EFFECTS OF INULIN OR YEAST CELL WALL EXTRACT ON NUTRIENT DIGESTIBILITY AND FECAL FERMENTATIVE END-PRODUCT CONCENTRATIONS OF HEALTHY ADULT DOGS FED RAW DIETS

BY

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THESIS

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ABSTRACT

The use of raw meat diets for pets continues to increase in popularity. Owners may choose to feed either homemade or commercially available raw meat-based diets. Raw meat-based diets often are fed because they do not contain preservatives, are highly digestible, and may improve stool quality or skin/coat quality, with many of these claims being substantiated by peer-reviewed research reports. Despite their potential benefits, raw diets also pose many potential risks. Raw meat-based diets have been shown to increase pathogen exposure, contain nutritional imbalances if not carefully formulated and monitored, and may be inconvenient for the pet owner to store or feed. Despite the proposed advantages and risks of feeding raw diets, little research has been performed to test their nutritional adequacy and safety. Due to the growing trend of pet owners choosing to feed raw meat-based diets, research on the nutrient composition and palatability of such diets, and testing their effects on stool characteristics, nutrient digestibility, fecal fermentative end-product concentrations, and fecal microbial populations is needed. The objective of this research was to determine the effects of inulin or yeast cell wall extract (YCW) on macronutrient digestibility, blood cell and metabolite concentrations, and fecal fermentative end-product concentrations in healthy adult dogs fed raw diets. Six adult female beagles (5.5 ± 0.5 yr; 8.5 ± 0.5 kg) were randomly allotted to the following diets using a 3 x 2 factorial in a Latin square design: 1) Beef control; 2) Beef + 1.4% inulin dry matter basis (DMB; Orafti HP, BENEO Group, Tienan, Belgium); 3) Beef + 1.4% YCW (DMB; Bio-Mos, Alltech Biotechnology, Nicholasville, KY); 4) Chicken control; 5) Chicken + 1.4% inulin (DMB); 6) Chicken + 1.4% YCW (DMB). All dogs maintained desirable stool quality characteristics, produced low stool volume, and diets were highly digestible (protein digestibility >88%; fat digestibility >97%). There were minor changes in fermentative end-product concentrations, but fecal short-chain fatty acid concentrations were increased (P<0.05)

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with inulin and YCW inclusion in dogs fed beef-based diets. Fecal spermine concentrations were increased (P<0.05) with inulin and YCW inclusion. In general, blood cell populations and metabolites were within the normal ranges for dogs. To evaluate the standardized amino acid digestibility of the six raw meat-based diets, a cecectomized rooster assay was conducted. Twenty-four, Single Comb White Leghorn cecectomized roosters were used in this study. Each rooster was crop-intubated and given an average of 24 g of each test diet. All excreta were collected and amino acid concentrations measured in each sample. The results of the cecectomized rooster assay indicate that the standardized amino acid digestibility was high for all diets; however, differences in amino acid digestibility existed between protein sources. The beef control diet had the lowest total essential amino acid (TEAA), total non-essential amino acid (TNEAA), and total amino acid (TAA) digestibilities (90.2, 88.7, and 85.9%, respectively) and the chicken + inulin diet had the highest TEAA, TNEAA, and TAA digestibilities (95.6, 95.2, and 92.2%, respectively). Our results agree with those from feline studies demonstrating a high nutrient digestibility of raw diets. Inulin and YCW inclusion in raw meat-based diets had similar effects on large intestinal fermentation as extruded diets containing inulin and YCW. More research is needed to confirm our data and study such diets when fed long term.

DEDICATION

This thesis is dedicated to my parents, Jack and Sandra Beloshapka, and my brother, Nicholas. Thank you for teaching me the value of hard work and always encouraging me to never give up.

