeptidases and exopeptidases) function collectively to cleave peptides bonds into tri- or di-peptides or free amino acids (FAA) to allow them to be absorbed in the small intestine and then metabolized. Endopeptidase enzymes (e.g., trypsin, chymotrypsin, and elastase) cleave internal peptide bonds in a protein. Trypsinogen, activated by enterokinase into trypsin, cleaves peptide bonds, carboxylic acid group, of basic amino acids and release arginine and lysine. Chymotrypsinogen, activated by trypsin into chymotrypsin, cleaves peptides, carboxylic acid group, of aromatic AA and may release phenylalanine, tryptophan, and tyrosine. Elastase, also activated by trypsin, cleaves the carboxyl end of aliphatic AA which releases glycine, alanine, valine, leucine, and isoleucine. Exopeptidases (carboxypeptidases) cleaves the carboxyl ends of basic, aromatic, or aliphatic AA and produces small peptides and FAA. Tri-/di-peptides can be absorbed into the enterocyte but must be further cleaved within the enterocyte into a FAA in order to cross into vasculature.

Based on taxonomic order, dogs are classified as carnivores. Metabolically, however, dogs are omnivores. In the National Research Council (NRC) for Dogs and Cats (NRC, 2006), dogs have a dietary minimum requirement of 8% dietary CP, while the Association of American Feed Control Officials (AAFCO, 2016) recommends that dogs consume 18% dietary CP. The reason why there is such a difference between the two sources is because the NRC references are

generated from a scientific standard to sustain nutritional adequacy of an animal, whereas AAFCO's value is from an industry standard to avoid macronutrient deficiencies in diets.

There are a large number of protein sources available to the pet food industry that are derived from animal, plant and marine sources; however, there are some considerations when selecting a proteinaceous ingredient such as nutritional quality, uniformity, appearance, palatability, shelf-life and social sustainability. In the pet food industry, ingredients are categorized in a number of ways, including primary and secondary products, animals vs. plant ingredients and processing methods. Primary and secondary products are ingredients relative to 'human-grade' status. Primary products are directly consumed by humans (e.g., beef and chicken) in the food industry. In contrast, secondary products are not utilized in the human food industry for consumption. Many of these products provide key nutrients needed in pet food diet formulations, but carry negative stigma with consumers who perceive these ingredients to be of poor quality. This creates a divide in the pet food industry between industry knowledge of nutrient targets and consumer misconceptions about ingredient selection.

Animal- and marine-based proteins have variable nutrient compositions when compared among sources and within species of that source thus making it difficult to have a consistent AA composition (Dust et al., 2004). Animal and marine sources can be divided into fresh products, by-products or meals. Simply put, the difference between fresh, by-product, and/or meal products is the processing method. According to AAFCO (2016), fresh meats are considered to be muscle tissue of the animal, but can also include fat, gristle, and other tissues that accompany the muscle. By-products include other parts of the animal that do not include the muscle tissue, but may also include internal organs and bones; and meals are rendered products from mammalian tissues that exclude any blood, hair, hoof, horn, hide trimmings, manure, and

stomach and rumen contents. The sources may come from a variety of animals including beef, swine, poultry, and fish species like cod or herring. In the literature, there are articles that show various compositional differences among protein-based sources fed to dogs (Dust et al., 2005; Faber et al., 2010) and some of those sources were evaluated for quality (Dust et al., 2005). A recurring trend in the pet food market is to humanize pet foods; therefore, humans want to see higher quality protein sources in their pet's diet such as chicken, beef and salmon instead of by-products or meals. Faber et al. (2010) evaluated the dietary CP composition and quality of several protein sources: beef loin (30.7% CP on DM basis), pork loin (31.2% CP on DM basis), chicken breast (30.0% CP on DM basis), pollock fillet (32.0% CP on DM basis) and pink salmon fillet (30.8% CP on DM basis). He also evaluated the acid hydrolyzed fat chemical composition the protein sources: beef loin (21.1% fat on DM basis), pork loin (21.4% fat on DM basis), chicken breast (21.4% fat on DM basis), pollock fillet (20.1% fat on DM basis), and pink salmon fillet (20.4% fat on DM basis). In conclusion of that study, protein sources were both variable in dietary CP and fat; therefore, macronutrient composition of ingredients should always be evaluated for diet formulation.

Dust et al. (2005) performed a compositional analysis of various protein sources as well, but also determined protein quality of chicken protein sources including three spray dried ingredients (cooked chicken from deboned chicken parts, with a CP (DM basis) of 49.2%, chicken liver with a CP (DM basis) of 69.0%, and processed pasteurized whole egg with a CP (DM basis) of 52.7%), chicken by-product meal with a CP (DM basis) of 62.8%, and poultry by-product meal with a CP (DM basis) of 64.1%]. There were also other protein sources such as processed blood cells (95.3% CP on DM basis), spray dried plasma (84.4% CP on DM basis), spray dried whole beef blood (95.5% CP on DM basis), enzyme-hydrolyzed fish protein

concentrate (62.3% CP on DM basis), soybean meal (51.0% CP on DM basis), and spray dried pork liver (69.7% CP on DM basis). The assays used to determine protein quality were protein solubility index, IDEA (immobilized digestive enzyme assay), and PER (protein efficiency ratio). The processed blood cells (23.9%) had the lowest and spray the dried plasma (92.9%) had the highest protein solubility index values. The poultry by-product meal (0.43) had the lowest and soybean meal (0.79) had the highest IDEA values. Chicken by-product meal (3.42) had the highest and poultry by-product meal (2.73) had the lowest PER values, suggesting that chicken by-product meal had better protein quality than the other dietary treatments.

Lipids are nonpolar heterogeneous compounds that include a subgroup called fatty acids long chain hydrocarbons that are a part of glycerides (e.g., triglycerides), phospholipids (e.g., lecithin), sterols (e.g., cholesterol) and waxes (Camire et al., 1990; Singh et al., 2007). Lipids are concentrated sources of energy for dogs and aid in the absorption of other essential nutrients like fat-soluble vitamins (e.g., A, D, E and K) and supply essential fatty acids (Frankel, 2014; Koppel et al., 2014). Lipids are hydrophobic; therefore, the enzyme, pancreatic lipase, will only function efficiently when fats are emulsified into smaller components. They are digested into smaller components called monoglycerides and fatty acids. Along with bile salts and phospholipids, micelles are formed. Once digested, monoglycerides and fatty acids diffuse across the plasma membrane of the enterocytes and, once inside, they are re-synthesized into triglycerides and form chylomicrons, which are transported with other lipids through circulation. In each ingredient, a variety of lipid types including saturated and unsaturated fats are present. Saturated fats are joined together by only one bond whereas unsaturated fats are joined together by one or more than one double bond; an example is polyunsaturated fat or fatty acids. Polyunsaturated fatty acids (PUFA) include different classes, for example, omega-3 and omega-6 fatty acids and, like

essential AA, these fatty acids are needed in the body and are only obtained in the diet. The major essential fatty acids for dogs and cats are: alpha-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are omega-3 fatty acids, and linoleic acid and arachidonic acid, which are omega-6 fatty acids. Higher consumption of these fatty acids has been reported to improve skin and coat quality, decrease inflammation, increase immune system response, treat and prevent cancer and retinal degeneration, enhance development of the nervous system, and improve overall cognition (Lenox and Bauer, 2013; Frankel, 2014).

According to the NRC (2006), dogs have a minimum requirement of 5.5% dietary fat and similarly, AAFCO recommends 5.5% of fat in the diet to maintain overall health of adult dogs. Lipids also can come from proteinaceous ingredients at different concentrations. For example, in plant products, soybean meal contains 1.4% fat, while wheat germ meal contains 7.0% fat. For animal products, chicken (meat and skin, raw) contains 20.3% fat, chicken liver contains 3.9% fat, beef (mechanically separated) contains 23.5% fat, poultry by-product contains 13.5% fat, and fish meal (tuna) contains 11.0% fat (NRC, 2006). Under good conditions, lipids enhance palatability and texture of pet foods. However, they can reduce shelf-life of pet foods through physical and chemical reactions, such as lipid oxidation, during heat processing and even storage (Barden and Decker, 2016). The essential fatty acids that are most reactive are PUFA. Due to their health benefits, consumers want to see these more often in their pet foods; unfortunately, PUFA are more prone to undesirable chemical reactions. There are two types of oxidation reactions in foods, the most common being auto-oxidation and it occurs when oxygen is present and the fat forms a free radical. Essentially when a hydrogen atom is in the presence of light, heat, metals or oxygen, and is absent from a fatty acid, an alkyl radical will form and change into a hydroperoxide. Hydroperoxides like to obtain hydrogen molecules from other molecules and

then create secondary oxidation products, like aldehydes and ketones (Johnson and Decker, 2015), which can affect food quality and produce off-odors and flavors, odd texture, color changes and nutrient losses lead to a poor and short shelf-life of the product (Tian et al., 2013). Because lipid peroxidation results in poor quality ingredients, it is a good measurement to determine overall quality of a pet food. Sensory tests are the most popular tests to determine if a food product has gone rancid but oxidation products, such as peroxides and malondialdehydes, can be measured by the following techniques in research: volumetric method (AOAC, 1965), vis-UV spectroscopy methods, ferrous oxidation method, iodide oxidation method and chromatography (e.g., standard, liquid and gas; Barriuso et al., 2013). To avoid pet food losses, processing with the addition of antioxidants, and packaging techniques can deliver better product quality.

Carbohydrates are organic compounds composed of carbon, hydrogen and oxygen and may be found in the cytoplasm of cells or in the plant cell wall. Simple sugars are known as monosaccharides (e.g., glucose, fructose and galactose) and complex sugars are disaccharides (e.g., lactose), oligosaccharides and polysaccharides. Carbohydrates are digested at varying rates and will influence where nutrients will be absorbed in the host. Rapidly and slowly digestible starches have a variable extent of digestion and the resulting glucose is primarily absorbed in the small intestine. In order to be absorbed, digestible carbohydrates must be broken down by enzymes into monosaccharide units. Glucose is absorbed by the enterocyte through transporters such as sodium-dependent glucose transporter-1 (SGLT-1) and glucose transporter 5 (GLUT 5) to later be utilized in the body. The SGLT-1 transports the monosaccharides, glucose and galactose, and GLUT 5 transports the monosaccharide, fructose, into the enterocytes. Glucose transporter 2 (GLUT 2) transports all three monosaccharides into the blood stream. Dietary

glucose is utilized for a number of tasks, including glycolysis, the citric acid cycle, electron transport chain, and Cori cycle, and to the synthesis of dispensable AA, lactose, and nucleic acids. Unlike dietary CP and fat contents, there are no dietary recommendations or requirements for carbohydrates for the dog. Ingredients that contain starch include cereal grains (e.g., corn, rice) and tubers (e.g., potato). There is a portion of these starches that are resistant to pancreatic alpha-amylase (Dust et al., 2004) and are not absorbed through the small intestine, but fermented in the colon. These are known as "resistant starches."