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Chapter 1

Introduction

The use of raw meat diets for pets continues to increase in popularity. Owners may choose to feed either homemade or commercially available raw meat-based diets. Raw meat-based diets often are fed because they do not contain preservatives, are highly digestible, and may improve stool quality or skin/coat quality, with many of these claims being substantiated by peer-reviewed research reports (Michel, 2006; Kerr et al., 2010b; Vester et al., 2010a; 2010b). Despite their potential benefits, raw diets also pose many potential risks. Raw meat-based diets have been shown to increase pathogen exposure, contain nutritional imbalances if not carefully formulated and monitored, and may be inconvenient for the pet owner to store or feed (Freeman and Michel, 2001; LeJeune and Hancock, 2001; Weese et al., 2005; Michel, 2006; 2008). Despite the long list of proposed advantages and risks of feeding raw diets, little research has been performed to test their nutritional adequacy and safety. The variability issues associated with animal-based protein sources exist for commercial formulators and pet owners preparing their own diets; however, they may be more apparent to those feeding raw and/or homemade diets. Recent reports in the literature have shown that there are many compositional differences among animal-based protein sources fed to dogs and cats (Murray et al., 1997; Dust et al., 2005; Husak et al, 2008; Faber et al., 2010; Kerr et al., 2010a). Previous nutrition studies testing raw meat-based diets have been conducted in cats, but not dogs.

A stable and balanced gut microbial population is important for gut and overall host health of both humans and pets. This gut microbial environment may be improved through the use of prebiotics or other fermentable fibers. There are three established prebiotics: fructans, galactooligosaccharides (GOS), and lactulose (Mussatto and Mancilha, 2007). Given that inulin

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is a fructan, a group of carbohydrates known to possess prebiotic characteristics, it is expected to beneficially alter the gut microbial populations of the host. Yeast cell wall extracts (YCW) are moderately fermentable substrates containing a mixture of carbohydrates and proteins that have been shown to stimulate immune function and modify gut microbes in healthy adult dogs (Hussein and Healy, 2001; Vickers et al., 2001; Swanson et al., 2002a; 2002b; Middelbos et al., 2007a; 2007b). One objective of this thesis was to evaluate the effects of feeding raw meat-based diets on total tract apparent macronutrient digestibility, fecal fermentative end-product concentrations, and blood metabolite concentrations on healthy, adult dogs fed raw meat-based diets. Another objective of this research was to evaluate the use of inulin and YCW supplementation on these outcomes in dogs fed raw meat-based diets. Another objective was to evaluate the standardized amino acid digestibility by cecectomized roosters dosed with the raw meat-based diets.

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Chapter 2

Literature Review

In 2009, the sales of pet food and pet care supplies continued to increase despite the recession, reaching nearly $27.5 billion in the United States. Pet owners have remained committed to the care of their pets, often considering them as part of the family (Taylor, 2009). It was estimated that in 2010, the total U.S. pet product and service retail sales was $52.69 billion, with $17.77 billion being sales of pet food alone (Packaged Facts, 2010). Because companion animals are considered part of the family, owners are always looking for ways to improve the quality of life for them. One way this can be achieved is through improved nutrition and diet quality, such as using human-quality ingredients (Bond, 2008). Many pet owners are choosing "natural" or "organic" diets for their dogs and cats especially in lieu of the increased number of recalls due to contaminated pet food in the past few years. Others are confused and distrustful of pet foods and the industry (Phillips, 2008), causing them to look for alternatives, such as raw diets that are either homemade or commercially available. In one year (October 2009-October 2010), U.S. sales of frozen/refrigerated dog food increased 13%, reaching nearly $39 million (Packaged Facts, 2011). Due to the ever-growing pet food market, emphasis on nutrition and health, and the use of new diet formats, more research is needed in this area.

Raw Meat Diets for Dogs

The use of raw meat diets for pets continues to increase in popularity. Owners may choose to feed either homemade or commercially available raw meat-based diets. Homemade diets, while giving the owner a great deal of control over the inclusion of ingredients, can have several drawbacks. Many homemade diets are time-consuming to prepare, expensive, and

potentially nutritionally inadequate (Michel, 2006). The use of raw meat diets was originally targeted for sled dogs or racing dogs, such as greyhounds, due to the high energy content that many of these diets possess. However, these are likely perceived benefits, with little support from published scientific data.