Unlike rapidly and slowly digestible starches, resistant starches are carbohydrates that cannot be hydrolytically digested and are fermented in the colon by microbiota. They display physiological benefits and are fermentable (Haralampu, 2002). Dietary fibers also are a category of carbohydrates. According to the U.S. Food and Drug Administration (FDA, 2016), dietary fiber is described as "non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health." Fibers vary greatly in regards to their physico-chemical properties; including solubility, viscosity, and fermentability, which influence the properties of the diet, processing conditions, and their physiological impacts on the body (Dikeman and Fahey, 2006). Both soluble (e.g., pectins, gums) and insoluble fibers (e.g., cellulose, soy hulls) have different roles in the digestive and absorptive processes within the gastrointestinal tract. The ratio between the two can affect overall utilization of the diet (Burkhalter et al., 2001). Soluble or viscous fibers have been associated with prolonged gastric emptying and slower transit time in the small intestine, while fermentability is primarily associated with large intestinal events. Rapidly fermented fiber sources result in the production

of short-chain fatty acids (SCFA), while other less fermentable fibers improve bowel health by promoting laxation, reducing transit time, and increasing stool weight (Dikeman and Fahey, 2006).

Macronutrient Digestibility

Macronutrient quality is evaluated by the source's nutrient content, bioavailability and digestibility values. Macronutrient digestibility is a measurement of how much a nutrient given to an animal was absorbed and this value is obtained by subtracting the amount of nutrient excreted from the amount ingested and divided by the amount ingested. This is very important because if an animal cannot digest what it consumes, it indicates low ingredient value and will probably result in poor fecal quality. There are a number of ways to determine macronutrient digestibility, however, two of them are measuring ileal and apparent total tract digestibility (ATTD). Intake and fecal excretion are calculated to determine ATTD, which is no value in the case of AA due to the microbial metabolism of AA in the hindgut. Ileal digestibility is the gold standard for measurement of AA digestibility in dogs (Bednar et al., 2000; Hendricks et al., 2013).

There are studies in the literature that discuss and evaluate fecal quality and nutrient digestibility values in extruded and canned diets fed to dogs and cats (Kane et al., 1981; Meyer et al., 1999; Bednar et al., 2000; de Oliveira et al., 2012; Hendricks et al., 2013). Fecal quality is commonly evaluated on a numerical scoring scale with a score of 1 being a hard, well-formed stool to a score of 5 or 7 being watery diarrhea (Moxham, 2001; Greco, 2016). Scoring helps determine how well the diet is being digested and can be an indication of overall gut health. Depending on the score chart, a 2 or 3 on a 5-point scale, or a 3 or 4 on a 7-point scale, is a good

sign of overall gut health. Apparent total tract digestibility values should be above 75-80% in order for a macronutrient to be considered well absorbed or have an acceptable digestibility content.

There are differences between animal- and plant-based proteins in AA digestibility. Bednar et al. (2000) evaluated the AA composition and the ATTD of animal and plant protein-based meal diets fed to ileal cannulated dogs. In that study, 4 female dogs with an average body weight of 25.5 ± 3.9 kg were used in a replicated 4 x 4 Latin square design. The 4 protein treatments evaluated during that study were: soybean meal (SBM) with a CP (DM basis) of 25.5%, poultry meal (PM) with a CP (DM basis) of 26.9%, poultry by-product meal (PBPM) with a CP (DM basis) of 24.4%, and beef and bone meal (BBM) with a CP (DM basis) of 24.5%. Corn gluten meal was included at a constant percentage to provide complementary AA in each treatment. There were no significant differences among the dietary treatments with an average of 74.7% of CP ileal digestibility; however total tract CP digestibility for PM (87.5%) was greater ($P<0.05$) than SBM (82.7%), PBPM (81.6%) and BBM (82.5%). When comparing apparent ileal and total tract digestibility values, there was a pronounced difference between the two, indicating that CP is influenced by microbial fermentation in the hindgut. The BBM and PBPM generally had the higher concentration of essential AA when compared to the other diets. There were no significant differences in ileal AA digestibilities among the treatments; however, numerically, ileal digestibilities of total AA for both groups were highest for the SBM treatment, intermediate for the PBPM and PM treatments and lowest for the BBM treatments. Fecal scores were greater ($P<0.05$) for SBM (2.6) than in PBPM (2.2), PM (2.3) and BBM (2.1), which suggests that higher intake of SBM may result in higher wet fecal output even though it is well digested.

Meyer et al. (1999) evaluated digestibility of commercial dry and canned diets in different sizes and breeds of dogs. There were a total of 66 dogs in that study, 10 different breeds and weighing between 4.2-52.5 kg. The breeds in this study were: Yorkshire Terrier, Miniature Poodle, Dachshund, Miniature Schnauzer, Cairn Terrier, West Highland White Terrier, Beagle, English Springer Spaniel, Labrador Retriever and Irish Wolfhound. The fecal score chart used in that study had a different scoring system compared to popular scoring charts. This one used a 10-point scoring chart, with a 0 associated with a diarrhea appearance and a 10 associated with dry and clumpy stools. A score of 7.5 was considered well-formed. There were two diets in that study that were fed consecutively, starting with the canned diet and followed by the dry diet that lasted for 15 days each. The CP composition of the dry diet was 29.4 g/kg (DM basis) and the canned diet had a CP of 45.9 g/kg (DM basis). The ATTD of all the macronutrients for both the dry and canned diets demonstrated that both diets were well digested and comparable. Among 10 breeds, the dry diet had an average CP digestibility value of 86.0% with a range of 84.5-88.2%. The canned diet had a CP digestibility average of 85.9% with a range of 84.8-89.4%. There were no significant CP digestibility differences between the breeds fed the canned diets; however, the Miniature Poodles had greater ($P<0.05$) CP digestibility than the Dachshunds and the West Highland White Terriers, while the other breeds remained intermediate.

Fecal quality of the two treatments were significantly different among breeds. The average fecal score for the dry diet was 6.6 with a range of 5.4-7.3 and for the canned diet the average fecal scored was 6.1 with a range of 4.8-7.3. The English Springer Spaniels had lower ($P<0.05$) fecal scores compared to all the other breeds. Beagles and Irish Wolfhounds had lower ($P<0.05$) fecal scores than the Labrador Retrievers, West Highland White Terrier, Cairn Terriers, Miniature Schnauzers, Miniature Poodles and Dachshunds. The Labrador Retrievers had lower

($P<0.05$) fecal scores compared to Cairn Terriers and Miniature Schnauzers while West Highland White Terriers, Dachshunds, Miniature Poodles and Yorkshire Terriers remained intermediate. The average fecal DM for dry diets was 40% and for canned diets 26.6%. Thus, both diets were digested and absorbed well; however, the average fecal score is a bit lower than what an ideal fecal score should be according to the study's fecal score chart. This may suggest there are different digestive sensitivities among breeds and perhaps size and weight.

A review written by Clauss et al. (2010) suggested that the digestive physiology and efficiency between different carnivore groups (canids, felids, hyenids, mustelids, ursids and pinnipeds) were not different with respect to protein and fat absorption. This can be observed in dogs and cats who have similar gastrointestinal tracts with high absorptive macronutrient rates; however, the difference in dogs is that they have a longer tract and thinner and thicker mucosal membranes in the proximal and distal parts of the stomach, respectively (NRC, 2006). Vester et al. (2010) evaluated ATTD of a high protein extruded kibble diet and a commercial raw meat-based diet fed to captive African wildcats. There were 5 cats total with an average body weight of 3.5 kg and they were randomly given one of the two diets per period. The raw beef-based diet contained 48% protein and 32% fat and the kibble diet contained 55% protein and 20% fat. The ATTD of CP was greater ($P<0.05$) in the raw meat diet than the kibble; however the ATTD was not significantly different between treatments. The fecal score for the animals fed both diets was a 2.2/5.0. Those results indicate that both the raw meat diet and the kibble diet were well digested and nutrients were absorbed. Kane et al. (1981) evaluated diets containing different fat concentrations (10, 25 and 50%) and sources (bleached tallow, butter, lard and yellow grease) fed to cats. They reported high digestibility values (97.2 to 98.5%), for all the diets containing 25 or 50% fat, indicating that cats can utilize high concentrations of fat and those that are much

higher that what is present in commercial diets. Fatty acids also were determined and they also had high digestibility values.

Processing Techniques

Heat Processing: Extrusion and Retort

The majority of dogs and cats in the U.S. are fed commercial pet foods that have undergone heat processing. These processes are used to improve the safety and nutritive properties of pet foods and increase protein and starch digestibility, destroy undesirable enzymes, inactivate some anti-nutritional inhibitors, sterilize the finished product and retain flavors (Singh et al, 2007). Generally, heat is required to produce microbiologically safe products of acceptable eating quality (Holdsworth et al., 2008) and, in pet foods, it is one of the most used methods for preservation and product safety for high moisture and low acid pet food products (Santana et al., 2013). These processing methods are time- and temperature-dependent, meaning the cooking process is determined by the length of time and the amount of heat required to cook the product completely. In order for these processes to be successful, the thermal process design has to be taken into consideration, which includes heating and cooling equipment, chemical, physical and microbiological properties of the pet food, nutritional changes, and packaging (Augusto et al., 2009; Santana et al., 2013).

The primary heat processing treatments used include extrusion and retort sterilization. Extrusion is a continuous process that uses a combination of moisture, temperature, pressure and mechanical shear to mix, knead and cook raw materials to form a favorable low moisture product (Tran et al., 2008). Extruders have the advantage of cooking to produce a range of products in a short amount of time (Riaz, 2003). The extrusion process was first applied to food technology in

the 1930's to produce human cereals and snack foods (Gibson and Sajid, 2014). It was adopted by pet food manufacturers in the 1950's, as pet food producers noticed that extruded food products did not immediately crumble or become soggy in the presence of high moisture. They realized this was an opportunity to create a durable pet food product that resulted in improved digestion and fecal quality (Fapojuwo et al., 1987). Currently, several extruder types and options exist for pet food manufacturers. Single and twin screw extruders are primarily used to produce pet foods. Both extruder types have segmented barrels that contain a variety of shafts, screws and shear locks for cooking and processing of pet foods and force the food material through a dye to create the finished product; however, the difference between the two is the actual screw. Single screw extruders have one compressive screw that increases shear and mechanical energy input for heating and is commonly used for high energy and expanded pet foods (Riaz, 2003). In order to have better efficiency and capacity, it is common to preheat the ingredients in a preconditioner. This adds moisture and steam to partially cook the ingredients before entering the extruder, which provides less wear and tear on the extruder. Unlike single screw extruders, twin screw extruders have parallel screws that produce a more uniform and homogenous product and is used for varying particle size, or for diets having greater viscosity, oil content, and/or high protein concentrations (Riaz, 2003).

After the materials are extruded, they are forced through dyes at the end of the extruder barrel that shape the product. Knives are used to cut and give the kibble a desired size and length. However, raw materials and nutrient content must be considered before adding to the process because it will affect extruder conditions to produce an appropriate product. Extrusion causes ingredients to go through physical and chemical changes and different reactions (Dust el al.,

2004). Because of this, there are considerations to be aware of such as nutrient, ingredient, and processing functionalities.

Retort processing is another heat processing method. Retort packaging materials include metal containers (cans), which are the most popular, plastic containers (trays) and retortable pouches made of laminate and metal foils. Although cans are the most popular packaging material, trays and pouches are becoming quite popular for their convenient size (e.g., single serving portions) and freshness (Chen and Ramaswamy, 2007; Bohrer, 2011; Zajko and Klimant, 2013). Once containers are hermetically sealed, heat processing prevents unwanted growth of microorganisms such as *Clostridium botulinum*, a heat-resistant pathogen (Awuah et al., 2007; Zajko and Klimant, 2013). There are a variety of retort options used for processing of conventional cans, including agitated batch systems, hydrostatic sterilizers and high speed continuous retorts; however, the vertical batch retort is the most widely used system in the human food industry (Holdsworth et al., 2008). Once the cans are inside the batch retort, sterilization occurs using a combination of steam, time, temperature and pressure that are known to be adequate for the product and container type in question. Most microorganisms are destroyed at temperatures of 121.1^{o}C, so temperatures between 100 ^{o}C and 140^{o}C are often used as a kill step to destroy pathogens (Holdsworth et al., 2008; Edley et al., 2003). After sterilization is complete, the retort chamber cools down and becomes depressurized. Canned products then are ready for storage and distribution.

Non-Heat Processing: Heat Pressure Processing and Freeze Drying

Although thermal processes are popular, there is a growing demand for minimally processed foods and more natural products that do not contain preservatives or chemical substances (Mújica-Paz, 2011). Food processing methods include raw and/or dehydrated diet

manufacture such as freeze drying (lyophilzation). In addition commercial raw diets often go through a process called high pressure pasteurization (HPP). This method uses an innovative technology that controls the incidence of food contamination, like *Listeria monocytogens,* in meat and poultry products in the food industry (Sun et al., 2010). HPP meets the requirements for delivering safe foods and extending the quality of meat with a longer shelf-life (Rendueles et al., 2011). The pet food industry has adopted this method to prevent contamination and enhance freshness in commercial raw pet diets. The HPP process is simple. Raw food is subjected to pressure (400-700 MPa) within a confined space containing water (san Martin et al., 2002). Pressure is applied isostatically so the raw diet or its containers do not collapse and become disfigured under heavy forces. The duration is dependent on product type and the desired result. Even though this format is popular in the pet food industry, there has been minimal research conducted pertaining to canine performance and overall health.

Another non-thermal processing technique that is popular and where minimal research has been conducted in dogs is freeze drying. Unlike HPP, dehydration methods remove most of the moisture from an ingredient or food. It is the most common and most energy-consuming food preservation process in the pet food industry (Ratti, 2001; Ciurzyńska and Lenart, 2011). Additionally, freeze drying is known to be the best method of water removal from foods (Ratti, 2001). It is based on the dehydration by sublimation of a frozen product. Due to the absence of liquid water and low temperatures required for this process, most deterioration and microbial reactions are prevented, which gives the product good quality (Ratti, 2001). This is ideal from a food safety perspective, and pet owners may prefer this diet over other diets because of microbial control. Unfortunately, this process is the most expensive out of the dehydration processes.

Processing and Macronutrient Digestibility

Thermal heat processes can improve macronutrient digestibility by allowing nutrients to be more bioavailable to the host. This is observed by denaturation of protein secondary, tertiary and quaternary structures (Awuah et al., 2007) and in extrusion, by the reduction of hydrolytic enzymes, lipase activity, and moisture concentrations because of the high temperatures used in the process (Camire et al., 1990; Singh et al., 2007). Even though heat is able to break down resistant starches to increase starch digestibility, they can also be formed through the retrogradation process. Essentially what happens during this process is that amylose and amylopectin chains are cooled and the gelatinized starch realigns itself. The gelatinization process uses heat and moisture to disrupt the starch granule's crystalline structure, which then causes free hydroxyl groups to bind to water, causing the granule to swell (Camiere et al., 1990). This increases the accessibility for digestion by enzymes such as alpha-amylase (Tran et al., 2008) and creates a viscous mass once all the granules have collapsed, which then increases starch digestibility and glucose aborption (Gibson and Alavi, 2014). Even though heat can improve macronutrient digestibility, excess time, temperature, or pressure may degrade essential nutrients such as AA and fatty acids, therefore, negatively influencing food quality and the pet's overall performance.

Animal-based protein sources often are rendered before they are extruded to kill pathogens. This doubled amount of processing may compromise the protein quality or nutritional value of protein sources; however, there are studies that show there is no significant loss due to the variation in chemical composition of by-products (Parsons et al., 1997; Hendriks et al., 2002). There is a similar trend for AA digestibility of protein-based ingredients (Wang and

Parsons, 1998; Ravindran et al., 2002). de Oliveira et al. (2011) compared four diets [all with a similar chemical composition, although protein and fat varied due to variability in meat and bone meal (MBM) composition] and associated each one with an extrusion application with or without rendered (one of two methods) MBM as a protein source. In other words, two out of the four diets either had MBM rendered through the conventional method (parameters: time (120 min), temperature (<120^{o}C), and pressure (0-2 bar)) or the high pressure, high temperature method (HPT) (Parameters: time (20 min.), temperature (135^{o}C), and pressure (3 bar)). Both conventional (MBM CP: 346 g/kg) and HPT (MBM CP: 357 g/kg) processes had either the whole diet extruded or all the ingredients except MBM extruded. The ATTD of the diets were assessed for dogs and cats and true digestibility of AA was assessed using cecectomized roosters fed the same diet. The HPT process increased ATTD of CP by cats and true digestibility of AA by cecectomized roosters. The extrusion process did not change the ATTD of MBM in dogs or their AA digestibility in roosters; however, digestibility increased in cats.

Another way excess heat can damage the overall quality of an animal diet is through Maillard product formation. The Maillard reaction is a non-enzymatic browning and flavoring reaction that can occur during processing and storage of foods (van Rooijen et al., 2014) and the complex is formed with the aid of free AA and peptides. Lysine is the most reactive AA in the Maillard reaction (Manzacoo et al., 2000; van Boekel, 2006) and its structure contains an alpha-amino group, an alpha carboxyl group and an ε-amino group. The alpha amino and carboxyl groups are involved in peptide bond formation in protein sequences, leaving the ε-amino group available to react with other molecules. Even though Maillard reactions could compromise the availability of essential AA, the pet food industry includes enough protein in the diet to avoid this from occurring. However, Maillard reactions do create flavor in pet foods and generate a

more palatable diet. Williams et al. (2006) determined the total and reactive lysine content of commercial dog foods. There was a total of 33 extruded diets (14 canine growth and 19 maintenance) and the bound lysine was calculated by the total and O-methlisourea (OMIU) reactive lysine method. The OMIU is a component that binds only to the reactive free e-amino groups of lysine (van Rooijen, 2015). The 33 extruded diets were highly variable in the proportion of total lysine that was bound (0 to 56%). In commercial dog diets, lysine and other essential AA appear to meet AA requirements; however, it also appears that there is a large amount of lysine that had been damaged and therefore, unavailable to the dogs.

During extrusion, the fat inclusion may affect the processing method. A stable extrusion is facilitated, and improved texture and palatability is achieved, through the use of low concentrations of fat; however, fat content that is greater than 6% can cause excess lubrication of the extruder barrel, causing a decrease in torque. Therefore, the product cannot expand due to insufficient pressure (Camire et al., 1990; Singh et al., 2007). To avoid use of high fat within the diet matrix during extrusion, kibbles may be coated with fat by spraying. B-vitamins and minerals also are added after extrusion due to their high degradation rate when heat is applied and, like vitamins, fat is sensitive to different levels of heat.

Elevated temperatures and long duration times correspond to higher oxidation rates by promoting hydroperoxide breakdown in a process that generates free radicals (Johnson and Decker, 2015). The PUFA are most susceptible because of their high level of unsaturation; therefore, heat processing (like retort sterilization), may not be the best technique to sustain fat quality. To minimize the damage, there have been other improved thermal technologies, such as high pressure thermal sterilization (HPTS), to implement the same conditions as in retort sterilization (rapid and uniform heating), but to reduce the negative effects. The HPTS method to

may extend shelf life; however, the amount of pressure needed with meat products have been found to accelerate the rate of lipid oxidation during storage.

Mesías et al. (2015) evaluated the fatty acid composition of two protein sources, tuna and sardine, canned in three different substrates (brine, sunflower oil, or olive oil) following retort sterilization and HPTS processing. The retort sterilization process was for 60 min at 116^{o}C and the HPTS process was for 28 min at 115°C at a pressure of 600 MPa. Tuna subjected to retort sterilization and HPTS presented similar fatty acid profiles with no significant differences in EPA and DHA contents; however, there were significant differences in the sardine with olive oil sample. The PUFA, EPA, and DHA content were lower ($P<0.05$) in these samples treated by HPTS. This suggests that the species of fish could be affected by different processing methods and the combination of high pressure and high temperature could promote lipid oxidation and, therefore, quality.

As regards heat processing, high temperatures are inevitable; therefore, industries create solutions to reduce lipid oxidation reactions during food storage using antioxidants and special packaging. There are various kinds of antioxidants, some that are synthetic such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and some that are natural such as flavonoids, carotenoids, and vitamin E. Packaging extends pet food shelf-life and improves food safety and quality, and there are a number of materials that help block the exterior from the interior environment. Some popular materials that are used to package foods are tin-free steal, polyolefin, and paper laminates (Marsh and Bugusu, 2007).

Fecal Metabolites and Fermentation

The final stages of the digestive process are moderated by microbiota in the colon, where fermentation occurs. The microbiome is complex and their activities are mostly determined by properties of substrates entering the colon. Carbohydrates that enter the colon primarily are in the form of resistant starches contained in beans, potatoes or grains, non-starch polysaccharides (e.g., cellulose, hemicelluloses and pectins), and oligosaccharides (e.g., fructans, inulin and raffinose) (Cummings and MacFarlane, 1991).