Exercising dogs, such as greyhounds and sled dogs, have unique nutritional requirements (Hill, 1998). For such dogs, it is important to build or maintain lean muscle mass and maintain body temperature, especially in harsh environments in which sled dogs reside. Sled dogs also have an increased energy requirement to sustain themselves at such low temperatures. Racing sled dogs require a high protein diet to prevent the development of anemia during training (Hill, 1998). High-fat raw diets are thought to improve racing performance because of the dog's efficient use of fat for energy. In dogs, fat oxidation provides most of the energy at low rates of energy expenditure. During times of high intensity exercise, a change to glucose oxidation occurs (Weibel et al., 1996). For the dog, however, the amount of energy derived from fat oxidation at rest and during exercise is twice that of less aerobic species such as humans (Meyer and Doty, 1988; McLelland et al., 1994). Given the dog's high capacity to burn fatty acids for fuel, many trainers feed their sprint racing dogs or sled dogs high-protein, high-fat diets that consist mainly of raw meat. However, some raw meats may not be of the highest quality, deeming it unfit for human consumption. Feeding low quality protein sources can increase the risk of pathogenic microorganisms to both the dogs and handlers (Chengappa et al., 1993; Stone et al., 1993; Cantor et al., 1997; Joffe and Schlesinger, 2002; Weese et al., 2005; Strohmeyer et al., 2006). Because it is still common for raw meat-based diets to be fed to racing dogs, it is important to research the use of such diets to potentially decrease the exposure to pathogenic microorganisms and to ensure nutrient balance.

As pet owners are becoming more health conscious, they are applying their knowledge of nutrition to what they feed their pets, often opting for "natural", "organic", or "raw" diets. Raw meat-based diets often are fed because they do not contain preservatives, are highly digestible, and may improve stool quality or skin/coat quality, with many of these claims being substantiated by peer-reviewed research reports (Michel, 2006; Kerr et al., 2010b; Vester et al., 2010a; 2010b). Despite their potential benefits, raw diets also pose many potential risks. Raw meat-based diets have been shown to increase pathogen exposure, contain nutritional imbalances if not carefully formulated and monitored, and may be inconvenient for the pet owner to store or feed (Freeman and Michel, 2001; LeJeune and Hancock, 2001; Weese et al., 2005; Michel, 2006; 2008).

Despite the long list of proposed advantages and risks of feeding raw diets, little research has been performed to test their nutritional adequacy and safety. Due to the growing trend of pet owners choosing to feed raw meat-based diets to their pets, research on the nutrient composition and palatability of such diets, and testing their effects on stool characteristics, nutrient digestibility, fecal fermentative end-product concentrations, and fecal microbial populations in dogs is needed.

Pet Food Ingredient Variability

There is a wide array of animal-based protein sources available for use in commercial pet foods. For example, in AAFCO, the list of poultry-based products alone includes: poultry by-product meal (PBPM), poultry hatchery by-product, poultry by-products, hydrolyzed poultry feathers, poultry, hydrolyzed whole poultry, hydrolyzed poultry by-products aggregate, egg shell meal, poultry meal, and egg product (AAFCO, 2010). These poultry-based products, along with other animal-based protein sources, are available to pet food formulators interested in

formulating extruded, canned, or raw meat-based diets. The variability issues associated with animal-based protein sources exist for commercial formulators and pet owners preparing their own diets; however, they may be more apparent to those feeding raw and/or homemade diets. This may be due to differences in diet preparation of raw and (or) homemade diets. Additionally, many raw or homemade diets are prepared in small batches and/or are not a homogenous product, allowing pets to sort out preferred ingredients and refusing others. The major issues with animal-based protein sources include the variability of species of animal used, the "parts" of the animal used, and the processing method of the diets.