Short-chain fatty acids and gases are produced from microbial action on carbohydrates. They can influence epithelial cell transport, cell growth, and provide energy to host tissues (Cummings and MacFarlane, 1991). Short-chain fatty acids produced in the greatest quantity are acetate, propionate, and butyrate. Acetate is produced by microbiota such as bacteroides, bifidobacteria and lactobacilli (Macfarlane and Macfarlane, 2012) and is quickly absorbed and transported to the liver where it is primarily used as a substrate for cholesterol synthesis. When acetyl-CoA synthetase is present in the cytosol, acetate is then used for lipogenesis and concentrations in peripheral blood samples can be used to monitor colonic events in humans, such as liver disease (Wong et al, 2006). Propionate can be produced by microbes such as bacteroides, propionibacteria, and veillonella (Macfarlane and Macfarlane, 2012) and is not well understood in monogastrics. However, in ruminants, propionate constitutes a major energy source for the animal because glucose uptake is minimal due to the microbiota present in the rumen. Propionate is a substrate for gluconeogenesis, which can also inhibit cholesterol synthesis (Venter et al, 1990; Wong et al, 2006). Butyrate can be produced from microbiota such as roseburia, faecalibacteria, and clostridia (Macfarlane and Macfarlane, 2012) and is considered the most important SCFA for intestinal health because 70-90% of it is metabolized by

colonocytes and it stimulates cell proliferation in normal colonocytes (Cook et al., 1998; Wong et al., 2006). Butyrate also regulates gene expression and cell growth for the colonic epithelium.

Amino acids enter the colon in a number of ways such as dietary residues (e.g., plant proteins and muscle proteins), small intestinal secretions (e.g., enzymes, sloughed intestinal cells, and mucins), and non-structural proteins (e.g., serum albumin and collagen) (Macfarlane and Macfarlane, 2012). Once these molecules are fermented, their individual components serve as carbon and nitrogen sources to the microbiota and, in return, will produce many putrefactive compounds that may cause inflammation and fecal odor.

Protein fermentation also produces branched-chain fatty acids (BCFA) and allows bacteria to utilize carbon skeletons and free N to form microbial protein. Isobutryate, 2-methylbutyrate and isovalerate are reduced carbon skeletons of the branched-chain AA valine, isoleucine, and leucine (Wong et al., 2006). When fermentation rate is high, AA breakdown increases, causing BCFA concentrations to be higher (Barry et al., 2011). Other metabolites that are produced from AA breakdown are ammonia, phenols, and indoles. Ammonia is primarily formed by the deamination of AA. Even in low concentrations, ammonia can alter and increase turnover in colonic epithelial cells and affect DNA synthesis by reducing its lifespan (Cummings and MacFarlane, 1991). Phenols and indoles are formed from deaminated aromatic AA, including tryptophan, phenylalanine, and tyrosine. Phenols are primarily produced from tyrosine and indoles are from tryptophan. Those metabolites have been associated with disease because they act as co-carcinogens (Windey et al., 2012). If carbohydrate fermentation is increased, then ammonia, phenol, and indole concentrations often decrease (Swanson et al., 2002; Barry et al., 2011).

Thesis Objective

The objective of this study was to determine ATTD, metabolizable energy, fecal characteristics and metabolites, serum chemistry, urinalysis, and voluntary physical activity levels of dogs fed extruded, mildly cooked, and raw diets. We hypothesized that the raw diets would have greater ATTD compared to the mildly cooked diets and the mildly cooked diets would have greater ATTD than the dry kibble control. We also hypothesized that there would be no negative effects due to treatment on fecal characteristics and metabolites, urinalysis, and serum chemistry profiles. Lastly, we hypothesized that the raw and mildly cooked diets would result in greater voluntary physical activity levels by dogs than the dry kibble control.

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Chapter 3

Apparent Total Tract Macronutrient Digestibility, Fecal Characteristics, Urinalysis, Fecal Metabolites, Serum Chemistry, and Voluntary Physical Activity Levels of Healthy Adult Dogs Fed Extruded, Mildly Cooked and Raw Foods

Abstract

The pet food market continues to be strong, with much of the growth coming from super-premium foods and those with novel formats or processing methods. In addition to canned and extruded diets, lightly cooked and raw diets are available today. Despite the increase in their popularity, little research has been performed on such diets. The objective of this study was to determine the apparent total tract macronutrient digestibility (ATTD), fecal characteristics and metabolites, serum chemistry metabolic profile, urinalysis, and voluntary physical activity levels of adult dogs fed the following commercial dog diets: 1) Purina Dog Chow (DC), as the control diet; 2) Freshpet Vital Balanced Complete Nutrition (CN); 3) Freshpet Roasted Meals (RM); 4) Freshpet Vital Raw (VR). All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to the study. Eight dogs (mean age = 3.6 yr ± 0.29; mean body weight (BW) = 13.0 kg ± 0.84) were used in a replicated 4x4 Latin square design. Each period consisted of 28 d, with a 14-d adaptation phase followed by a 7-d phase for measuring voluntary physical activity, a 1-d adaptation phase to metabolic cages, a 5-d phase for fecal and urine collection, and 1-d for blood collections. Fresh fecal samples were collected for pH, moisture, and metabolite measurements. Food was fed twice daily and at a rate to maintain BW. All data were analyzed statistically by mixed models of SAS. Dry fecal output and ATTD of dry matter (DM) and organic matter (OM) were not affected by treatment; however, ATTD of crude protein (CP) was greater ($P<0.05$) for dogs fed CN than dogs fed VR, with dogs fed RM being intermediate. Dogs fed CN or RM had greater ($P<0.05$) ATTD of CP than dogs fed DC.

ATTD of fat was greater (P<0.05) by dogs fed VR than dogs fed RM, with dogs fed CN being intermediate. Dogs fed CN, VR, or RM had a greater (P<0.05) ATTD of fat than dogs fed DC. Dogs fed CN had higher (P<0.05) fecal pH than dogs fed VR, with dogs fed RM and DC being intermediate. Dogs fed DC, CN or RM had higher (P<0.05) fecal DM% than dogs fed VR. Dogs fed VR had a higher (P<0.05) fecal acetate concentration than dogs fed RM, with dogs fed CN and DC being intermediate. Dogs fed RM had higher (P<0.05) fecal indole and total phenol and indole concentrations than dogs fed CN, VR and DC. Dogs fed VR had a higher (P<0.05) fecal ammonia concentration than dogs fed RM, CN and DC while dogs fed RM had a higher (P<0.05) ammonia concentration than dogs fed DC, with dogs fed CN being intermediate. All other fecal metabolites were not affected by treatment. Most serum metabolites and urinary measures were within reference ranges for dogs fed all dietary treatments and were not affected by diet (P>0.05). Serum triglycerides were within reference ranges, but greater (P<0.05) for dogs fed DC than dogs fed CN or VR, with those fed RM being intermediate. All diets were well tolerated and dogs remained healthy throughout the study. In conclusion, the lightly cooked and raw diets tested were highly palatable, highly digestible, reduced blood triglycerides, and maintained fecal quality and serum chemistry.

Introduction

The relationship between humans and their pets has become stronger throughout the years. It started as a mutually symbiotic relationship and grew into one of companionship as owners began to viewing pets as family members. Because of this human-animal bond, the pet industry has been positively impacted by increased purchases of pet commodities such as food, supplies and medicine, veterinary care, and live animal purchase and pet services (e.g., grooming

and boarding). In 2016, the pet products industry was valued at $66.8 billion in the U.S. market and a large portion came from pet food sales ($28.2 billion) (APPA, 2017).

Extruded and retorted foods remain the most popular formats of pet food, but diet formats continue to expand. Pet foods are being developed that cater to consumer desires. Some of the newer pet food formats include raw and dehydrated products. Unfortunately, little research has been performed on these novel pet food formats; therefore, they need to be evaluated for their nutritional value and adequacy and physiological benefits to pet animals. With that in mind, the objective of this study was to determine ATTD, metabolizable energy, fecal characteristics and metabolites, serum chemistry, urinalysis, and voluntary physical activity levels of dogs fed extruded, mildly cooked, and raw diets. We hypothesized that the raw diets would have greater ATTD compared to the mildly cooked diets, and the mildly cooked diets would have greater ATTD than the dry kibble control. We also hypothesized that there would be no negative effects on fecal characteristics and metabolites, urinalysis or serum chemistry profile. Lastly, we hypothesized that the raw and mildly cooked diets would result in greater voluntary physical activity levels than the low moisture kibble diet. We came to this hypothesis because there was a study conducted by Deng et al. (2014) that observed cats having greater voluntary physical activity when they were being fed a high moisture diet (70% hydration) compared to a dry kibble control (8% moisture).

Materials and Methods

All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation.

Animals:

Eight adult female beagles (mean age: 3.57±0.29 yr) were used in this study. Dogs were weighed (mean BW: 13.0±0.84 kg) once a week prior to the 0800 feeding during the adaptation phase and on the first and last day of the sample collection phase of each experimental period. During the first 21 d of each experimental period, dogs were housed individually in runs (1.0 x 2.1 x 1.8 m). From d 22-28, dogs were housed individually in stainless steel cages (0.9 x 0.9 x 0.8 m). Dogs were fed twice a day (0800 and 1700) and had access to fresh water at all times.

Treatments:

All dietary treatments were commercial diets formulated to meet all Association of American Feed Control Officials (AAFCO, 2016) nutrient recommendations. The treatments were as follows: Purina Dog Chow (DC; extruded kibble, low-moisture), Freshpet Vital Balanced Complete Nutrition (CN; roasted, high-moisture refrigerated product), Freshpet Roasted Meals (RM; roasted, high-moisture refrigerated product), and Freshpet Vital Raw (VR; raw meat patties, high-moisture refrigerated product, Table 3.1). Freshpet dietary treatments were manufactured using novel processing methods. The mildly cooked diets, CN and RM, contained protein sources (meats) that were ground and emulsified and mixed into a homogenous blend with dry ingredients such as the pea protein, vitamin mix, and mineral mix. The blend then was formed into small meatball sized chunks that were pasteurized and chilled at approximately 4°C. Once cooled, the chunks were mixed with whole vegetable pieces, packaged using a gas flush and stored under refrigeration (1-4°C) until used. The raw diet, VR, also had meat components ground and emulsified and mixed with the fiber source, vegetables, vitamin mix, mineral mix, and *Pediococcus acidilacticii* fermentation product. The log format was incubated at room temperature (29-38°C) and chilled at approximately 4°C. The diet was packaged using a

gas flush and refrigerated (1-4°C) until used. Once the refrigerated packages are opened, shelf life was 7 d.