The term "quality" often is used when referring to protein sources. Quality of such ingredients can be affected by the amino acid profile and (or) processing. "High quality" or "good-quality" ingredients have been defined as those having a relatively low fat content, that avoid the use of additives, such as salt or antioxidants (commonly used for preservation), and that are of human-grade (ingredients that could be used for human consumption) (Faber et al., 2010). Specifically, protein quality may be defined as the ability of a protein source to meet the nitrogen and amino acid requirements of an animal. Protein quality may be assessed in many ways, including the amino acid profile (standardized amino acid digestibility) by using the cecectomized rooster assay, by using a protein solubility in potassium hydroxide assay (Araba and Dale, 1990), the immobilized digestive enzyme assay (IDEA; Schasteen et al., 2002), and protein efficiency ratio (PER) assay (Johnson and Coon, 1979). Egg often is used as the standard comparison among protein sources for the protein solubility assay because it is very close to being an ideal protein, having a 95% biological value (Food and Agriculture Organization, 1970). The IDEA is used to predict crude protein (CP) and amino acid digestibility, most often

lysine digestibility. Casein often is used as the standard reference protein for the PER assay, with a PER value of 2.5 (Munro and Allison, 1969).

Recent reports in the literature have shown that there are many compositional differences among animal-based protein sources fed to dogs and cats (Murray et al., 1997; Dust et al., 2005; Husak et al, 2008; Faber et al., 2010; Kerr et al., 2010a). Variability in diet composition is dependent on the source and quality of the animal ingredients (e.g., skeletal muscle, organ meats, offal, etc) and differences among and within species of animal (e.g., beef, pork, poultry, fish) used. Murray et al. (1997) measured the chemical composition and nutrient digestibilities (ileal and total tract) of various animal products used in dog food, including rendered beef meat and bone meal (RMBM), PBPM, fresh beef (FB), and fresh poultry, which included fresh poultry necks and backs (FPNB) and fresh poultry viscera (FPV). These ingredients were incorporated into diets ranging from 6.5% to 12.4% of the diet, depending on the protein source. Whole egg (WE) was used in the animal protein-based control diet and defatted soy flour (DS) was used in the plant protein-based control diet. Five mature, ileal-cannulated hounds (25 ± 5 kg) were used to study these six diets using a 5 x 6 Youden square (incomplete Latin square) design. The researchers found that CP concentration ranged from 30.4 to 67.6% and the fat concentration ranged from 11.6 to 50.7% among the protein sources studied. Overall, the researchers reported that these diets were highly digestible (total tract digestion: CP digestibility = 88.2% to 89.9%; fat digestibility = 92.9% to 93.7%). However, the diet containing the RMBM tended to have lower total tract CP digestibility (88.2%) than the WE control diet (91.2%). The researchers attributed the lower digestibility to the increased collagen in the RMBM diet, which was also noted by Eastoe and Long (1960).

Chicken-based protein sources may be highly variable due to the use of different body parts and their nutritive value. Dust et al. (2005) measured the chemical composition and protein quality of several alternative protein sources including: spray-dried cooked chicken (from deboned USDA-inspected chicken parts; spray dried to form a powder); spray-dried cooked chicken liver (produced from USDA-inspected facilities using chicken livers that were ground, cooked, and spray-dried); spray-dried egg (processed from pasteurized whole egg solids and then spray dried to form a granulated powder); chicken-by-product meal (comprised of ground, cleaned, rendered carcass of chicken with trace amounts of feathers and blood); PBPM (comprised of ground, cleaned, rendered carcass of poultry including heads, feet, and viscera, to include trace amounts of feathers and blood); processed red blood cells; spray-dried plasma; spray-dried whole beef blood; enzyme-hydrolyzed fish protein concentrate; soybean meal; and spray-dried pork liver, all of which may be included in pet food. Protein quality was assessed by using a protein solubility in potassium hydroxide assay (Araba and Dale, 1990), the IDEA assay (Schasteen et al., 2002), and the PER assay (Johnson and Coon, 1979). They reported that CP concentration among the chicken-based protein sources was highly variable, ranging from 49.2 to 69.0% for spray-dried cooked chicken and spray-dried cooked chicken liver, respectively. The fat concentration of the chicken protein sources also was highly variable, ranging from 18.3 to 49.5% for chicken by-product meal and spray-dried cooked chicken, respectively. Additionally, Dust et al. (2005) reported that the chicken by-product meal and PBPM used in their study were highest in glycine, proline, and hydroxyproline contents, which may be indicative of the increased amount of connective tissue in these by-products compared with other chicken-based protein sources. The protein solubility index value was lowest for processed red blood cells (23.9%) and highest for spray-dried plasma (92.9%). The IDEA values also varied