Because the dietary treatments tested were very different in terms of macronutrient composition and format, dogs were slowly adapted to the new dietary treatments at the beginning of each experimental period to avoid gastrointestinal distress. The following feeding protocol was used in each experimental period: d 1-3: 75% kcal from prior dietary treatment + 25% kcal from new dietary treatment; d 4-6: 50% kcal from prior dietary treatment + 50% kcal from new dietary treatment; d 7-9: 25% kcal from prior dietary treatment + 75% kcal from new dietary treatment; and d 10-28: 100% kcal from new dietary treatment.

Experimental Design and Timeline:

The study used a replicated 4x4 Latin square design. The experiment was composed of four, 28 d-periods, with each consisting of a 14-d adaptation phase, a 7-d phase for measuring voluntary physical activity using activity monitors, a 1-d adaptation to metabolic cages, a 5-d total fecal and urine collection phase, and 1 d for blood collection.

Fecal Sample Collection and Scoring:

To monitor adaptation to diet, fecal samples were scored during the first 14 d of each experimental period using a 5-point scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool, retains shape; 4 = soft, unformed stool, assumes shape of container; and 5 = watery, liquid that can be poured. During the fecal collection phase, total fecal samples were collected, weighed, and frozen at-20°C until further analysis. Fresh fecal samples were collected within 15 min of defecation, weighed, and scored. Once fresh samples were collected, pH was measured using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman

Instruments Inc., Fullerton, CA) and then aliquoted for measurement of short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), ammonia, phenols, indoles, and DM content. Fecal aliquots for analysis of phenols and indoles were frozen at -20°C immediately after collection. One aliquot was collected and placed in 2 N hydrochloric acid for SCFA, BCFA, and ammonia analyses.

Urine Collection:

During the urine collection phase, total urine output was measured. A fresh urine sample was collected for measurement of pH using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman Instruments Inc., Fullerton, CA) and specific gravity and total protein. Specific gravity was measured by the University of Illinois Veterinary Medicine Diagnostics Laboratory using a refractometer (Leica TS Meter Refractometer, Leica Microsystems Inc., Buffalo, NY). Fresh samples were collected into sterile cryogenic vials (Nalgene, Rochester, NY) and stored at 4^{o}C until analysis. Total urine samples were collected into vessels containing 2 N hydrochloric acid for immediate acidification upon urination to prevent loss of nitrogen. Acidified urine samples were subsampled (25% of each sample) and stored at -20^{o}C until analysis.

Blood Sample Collection:

On d 28, 5 mL of blood was collected for serum metabolite concentrations and complete blood count via jugular and/or cephalic venipuncture. Samples were transferred immediately to appropriate vacutainer tubes [#367841 BD Vacutainer Plus plastic whole blood tube (lavender) with K_2EDTA additive; #367974 BD Vacutainer Plus plastic serum tube (red/grey) with clot activator and gel for serum separation; BD, Franklin Lakes, NJ]. Red/grey tubes then were centrifuged at 1,200 x g for 10 min at 4°C for serum collection. Samples then were transported to

the University of Illinois Veterinary Medicine Diagnostics Laboratory for serum chemistry and complete blood count analysis using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Physical Activity:

On d 15-21 (0800-0800), voluntary physical activity was evaluated using activity monitors (Actical monitors; Mini Mitter, Bend, OR), which were placed on collars and worn around the neck of the dogs. Commercial software (Mini Mitter, Bend, OR) was used to analyze the data compiled by the monitor and was expressed as activity counts per epoch (epoch length = 15 s). Values represent the mean epoch activity count over the 7-d measurement period during light hours (0700-2000), dark hours (2000-0700) and an average of the daily activity. Dogs wore the same monitor throughout all four periods to restrict variability. Human interaction was limited as much as possible during the measurement week. Data were most variable during feeding times (0800 and 1700) and sanitary maintenance that occurred between 1200 and1400 each day.

Chemical Analyses:

To avoid nutrient degradation, high-moisture dietary treatments were lyophilized using a corrosion resistant Dura-Dry MP (FTS System; Stone Ridge, NY). Fecal samples were dried at 55°C in a forced-air oven. All dietary treatments and dried feces were ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen. Diet and fecal samples were analyzed for dry matter (DM), organic matter (OM), and ash according to Association of Official Analytical Chemists procedures (AOAC, 2006; methods 934.01 and 942.05). Nitrogen content of diets, feces, and urine was determined using a Leco Nitrogen/Protein Determinator (FP-2000, Leco Corp., St. Joseph, MI) (AOAC 2006, method 992.15). Total lipid content (acid-

hydrolyzed fat) was determined according to the methods of the American Association of Cereal Chemists (AACC; 1983) and Budde (1952). Total dietary fiber was determined according to Prosky et al. (1992). Gross energy content of diets, fecal, and urine samples was measured using an oxygen bomb calorimeter (model 1261; Parr Instruments; Moline, IL).

Fecal SCFA and BCFA concentrations were determined by gas chromatography according to Erwin et al. (1961) used a gas chromatograph (Hewlett-Packard 5890A series II, Palo Alto, CA) and a glass column (180 cm x 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. Fecal ammonia concentrations were determined according to the method of Chaney and Marbach (1962). Fecal phenol and indole concentrations were determined using gas chromatography according to the methods described by Flickinger et al. (2003).

Calculations:

Apparent total tract digestibility values were calculated using the equation as follows: [nutrient intake (g/d) – fecal output (g/d)/nutrient intake (g/d)] x 100. Dietary metabolizable energy was calculated by the following equation: [gross energy (GE) intake (kcal/d) – fecal GE (kcal/d) - urinary GE (kcal/d)]/dry matter intake (DMI) (g/d). Data normality was checked using the univariate procedure and Shapiro-Wilk statistic, with log transformation being used when normal distributions were lacking.

Statistical Analysis:

All data were analyzed using SAS (version 9.4, SAS Institute, Cary, NC) using the Mixed Models procedure with dietary treatment being the fixed effect and animal being the random effect. Data normaility was checked using the univariate procedure and Shapiro-Wilk statistic,

with log transformation being used when normal distribution were lacking. When a main effect was significant, post hoc pairwise comparisons were performed using Tukey's multiple comparison tests. Data were reported as means ± SEM with $P<0.05$ considered significant and $P<0.10$ considered a trend.

Results

The ingredient composition, chemical composition, and energy content of the experimental treatments are presented in Table 3.1. The experimental diets were dramatically different in terms of ingredient and chemical composition. Dog Chow had the highest DM percentage and was much higher compared to CN, VR, and RM that were similar to one another. On a DM basis, the diets had similar OM percentages; however, CN and VR had higher CP concentrations (DMB) compared to DC and RM. Dog Chow had a lower fat concentration (DMB) than the other three treatments. Total dietary fiber (DMB) content was greater in VR than DC, CN and RM. Due to the low moisture content, as-is GE, DE, and ME were greater for DC than CN, VR and RM. On a DMB, however, CN, VR and RM had greater GE, DE, and ME than DC.

Food intake, fecal characteristics, and apparent total tract macronutrient and energy digestibility data of are presented in Table 3.2. As-is food intake (g/d) was greater ($P<0.05$) for dogs fed RM than dogs fed CN or DC, but not VR. The DM and OM intake (g/d) by dogs fed VR or RM was greater ($P<0.05$) than for those fed CN. The CP intake (g/d) by dogs fed CN was greater ($P<0.05$) than for those fed VR or DC, while CP intake (g/d) by dogs fed RM was greater ($P<0.05$) than for those fed DC. The fat intake (g/d) by dogs fed VR was greater ($P<0.05$) than for dogs fed CN, RM or DC, whereas dogs fed CN and RM were intermediate. Gross energy

intake (kcal/d) by dogs fed VR was greater (P<0.05) than for those fed DC or CN, whereas dogs fed DC, CN and RM were not different from one another. Fecal output, on an as-is basis, was greater (P<0.05) for dogs fed VR than for those fed CN, whereas dogs fed DC and RM were intermediate. The ATTD of CP was greater (P<0.05) for dogs fed CN than for those fed VR and DC, whereas dogs fed VR and RM were intermediate. The ATTD of fat was greater (P<0.05) for dogs fed VR than for those fed DC or RM, whereas fat digestibility by dogs fed CN was greater (P<0.05) than for those fed DC, but not for those fed VR or RM. Digestible energy was greater (P<0.05) for dogs fed CN than for those fed DC, whereas those fed VR and RM were intermediate. Fecal output (DMB), the ratio of as-is fecal output and DM intake, and apparent total tract DM and OM digestibility were not different among treatments.

Fecal and urine characteristics of dogs are presented in Table 3.3. The fecal pH of dogs fed CN was higher (P<0.05) than for those fed VR, whereas dogs fed DC and RM were intermediate. Fecal DM percentage was greater (P<0.05) for dogs fed DC, CN or RM than those fed VR. Daily stool number during the collection period (d 23-d 28) was greater (P<0.05) for dogs fed VR than for those fed DC, CN and RM. Fecal acetate concentration was greater (P<0.05) for dogs fed VR than for dogs fed RM, whereas dogs fed DC and CN were intermediate. Fecal indole and total phenol and indole concentrations were greater (P<0.05) for dogs fed RM than for those fed DC, CN or VR. Fecal ammonia concentrations were greater (P<0.05) for dogs fed VR than for those fed DC, CN and RM, whereas dogs fed RM had higher (P<0.05) fecal ammonia concentrations than for those fed DC. Fecal scores, daily stool number during the adaptation period (d 10-d 14); fecal propionate, butyrate, total SCFA, isobutyrate, isovalerate, valerate, total BCFA and phenol concentrations; and urinary specific gravity, pH and total protein were not different among treatments.

Serum metabolites of dogs are presented in Table 3.4. Blood chloride concentrations for dogs fed VR were greater ($P<0.05$) than for those fed DC, whereas dogs fed CN and RM were intermediate. Blood chloride concentrations for dogs fed VR were greater ($P<0.05$) than for those fed DC, whereas dogs fed CN and RM were intermediate. Blood triglyceride concentrations were greater ($P<0.05$) for dogs fed DC than for those fed CN or VR, with dogs fed RM being intermediate. Blood alkaline phosphatase (ALP) concentrations were greater ($P<0.05$) for dogs fed DC than for those fed CN, with dogs fed VR and RM being intermediate. Blood albumin:globulin ratio tended to be greater ($P<0.10$) for dogs fed CN or VR than for those fed DC, but not for those fed RM. Blood creatinine, blood urea nitrogen (BUN), total protein, total bilirubin, albumin, globulin, calcium, phosphorus, sodium, potassium, sodium: potassium ratio, bicarbonate, anion gap, corticosteroid-induced alkaline phosphatase (C-ALP), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), glucose, and cholesterol concentrations were not different among treatments. Despite differences observed among some dietary treatments, all serum metabolites were within reference ranges except for creatine (0.48 mg/dL), which was just slightly out of range for CN (0.5-1.5 mg/dL). Blood cell counts (Table 3.5) were not different among treatments and were all within reference ranges.