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among protein sources, being lowest for PBPM (0.43) and highest for soybean meal (0.79), with the largest variation occurring for the chicken protein sources. Chicken by-product meal had a higher PER value (3.42) than PBPM (2.73), which was likely due to the chicken by-product meal being ground, clean, rendered carcass of the chicken with only trace amounts of feathers and blood, whereas PBPM also can include heads, feet, and viscera.

Variability may also exist within a protein source. For example, the inclusion of whole chicken or particular body parts in the formulation provides a great deal of variability as it pertains to CP and fat contents. Husak et al. (2008) evaluated the composition of raw versus cooked organic, free-range, and conventional poultry parts, including breast meat, thigh meat, and skin. Eight whole broilers were used for this portion of their study. The researchers reported that raw breast meat from organic and free-range broilers was significantly higher in CP (23.31 and 23.26%, respectively) than raw breast meat from conventional broilers (22.26%). Raw thigh meat from organic and free-range broilers also had higher CP (19.25 and 19.49%, respectively) than raw thigh meat from conventional broilers (17.82%). Cooked breast meat from conventional broilers had higher fat content (4.78%) than that from organic and free-range broilers (3.31 and 3.95%, respectively). Cooked conventional breast meat had lower CP (25.37%) than cooked organic breast meat (26.95%). While the differences reported here are not as variable as were the data of Dust et al. (2005), management of the animal prior to processing also may affect the nutrient composition.

Processing of animal ingredients can affect protein quality and amino acid digestibility of protein sources. Pérez-Calvo et al. (2010) evaluated the effect of rendering on protein value and fat quality of 12 batches of raw animal by-products and the corresponding animal by-product meals from two rendering plants. They used the following rendering process: raw material was

minced and then passed to the cooker where the material was heated (average temperature for plant 1 = 150ºC; average temperature for plant 2 = 141.8ºC) and the liquid fat was removed and then centrifuged; the remaining material was dried in a cooker. They reported that the rendering process decreased the fat content of all samples [40.8 to 13.6% DM (plant 2); 49.9 to 30.9% DM (plant 1)]. As a result, both ash and protein concentrations increased [ash = 13.09 to 21.93% DM (plant 1) and 21.78 to 32.42% DM (plant 2); CP = 35.88 to 46.26% (plant 1) and 36.56 to 53.14% DM (plant 2). Additionally, rendering negatively affected the total and essential amino acid content [total = 91.80 to 87.76% CP (plant 1) and 91.46 to 86.85% CP (plant 2); essential = 38.77 to 35.80% CP (plant 1) and 36.57 to 33.38% CP (plant 2)]. Of the essential amino acids, lysine was the most affected by the rendering process, which was decreased by 18% (plant 1) and 15% (plant 2). Rendering also caused a decrease in *in vitro* protein digestibility (from 94.6 to 78.5%) in plant 1. The rendering process resulted in an increase in the saturated to unsaturated fatty acid ratio [from 0.76 ± 0.014 to 0.88 ± 0.006 (plant 1) and from 0.71 ± 0.016 to 0.88 ± 0.037 (plant 2)].

Shirley and Parsons (2000) evaluated the effects of various processing pressures and times of processing on the digestibility of amino acids in meat and bone meal. The following processing treatments were used: (1) 0 psi (94°C) for 20 min; (2) 15 psi (121°C; 103 kPa) for 20 min; (3) 15 psi (121°C; 103 kPa) for 30 min; (4) 30 psi (133°C; 207 kPa) for 20 min; (5) 30 psi (133°C; 207 kPa) for 30 min; (6) 45 psi (147°C; 310 kPa) for 20 min; and (7) 60 psi (144°C; 413 kPa) for 20 min. They concluded that pressure processing of meat and bone meal will likely decrease its protein quality. When meat and bone meal samples were processed at 60 psi for 20 min, total concentrations of most amino acids were reduced. This was especially apparent with cysteine concentration, which decreased from 0.51 to 0.26% as pressure increased from 0 to 60

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psi. Processing meat and bone meal at 15 psi for 20 min resulted in decreased digestibility of most amino acids when compared to the control meat and bone meal.

Due to the increased humanization of pets, more pet owners are searching for "high quality" ingredients to feed their pets. Many owners attribute human-grade ingredients to be of higher quality. Interestingly, AAFCO has no definition of "human-grade", a term that is not allowed by FDA on pet food labels. Despite the lack of legal terminology in this regard, many owners desire to feed cuts of meat that are commonly consumed by humans. Few studies performed in pets, however, have evaluated such protein sources. Faber et al. (2010) evaluated the chemical composition and ileal and total tract apparent protein digestibility of mildly processed, high-quality protein sources in dogs, including beef loin, pork loin, chicken breast, pollock fillet, and salmon fillet, for their application to the pet food industry. They reported that CP concentrations ranged from 82.7% (beef loin) to 96.9% (pollock fillet) and fat concentrations ranged from 4.5% (pollock fillet) to 16.4% (beef loin). Ileal CP digestibility ranged from 89.7% (beef loin) to 90.5% (pork loin and pollock fillet). Total tract CP digestibility ranged from 94.4% (beef loin) to 94.8% (pollock fillet). Protein digestibility also was assessed by using IDEA and cecectomized rooster assays. The IDEA value was greatest for pollock fillet (0.71) and lowest for chicken breast (0.52). Similarly, all individual amino acid digestibility values from roosters were greatest for pollock fillet and lowest for chicken breast (Faber et al., 2010).

Even though many dog owners are following the newest trend of feeding raw meat-based diets, most of the research in this area has been done in cats. For example, Kerr et al. (2009; 2010a) evaluated the chemical composition, nutrient digestibility, and nitrogen (N) balance of four raw meat diets fed to domestic cats. The four diets were based on beef trimmings, bison trimmings, elk muscle meat, or horse trimmings, and included a vitamin and mineral premix and

Solka floc as a dietary fiber source. They reported that the CP and fat concentrations were highly variable, with CP ranging from 48.7% (bison) to 78.8% (elk), and fat ranging from 5.4% (elk) to 38.0% (bison). Researchers also reported that all diets were highly digestible (DM digestibility = 84.1% to 88.1%; CP digestibility = 96.6% to 97.3%) and that cats maintained BW and N balance on all treatments.

As it pertains to micronutrients, one of the most common inadequacies in raw diets is calcium (Ca), which may lead to an unbalanced Ca to phosphorus (P) ratio. For adult dogs, it is recommended that diets contain Ca concentrations between 0.6 and 2.5%, and P concentrations between 0.5 and 1.6%, with a Ca:P ratio of 1:1 to 2:1 (AAFCO, 2010). Most skeletal muscle meats, however, contain 20-30 times higher P than Ca concentrations. Therefore, feeding diets based on muscle meats may lead to serious macromineral imbalances. Some implications of an unbalanced Ca:P ratio include bone disease, decreased bone density, hyperparathyroidism, poor bone mineralization, and increased risk of fractures (Morris et al., 1971; DeLay and Laing, 2002; Taylor et al., 2009). By purchasing commercially prepared raw diets, many of these nutrient inadequacies may be eliminated with careful formulation by the manufacturer. If done properly, these diets are complete and balanced without the need for supplementation (Freeman and Michel, 2001). If a pet owner decides to feed their dog a homemade diet, it may be more difficult to balance these micronutrients with the use of natural nutrient sources, (e.g., whole chicken carcass versus balanced vitamin/mineral premixes). Additionally, there are risks associated with feeding bones, including obstruction or perforation of the gastrointestinal tract and potential transmission of bacteria or other infectious organisms (Laflamme et al., 2008).