Voluntary physical activity data (activity counts/epoch) are presented in Table 3.6 and Figure 3.1. Activity during the dark period was greater ($P<0.05$) for dogs fed DC than for those fed CN or VR, but was not different than for those fed RM. The light:dark activity ratio was greater ($P < 0.05$) for dogs fed CN or VR than for those fed DC, but was not different for dogs fed RM.

Discussion

Due to the growing trend of pet owners to feed more premium and super-premium diets, sales of novel formats (e.g., raw and freeze-dried) are growing, while traditional formats are decreasing. Extruded and retort sterilization methods cook raw materials at high temperatures and pressures to increase nutrient digestibility (e.g., starch and protein) and improve food safety; however, consumers are shifting towards more convenient, human-like, and fresher looking pet foods. In the current study, minimally processed and commercial raw diets were evaluated for their nutritional value.

Because animal-based protein sources are leading the ingredient list on premium and super-premium pet food labels, more protein studies on ingredient variability need to be performed. Concentration and composition of dietary protein and fat fed to dogs and cats can vary greatly. This variability is dependent on the sources and quality of the animal ingredients used (Faber et al., 2010). Dust et al. (2005) evaluated a number of protein sources (e.g., chicken protein sources, blood protein sources, enzyme-hydrolyzed fish protein concentrate, soybean meal and spray-dried pork liver) with a CP range of 49.2-95.3% (DMB) and a fat range of 1.6-49.5% (DMB). They concluded that protein sources are highly variable in regards to protein and fat concentrations. Given these variations in chemical composition, many possibilities for commercial diet manufactures exist. Diets tested in the current study were not only composed of different protein sources, but were formulated to contain different protein and fat concentrations and energy contents. Therefore, differences due to the dietary treatments cannot be attributed to any specific ingredients or nutrient concentrations, but the diets as a whole.

Diet nutrient composition and energy content can lead to differences in food intake and the amount needed to meet nutrient and energy needs. Even though the goal was to feed dogs to

maintain BW, food intake in the current study was highly variable and was impacted by palatability. Apparent total tract macronutrient digestibilities may be affected by many factors, including animal age, differences in ingredient source and format, and processing methods used to prepare the dietary treatments. While the authors are unaware of any data pertaining to the mild cooking procedures used to produce the CN and RM treatments tested in this study, a few researchers have published data pertaining to raw diets designed for dogs or cats (Crissey et al., 1997; Vester et al., 2008; Vester et al., 2010; Beloshapka et al., 2010; Kerr et al., 2012). The apparent total tract CP digestibility was lower for the raw diet tested in our study compared to Crissey et al. (1997; 90.26% in exotic cats), Vester et al. (2008; 92.94% in exotic cats), Vester et al. (2010; 91.7% in exotic cats), Beloshapka et al. (2010; raw beef: 91.35%; and raw chicken: 88.35% in dogs), and Kerr et al. (2012; 93.3% in domestic cats). However, the apparent total tract fat digestibilities for the raw diet tested in the current study and those tested in previous studies were similar and ranged from 93.0-97.8%.

Mild processing may increase nutrient digestibilities without damaging essential nutrients. Although the authors are unaware of publications pertaining to commercially available diets undergoing mild cooking, Kerr et al. (2012) compared raw vs. cooked beef-based raw diets formulated for cats. In that study, the ATTD were similar for raw and cooked treatments (cooked diet: 92.9% CP, 95.3% fat; raw diet: 93.3 CP% and 95.5% fat) and similar to the mildly cooked diets used in the present study. Apparent total tract CP digestibility is not a true representation of what the host digests because of microbial metabolism in the hindgut. To accurately measure CP digestibility, ileal-cannulated animals or the cecectomized rooster assay can be used. A recent study evaluated the macronutrient digestibility and nitrogen-corrected true metabolizable energy (TMEn) of chicken-based ingredients that had undergone different

processing conditions using the precision-fed cecectomized rooster assay (Swanson et al., 2017). The processing conditions tested in that study were similar to those used to produce the mildly cooked diets tested in this study (CN; RM). In that study, chicken meal had a lower digestibility of DM (60.0%) and OM (65.9%), but higher AHF digestibility (90.3%) than the raw (DM: 75.9%; OM: 80.5%; AHF: 88.3%), steamed (DM: 76.5%; OM: 80.6%; AHF: 86.5%), and retorted (DM: 73.5%; OM: 77.8%; AHF: 83.5%) ingredients. For all essential and non-essential amino acids (AA), steamed chicken had the highest digestibilities. For all essential AA and all but one non-essential AA (proline), raw and retorted chicken digestibilities were similar to one another and greater than that of chicken meal.

Along with diet composition and food intake, nutrient digestibility influences fecal output and characteristics such as consistency score and fermentative end-product concentrations. Fecal score is a good measure of fecal quality (Hernot, 2005; Nery et al., 2010). In the current study, dogs fed the raw diet had softer stools than dogs fed the other dietary treatments, but all were of acceptable quality. Fecal DM also be a good measure of fecal quality. In other studies comparing extruded and raw diets (Vester et al., 2010; Kerr et al., 2012), cats fed extruded and raw diets had similar fecal DM content; however, in the present study, dogs fed the raw diet had lower fecal DM than those fed the other treatments. Also, the raw diet contained citrus fiber and inulin, a non-digestible carbohydrate that stimulates the growth of beneficial bacteria (Roberfroid, 2001), which may cause higher fecal moisture and fecal volume (Diez et al., 1998).

Kerr et al. (2012) evaluated extruded, cooked and raw beef-based diets fed to domestic cats. Cats fed the extruded diet had greater fecal output compared to those fed the raw or cooked diets. This result also has been reported by Vester et al. (2010) who evaluated a high-protein extruded kibble diet with a commercial raw meat-based diet fed to captive African wildcats. In

that study, fecal output on an as-is basis was greater for cats that ate the kibble diet (32.0 g/d) compared with the raw diet (17.6 g/d). That result was likely due to the higher digestibility of raw diets compared to kibble diets. In the present study, it was observed that the extruded diet produced a greater fecal output compared to the mildly cooked diets; however, the raw diet resulted in even greater fecal output. Because dogs demonstrated different food intakes among diets, the food intake: fecal output ratio is probably the most appropriate comparison, which was similar among all dietary treatments.

Fecal quality also be evaluated by measuring the metabolites produced by gut microorganisms. Fermentable substrates largely come from dietary carbohydrate and protein sources, but their impact on gut microbiota and metabolite production are quite different. Carbohydrates such as resistant starches, non-starch polysaccharides, and non-digestible oligosaccharides are fermented by microbes and produce SCFA such as acetate, propionate and butyrate. The SCFA have a number of functions, which include serving as an important energy source to colonocytes, lowering pH to limit gut pathogen growth, gut signaling and gut peptide synthesis. In contrast, phenols, indoles, and BCFA are an indication of protein fermentation occurring in the large intestine, contributing to fecal odor and are associated with gastrointestinal diseases (Cummings and MacFarlane, 1991; Swanson et al., 2002; Macfarlane and Macfarlane, 2012). Fecal pH often coincides with SCFA and may be a good marker of SCFA production. According to Wong et al. (2006), a decreased pH indicates an increase in SCFA production that indirectly influences the composition of colonic microbiota (e.g., acidic pH reduces pathogens). In the current study, dogs fed a raw diet had a low fecal pH while also having high SCFA concentrations. While this may have been due to microbial fermentation of carbohydrates, this result may be due to the fact that it is an acidic product ($pH < 5$). The raw diet tested in this study

undergoes an acidification process through the addition of *Pedicoccus acidilacticii*, a bacterial taxa that is used in human and pet food products. Dogs fed the mildly cooked diets had higher fecal pH, indicating that lower SCFA were produced or that more of the SCFA were absorbed compared to the other dietary treatments. Beloshapka et al. (2010), who evaluated inulin and yeast cell wall supplements in raw diets fed to dogs, reported greater ($P<0.05$) fecal acetate concentrations in dogs fed diets containing inulin than those fed the control diet. In the current study, the two diets that resulted in greater fecal acetate concentrations contained inulin.

Branched-chain fatty acids, phenols, indoles and ammonia are produced through protein fermentation. There are many types of protein in the large bowel and they occur partly from dietary residues, such as animal and plant proteins, but the host also produces a significant amount of protein sources in the form of oral, gastric, pancreatic and small intestinal secretions (e.g., enzymes and glycoproteins; Macfarlane and Macfarlane, 2012). Phenols and indoles are deaminated aromatic amino acids, tyrosine, phenylalanine and tryptophan, and may act as co-carcinogens (Cummings and MacFarlane, 1991). In the current study, there were no differences in phenol concentrations among the dietary treatments; however, RM had high indole and total phenol and indole concentrations. This response may be due to lack of inulin in the diet. Therefore, to reduce protein fermentative metabolites, there needs to be more carbohydrates available for microbial fermentation rather than protein (Flickinger et al., 2003; Beloshapka et al., 2010).

Another harmful fermentative substrate is ammonia. It induces faster turnover of epithelial cells. When SCFA concentrations increase, pH decreases, which reduces the ammonia absorption dissociating, or separating, ammonia and other amines (Wong et al., 2006). This is

also observed in this study. Remaining metabolites were not affected in dogs by dietary treatment.

Blood metabolites indicated that animals were in good health throughout the study. Statistical differences were observed in blood chloride, ALP and triglyceride concentrations, and a trend was noticed in the albumin:globulin ratio. Because these metabolites remained within reference ranges, these differences likely did not affect the animal; however, the triglyceride results were intriguing. When animals eat excess food, the unused nutrients are converted into triglycerides and stored in the body until energy is needed. Triglycerides become high when there is excess calories in the body from the consumption of carbohydrates and fats. Kerr et al. (2012) and Vester et al. (2010) measured triglycerides in felines fed extruded and raw diets. Kerr et al. (2012), measured triglyceride concentrations in cats fed the following diet formats: measured extruded (26.7 mg/dL), raw (32.4 mg/dL) and cooked (37.3 mg/dL). In Vester et al. (2010), triglyceride concentrations in felines fed the following diet formats were: extruded (26.0 mg/dL) and raw (22.0 mg/dL). As noted, cats fed extruded diets had similar triglyceride concentrations with concentration being lower than that for raw and mildly cooked treatments. This may be expected because the raw diets contained a higher fat content than the kibble diets and provided excess calories. The triglyceride concentrations in felines fed the raw diets, however, were quite different between these two studies. Variance between the values could be due to ingredient sources and processing methods. In the current study, dogs fed extruded, raw and mildly cooked diets had higher triglyceride concentrations compared to either of those studies. Interestingly, the dogs fed the kibble diet (80.13 mg/dL) had higher triglyceride concentrations than dogs fed the raw (53.38 mg/dL) and mildly cooked (53.50; 60.26 mg/dL) diets. Similarly, Beloshapka (2011), also evaluated blood triglycerides in dogs fed raw diets.

Their values for dogs fed the raw chicken diet with inulin supplement was 37.5 mg/dL, and 37.7 mg/dL for dogs fed the chicken diet with yeast cell wall supplement. Values for the raw beef diet with inulin and yeast cell wall supplements were 46.3 mg/dL and 44.8 mg/dL, respectively. More research needs to be done to understand the mechanism behind these changes.

Similar to humans, obesity in animals is a nutritional disease that affects energy balance of the host due to excessive food intake and lack of physical exercise. Obesity can result in a number of adverse effects in dogs, including shortened life span and bone and joint inflammation. According to the American Pet Products Association (APPA, 2015), there is an estimated 53.8% of US dogs and 58.2% of US cats that are considered overweight, with a body condition score between 4-5 on a 5-point scale. However, there are studies that believe it is not only because of a lack of stimulating physical activity (Kienzle and Bergler, 2006), but a decrease in overall energy expenditure (Villaverde et al., 2008).

Deng et al. (2014) evaluated the effects of dietary water content on voluntary physical activity of cats. Cats were fed one of two diets in that study, either a commercial dry kibble feline diets in its original state (8% moisture) or the same diet that had water introduced to it (70% moisture). Interestingly, there was an increase in voluntary physical activity throughout the day and night, especially between 0600 and 1930 by cats fed the high moisture diet.

The Deng et al. (2014) study was used as a basis for the current study, testing mildly cooked and raw dietary treatments had an average moisture content of 57.29%. We noted peaks of physical activity, however, this was more likely due to human interaction due to feeding times and animal care. Unlike the study performed by Deng et al. (2014), dogs fed the kibble diet experienced higher physical activity levels during the dark period, and there was no difference among the mildly cooked and raw dietary treatments which may be due to satiety differences.

This could be because of the individual housing and small quarters for the dogs. Dogs remained in their kennels throughout this study, whereas the cats had access to rooms that allowed them to move about more freely. Other reasons could be the behavioral differences between dogs and cats. Felines are obligate carnivores and hunt and eat individually, obtaining most of their water from the carcasses they consume. Canines are omnivorous and share their prey with the whole pack and rely on drinking sources for hydration purposes. Further research needs to be done to understand the mechanism behind hydrated diet ingestion and voluntary physical activity in companion animals.

In conclusion, all diets tested in this study were well tolerated and dogs remained healthy throughout the study. Mildly cooked and raw diets were highly palatable, highly digestible, resulted in reduced blood triglycerides concentrations and maintained fecal quality and serum metabolite concentrations.

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Tables and Figure

Table 3.1. Analyzed chemical composition and energy content of experimental diets fed to dogs

Item	Treatment			
	Purina Dog Chow[1]	Freshpet Vital Balanced Complete Nutrition[2]	Freshpet Roasted Meals[3]	Freshpet Vital Raw[4]
Dry matter (%)	93.33	38.63	42.23	47.28
Organic matter (%), DMB[5]	92.47	88.58	89.51	93.23
Crude protein (%), DMB	24.07	45.69	31.08	25.13
Acid-hydrolyzed fat (%), DMB	13.30	30.30	27.82	33.90
Total dietary fiber (%), DMB	9.60	7.28	6.94	11.84
GE[1] (kcal/g; measured), as-is	4.58	2.32	2.39	3.07
GE (kcal/g; measured), DMB	4.91	6.01	5.66	6.50
DE[1] (kcal/g; measured), as-is	3.07	1.66	1.80	2.18
DE (kcal/g; measured), DMB	3.29	4.30	4.26	4.61
ME[1] (kcal/g; measured), as-is	3.02	1.63	1.77	2.15
ME (kcal/g; measured), DMB	3.24	4.22	4.19	4.55

[1]Purina Dog Chow: Whole grain corn, meat and bone meal, corn gluten meal, animal fat preserved with mixed-tocopherols, soybean meal, poultry by-product meal, egg and chicken flavor, whole grain wheat, animal digest, salt, calcium carbonate, potassium chloride, mono and dicalcium phosphate, choline chloride, L-Lysine monohydrochloride, zinc sulfate, Yellow 6, vitamin E supplement, copper sulfate, calcium pantothenate, garlic oil, pyridoxine hydrochloride, vitamin B-12 supplement, thiamine mononitrate, vitamin D-3 supplement, riboflavin supplement, calcium iodate, menadione sodium bisulfite complex (source of vitamin K activity), folic acid, biotin, sodium selenite.

Table 3.1 (cont.)

[2]Freshpet Vital Balanced Complete Nutrition: chicken, chicken liver, beef, salmon, eggs, cranberries, spinach, pea protein, natural flavors, minerals (dicalcium phosphate, calcium carbonate, zinc proteinate, iron proteinate, manganese proteinate, copper proteinate, sodium selenite, calcium iodate), pea fiber, vinegar, salt, peas, carrageenan, potassium chloride, inulin, beta-carotene, vitamins (choline chloride, vitamin E supplement, niacin, calcium pantothenate, biotin, riboflavin, thiamine mononitrate, vitamin B12 supplement, vitamin D3 supplement, pyridoxine hydrochloride, folic acid), celery powder.
[3]Freshpet Roasted Meals: chicken, chicken liver, ground oats, carrots, eggs, spinach, rice bran, natural flavors, minerals (dicalcium phosphate, calcium carbonate, potassium chloride, zinc proteinate, iron proteinate, manganese proteinate, copper proteinate, sodium selenite, calcium iodate), salt, vinegar, beta-carotene, vitamins (choline chloride, vitamin E supplement, niacin, calcium pantothenate, biotin, riboflavin, thiamine mononitrate, vitamin B12 supplement, vitamin D3 supplement, pyridoxine hydrochloride, folic acid), celery powder
[4]Freshpet Vital Raw: chicken, sweet potatoes, kale, citrus fiber, water, sea salt, dicalcium phosphate, dextrose, celery powder, vitamin and minerals (choline chloride, zinc proteinate, iron proteinate, vitamin E supplement, copper proteinate, manganese proteinate, vitamin A supplement, niacin, calcium pantothenate, biotin, sodium selenite, thiamine mononitrate, riboflavin, vitamin B12 supplement, calcium iodate, vitamin D3 supplement, pyridoxine hydrochloride, folic acid), inulin, dried *Pediococcus acidilacticii* fermentation product, cherry juice powder.
[5]DMB = dry matter basis; GE = gross energy (measured by bomb calorimetry); DE = digestible energy (gross energy – fecal energy); ME = metabolizable energy (gross energy – fecal energy – urinary energy).

Table 3.2: Food intake, fecal characteristics, and total tract apparent macronutrient and energy digestibility of dogs fed extruded, mildly cooked, and raw foods

Item	Treatment			
	Purina Dog Chow	Freshpet Vital Balanced Complete Nutrition	Freshpet Roasted Meals	Freshpet Vital Raw
Food intake				
g food/d (as-is)	176.3 ± 26.98[c]	342.0 ± 26.98[b]	426.0 ± 28.30[a]	391.3 ± 26.98[ab]
g DM/d[1]	164.5 ± 14.20[ab]	132.1 ± 14.20[b]	179.7 ± 14.90[a]	185.0 ± 14.20[a]
g OM/d[1]	152.1 ± 12.99[ab]	117.0 ± 12.99[b]	160.8 ± 13.6[a]	172.5 ± 12.99[a]
g CP/d[1]	39.6 ± 4.09[c]	60.4 ± 4.09[a]	55.8 ± 4.28[ab]	172.5 ± 12.99[a]
g fat/d	21.9 ± 3.55[c]	40.0 ± 3.55[b]	49.9 ± 3.73[b]	46.5 ± 4.09[bc]
GE, kcal/d[1]	806.9 ± 79.63[b]	794.5 ± 79.63[b]	1015.7 ± 83.46[ab]	62.7 ± 3.55[a]
Fecal output				
Fecal output, as-is (g/d)	84.3 ± 11.83[ab]	52.3 ± 11.83[b]	77.6 ± 12.62[ab]	101.6 ± 11.83[a]
Fecal output, DM[1] (g/d)	29.4 ± 3.54	19.9 ± 3.54	28.8 ± 3.76	29.6 ± 3.54
As-is fecal output (g/d)/DM intake (g/d)	0.48 ± 0.04	0.39 ± 0.04	0.44 ± 0.05	0.55 ± 0.04
Nutrient and energy digestibility				
DM (%)	82.6 ± 1.52	85.1 ± 1.52	84.1 ± 1.63	83.6 ± 1.52
OM (%)	87.8 ± 1.21	89.9 ± 1.21	89.1 ± 1.29	86.2 ± 1.21
CP (%)	85.1 ± 1.02[c]	94.6 ± 1.02[a]	92.0 ± 1.09[ab]	88.3 ± 1.02[bc]
Fat (%)	92.1 ± 0.38[c]	97.2 ± 0.38[ab]	95.8 ± 0.41[b]	97.5 ± 0.38[a]
Energy (%)	87.4 ± 0.93[b]	92.7 ± 0.93[a]	90.7 ± 1.00[ab]	90.8 ± 0.93[ab]

[1]DM = dry matter; OM = organic matter; CP = crude protein; GE = gross energy.

[a-c] Means in the same row without common superscript letters differ ($P<0.05$).