Dietary fiber concentrations often are quite low in raw meat-based diets (Kerr et al., 2010a; Vester et al., 2010a). Although large amounts are not necessary, fiber inclusion in such

diets is important to minimize constipation. Fiber sources and concentrations must be closely monitored. Higher concentrations of fiber in a diet may decrease the digestibility of other nutrients due to increased rate of passage through the colon or by physical hindrance. Additionally, fiber may appear artificially high in diets due to the presence of collagen. In raw meat-based diets, collagen analyzes as dietary fiber in the diet, but in the body, much of it is either broken down by host digestion or fermented in the large bowel (fermented as protein).

Nutrient Digestibility of Raw Meat-Based Diets

Recent studies performed in our laboratory have examined the nutrient digestibility and fecal characteristics resulting from feeding raw diets composed of novel protein sources in both domestic and exotic felids. In these studies, large variations in fecal quality, nutrient digestibilities, fecal fermentative end-product concentrations, and fecal microbial populations were observed (Kerr et al., 2009; Vester et al., 2010a). In general, raw meat diets may be expected to have a DM digestibility greater than 85% and OM and fat digestibilities greater than 95%. Of the nutrients, CP digestibility is commonly affected the greatest due to the variability and sources of protein used in raw meat-based diets. Total tract apparent CP digestibility can be affected and misleading due to microbial metabolism of CP in the hindgut. Crude protein digestibility was different between diets fed to exotic felids, with a horse-based diet being more digestible (94.7%) than a beef-based diet (92.2%) (Vester et al., 2010a). Vester et al. (2010a) discussed the difficulty of distinguishing whether digestibility differences were due to actual differences in CP digestibility among protein sources or due to variation in the fiber sources included in the diets. In that study, fiber sources differed between the two diets; the horse meat-based diet contained a nonfermentable fiber source (cellulose) and the beef-based diet contained a moderately fermentable fiber source (beet pulp). They concluded that composition and

fermentability of dietary fiber sources may have affected microbial metabolism and fecal protein

concentration and, thus, total tract CP digestibility, further stressing the need for controlled fiber

sources and concentrations when including them in raw meat-based diets.

Bacteria Risk

As it pertains to the bacteria risk of feeding raw diets, researchers and consumers are

primarily interested in two main issues: (1) risk of pathogen exposure directly from the food and

(2) effects of macronutrient composition on gut microbial populations. To reduce health risks to

both pet owners and their companion animals fed raw diets, the Food and Drug Administration's

(FDA) Center for Veterinary Medicine (CVM) has recommended specific guidelines to be

followed by the manufacturer and consumer (CVM, 2004). Guidelines for manufacturing

include the following: (1) all meat- and poultry-derived ingredients should be USDA/Food

Safety and Inspection Service (FSIS)-inspected and passed for human consumption; (2) it is

recommended that bones and other hard materials be ground; (3) all other ingredients should be

of an appropriate grade that qualified experts would agree are safe for use in raw food for

animals; (4) manufacturing facilities should take all precautionary measures to prevent

adulteration by irradiating the final packaged product, participating in the USDA voluntary

inspection program for Certified Products for Dogs, Cats, and Other Carnivora, following other

Good Manufacturing Practices, such as those for human foods, or implementing a Hazard

Analysis and Critical Control Point (HACCP) plan; and (5) product should be transported and

stored in a manner to avoid microbial contamination and growth (i.e., frozen at all times prior to

use, unless freeze-dried). They also recommend the use of clear storage and handling

instructions on the labels, including a recommendation to keep the product frozen until ready to

use, to thaw the product in a refrigerator or microwave, to keep the product separate from other

foods, washing working surfaces, utensils, and hands with hot soapy water, and to refrigerate leftovers immediately or discard them.