Table 3.3: Fecal and urine characteristics of dogs fed extruded, mildly cooked and raw foods

Item	Treatment			
	Purina Dog Chow	Freshpet Vital Balanced Complete Nutrition	Freshpet Roasted Meals	Freshpet Vital Raw
Fecal characteristics				
pH	6.22 ± 0.18^{ab}	6.78 ± 0.18^{a}	6.59 ± 0.18^{ab}	6.15 ± 0.18^{b}
Fecal score[1]	2.40 ± 0.13	2.21 ± 0.13	2.40 ± 0.13	2.08 ± 0.13
Fecal DM%	37.16 ± 2.19^{a}	37.68 ± 2.19^{a}	36.58 ± 2.34^{a}	28.82 ± 2.19^{b}
Stools/d (adaptation; d 10-14)	1.7 ± 0.14	1.4 ± 0.14	1.5 ± 0.15	1.8 ± 0.14
Stools/d (collection; d 23-28)	1.4 ± 0.16^{b}	1.1 ± 0.16^{b}	1.4 ± 0.17^{b}	2.0 ± 0.16^{a}
Fecal metabolites				
Acetate (umol/g DMB)	257.52 ± 24.18^{ab}	225.65 ± 24.18^{ab}	214.52 ± 24.18^{b}	312.09 ± 24.18^{a}
Propionate (umol/g DMB)	116.00 ± 15.93	106.16 ± 15.93	129.14 ± 15.93	127.33 ± 15.93
Butyrate (umol/g DMB)	43.59 ± 8.10	45.90 ± 8.10	58.17 ± 8.10	44.64 ± 8.10
Total SCFA[2] (umol/g DMB)	417.11 ± 38.44	377.71 ± 38.44	401.83 ± 38.44	484.07 ± 38.44
Isobutyrate (umol/g DMB)	5.33 ± 0.70	5.93 ± 0.70	6.68 ± 0.70	4.19 ± 0.70
Isovalerate (umol/g DMB)	9.66 ± 1.12	8.76 ± 1.12	9.84 ± 1.12	6.19 ± 1.12
Valerate (umol/g DMB)	0.73 ± 0.11	0.51 ± 0.11	0.83 ± 0.11	0.55 ± 0.11
Total BCFA[2] (umol/g DMB)	15.72 ± 1.81	15.21 ± 1.81	17.35 ± 1.81	10.94 ± 1.81
Phenol (umol/g DMB)	0.32 ± 0.23	0.51 ± 0.23	1.09 ± 0.23	0.50 ± 0.23
Indole (umol/g DMB)	1.18 ± 0.21^{b}	1.10 ± 0.21^{b}	2.26 ± 0.21^{a}	0.97 ± 0.21^{b}
Total P/I[2] (umol/g DMB)	1.50 ± 0.39^{b}	1.60 ± 0.39^{b}	3.35 ± 0.39^{a}	1.47 ± 0.39^{b}
Ammonia (umol/g DMB)	26.06 ± 4.23^{c}	36.26 ± 4.23^{bc}	44.18 ± 4.23^{b}	61.43 ± 4.23^{a}
Urine characteristics				
Specific gravity	1.042 ± 0.00	1.045 ± 0.00	1.039 ± 0.00	1.042 ± 0.00
pH	6.49 ± 0.16	6.56 ± 0.16	6.44 ± 0.16	5.97 ± 0.16
Total protein	1.19 ± 0.28	0.88 ± 0.28	0.81 ± 0.28	0.81 ± 0.28

Table 3.3 (cont.)

[1]Fecal scores: 1 = hard, dry pellets; small hard mass; 2 = hard formed, dry stool; remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.
[2]Total SCFA = acetate + propionate + butyrate; Total BCFA = valerate + isovalerate + isobutyrate. Total P/I = phenol + indole.
[a-c] Means in the same row without common superscript letters differ ($P<0.05$).

Table 3.4. Serum metabolites of dogs fed extruded, mildly cooked and raw foods

Item	Treatment				Reference Range[1]
	Purina Dog Chow	Freshpet Vital Balanced Complete Nutrition	Freshpet Roasted Meals	Freshpet Vital Raw	
Creatinine (mg/dL)	0.65 ± 0.15	0.48 ± 0.15	0.78 ± 0.15	0.60 ± 0.15	0.5-1.5
BUN (mg/dL)[2]	14.88 ± 2.40	12.50 ± 2.40	17.50 ± 2.40	11.13 ± 2.40	6-30
Total protein (g/dL)	6.14 ± 0.17	6.34 ± 0.17	6.19 ± 0.17	6.21 ± 0.17	5.1-7.0
Total bilirubin (mg/dL)	0.18 ± 0.02	0.16 ± 0.02	0.18 ± 0.02	0.16 ± 0.02	0.1-0.3
Albumin (g/dL)	3.28 ± 0.08	3.53 ± 0.08	3.39 ± 0.08	3.48 ± 0.08	2.5-3.8
Globulin (g/dL)	2.86 ± 0.11	2.81 ± 0.11	2.80 ± 0.11	2.74 ± 0.11	2.7-4.4
Albumin:globulin ratio	1.15 ± 0.04^{y}	1.28 ± 0.04^{x}	1.21 ± 0.04^{xy}	1.28 ± 0.04^{x}	0.6-1.1
Ca (mg/dL)	9.93 ± 0.18	9.89 ± 0.18	9.98 ± 0.18	9.85 ± 0.18	7.6-11.4
P (mg/dL)	4.51 ± 1.80	3.94 ± 1.80	4.58 ± 1.80	3.46 ± 1.80	2.7-5.2
Na (mmol/L)	144.12 ± 0.66	144.12 ± 0.66	144.13 ± 0.66	144.50 ± 0.66	141-152
Cl (mmol/L)	110.63 ± 0.67^{b}	112.25 ± 0.67^{ab}	111.38 ± 0.67^{ab}	113.50 ± 0.67^{a}	107-118
K (mmol/L)	4.73 ± 0.13	4.65 ± 0.13	4.81 ± 0.13	4.75 ± 0.13	3.9-5.5
Na:K ratio	30.63 ± 0.88	31.00 ± 0.88	30.25 ± 0.88	30.50 ± 0.88	28-36
Bicarbonate (mmol/L)	20.38 ± 0.79	19.38 ± 0.79	18.50 ± 0.79	18.75 ± 0.79	16-24
Anion gap	18.00 ± 0.96	17.38 ± 0.96	19.13 ± 0.96	17.13 ± 0.96	8-25
ALP (U/L)[2]	38.13 ± 0.08^{a}	12.88 ± 0.08^{c}	29.38 ± 0.08^{ab}	16.00 ± 0.08^{bc}	7-92
C-ALP (U/L)[2]	9.88 ± 2.89	2.50 ± 2.89	8.00 ± 2.89	4.00 ± 2.89	0-40
ALT (U/L)[2]	30.50 ± 2.28	24.38 ± 2.28	27.13 ± 2.28	25.25 ± 2.28	8-65
GGT (U/L)[2]	2.50 ± 0.38	2.63 ± 0.38	1.88 ± 0.38	2.38 ± 0.38	0-7
Glucose (mg/dL)	91.63 ± 6.29	91.38 ± 6.29	99.75 ± 6.29	90.38 ± 6.29	68-126

Table 3.4 (cont.)

Cholesterol (mg/dL)	227.63 ± 19.30	224.38 ± 19.30	251.75 ± 19.30	242.38 ± 19.30	129-297
Triglycerides (mg/dL)	80.13 ± 5.97[a]	53.50 ± 5.97[b]	60.25 ± 5.97[ab]	53.38 ± 5.97[b]	32-154

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.

[2]BUN: blood urea nitrogen; ALP: alkaline phosphatase; C-ALP: corticosteroid-induced alkaline phosphatase; ALT: alanine transaminase; GGT: gamma-glutamyltransferase.

[a-c] Means in the same row without common superscript letters differ ($P<0.05$).

[x-y] Means in the same row without common superscript letters differ ($0.05<P<0.10$).

Table 3.5: Blood cell counts of dogs fed extruded, mildly cooked, and raw foods

Item	Treatment				Reference Ranges[1]
	Purina Dog Chow	Freshpet Vital Balanced Complete Nutrition	Freshpet Roasted Meals	Freshpet Vital Raw	
Total white blood cells (10^3/ul)	8.95 ± 0.69	8.79 ± 0.66	8.62 ± 0.66	8.69 ± 0.66	6-17
Neutrophils (10^3/ul)	5.96 ± 0.66	5.90 ± 0.63	5.66 ± 0.63	5.75 ± 0.63	3.0-11.5
Lymphocytes (10^3/ul)	2.14 ± 0.25	2.06 ± 0.23	2.27 ± 0.23	2.21 ± 0.23	1.0-4.8
Monocytes (10^3/ul)	0.47 ± 0.09	0.36 ± 0.08	0.41 ± 0.08	0.41 ± 0.08	0.2-1.4
Eosinophils (10^3/ul)	0.34 ± 0.11	0.44 ± 0.10	0.23 ± 0.10	0.31 ± 0.10	0.1-1.0
Basophils (10^3/ul)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0-2
Neutrophils (%)	66.20 ± 3.29	65.59 ± 3.14	64.50 ± 3.14	65.38 ± 3.14	
Lymphocytes (%)	24.45 ± 3.45	25.25 ± 3.30	27.00 ± 3.30	26.25 ± 3.30	
Monocytes (%)	5.29 ± 0.96	4.04 ± 0.90	5.25 ± 0.90	4.63 ± 0.90	
Eosinophils (%)	3.95 ± 1.14	4.94 ± 1.07	3.13 ± 1.07	3.63 ± 1.07	
Basophils (%)	0.00 ± 0.04	0.06 ± 0.03	0.00 ± 0.03	0.00 ± 0.04	
Red blood cells (10^6/ul)	6.99 ± 0.27	7.33 ± 0.26	7.26 ± 0.26	7.58 ± 0.26	5.5-8.5
Hemoglobin (g/dL)	16.68 ± 0.66	17.58 ± 0.62	17.36 ± 0.62	18.18 ± 0.62	12-18
Hematocrit (%)	52.05 ± 1.90	54.43 ± 1.80	54.15 ± 1.80	56.24 ± 1.80	32-52
Mean corpuscular volume (fl)	74.55 ± 0.64	74.36 ± 0.61	74.60 ± 0.61	74.31 ± 0.61	60-77
Mean corpuscular hemoglobin (pg)	23.88 ± 0.25	23.96 ± 0.24	23.90 ± 0.24	23.99 ± 0.24	20-25
MCHC (g/dL)[1]	32.04 ± 0.21	32.25 ± 0.19	32.05 ± 0.19	32.28 ± 0.19	32-36
Platelets (10^3/ul)	382.66 ± 36.97	341.63 ± 34.33	372.50 ± 34.33	356.13 ± 34.33	200-900
Mean platelet volume (fl)	11.05 ± 0.91	12.40 ± 0.91	10.78 ± 0.75	9.66 ± 0.75	

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.

[2]MCHC: Mean corpuscular hemoglobin concentration.

Table 3.6. Physical activity (activity counts/epoch)[1] by dogs fed extruded, mildly cooked, and raw foods

	Treatment			
Item	Purina Dog Chow	Freshpet Vital Balanced Complete Nutrition	Freshpet Roasted Meals	Freshpet Vital Raw
Total activity	30.56 ± 3.79	26.70 ± 3.72	29.27 ± 3.72	29.58 ± 3.72
Light period	43.30 ± 6.27	38.68 ± 6.19	43.09 ± 6.19	43.67 ± 6.19
Dark period	13.39 ± 1.49[a]	10.40 ± 1.46[b]	11.55 ± 1.46[ab]	10.21 ± 1.46[b]
Light:dark ratio	3.55 ± 0.79[b]	4.39 ± 0.79[a]	4.03 ± 0.79[ab]	4.49 ± 0.79[a]

[1]Epoch: 15 seconds.

[a-b]Means in the same row without common superscript letters differ (P<0.05).

Figure 3.1. Voluntary physical activity patterns for dogs fed the dietary treatments. Lights were on from 07:00 to 21:00 hours. Dogs were fed at 08:00 and 17:00 hours each day.