Because dogs and cats are a large part of pet owners' lives, they often live freely in households. Because of this close interaction between humans and pets, it is imperative to understand the risks of bacterial contamination that pets may bring into homes. Humans may become contaminated through fecal shedding or even oral bacteria. Bacterial contamination and fecal shedding are big concerns when feeding a raw meat diet to dogs, especially for owners with young children, the aged, or other people that may have compromised immune systems (Ngaage et al., 1999; Sato et al., 2000; Joffe and Schelsinger, 2002; Behravesh et al., 2010). Joffe and Schlesinger (2002) evaluated fecal *Salmonella* spp. in twenty client-owned dogs, ten of which were fed a homemade bones and raw food (BARF) diet and the other ten were fed various commercial dry dog foods as controls. The owners were instructed to collect one meal-sized sample of food and one fresh stool sample from each test subject for evaluation. Researchers reported that all food and stool samples from the ten controls were negative for *Salmonella* spp., but 80% of the BARF-diet samples and 30% of the stool cultures from dogs fed a BARF diet were positive for *Salmonella* spp. The results of this small study suggest that some dogs fed a BARF diet shed *Salmonella* spp. in their stools. Pet owners are also at risk of becoming infected with *Salmonella* spp. if they handle contaminated meat products intended for dogs, such as bones and pig ear dog treats (Clark et al., 2001; Finley et al., 2006; 2008).

Feeding raw meat or high-protein diets also may provide a higher risk of bacterial contamination by promoting the growth of potential pathogenic species in the colon. Vester et al. (2009) evaluated differences in fecal microbial populations of kittens fed moderate- or high-protein diets. Researchers concluded that *Bifidobacterium* spp., *Lactobacillus* spp., and

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Escherchia coli concentrations were greater in kittens fed the moderate-protein versus the high-protein diet. Additionally, denaturing gradient gel electrophoresis (DGGE) identified *Clostridium difficile* as a distinguishing microbe of the high-protein treatment, having a much higher prevalence in that group. Zentek et al. (2003) compared two extruded dog diets with or without added non-digestible oligosaccharides (NDOs) from chicory with a high-protein diet, which was rich in protein from low quality animal protein sources. They concluded that when dogs were initially switched from the extruded diets to the high-protein diet, there was a significant increase in fecal *Clostridium perfringens* concentrations.

Other Potential Health Effects

While nutrient balance and bacterial load are the primary concerns with raw diets, long-term testing is needed to verify their safety. Alanine aminotransferase (ALT) is a cytosolic enzyme present in the liver. Elevation of serum ALT can be indicative of liver dysfunction or toxic insult (Duncan et al., 1994; Merck, 2005). Alanine aminotransferase levels were elevated when African wildcats consumed a commercial raw meat diet vs. a kibble diet (Vester et al., 2010b). Although all animals remained healthy on that experiment, researchers concluded that ALT levels should be closely monitored when feeding raw meat diets to companion animals.

Prebiotic Supplementation

A stable and balanced gut microbial population is important for gut and overall health of both humans and pets. One's diet can promote beneficial bacteria and fermentation profiles to help improve or maintain a healthy gut. Short-chain fatty acid (SCFA) production is important as an energy source of colonocytes (Roediger, 1980) and pH control, and can be increased by the inclusion of fermentable fiber(s) in the diet. Therefore, an increase in SCFA is expected to be an

indication of a healthy intestinal environment (Vickers et al., 2001). Some fermentable fibers

not only increase SCFA production, but positively manipulate microbial populations. The term

'prebiotic' has been used for such ingredients since the mid 1990's.

A prebiotic must (1) be resistant to gastric activity, enzymatic hydrolysis, and

gastrointestinal absorption (non-digestible); (2) be fermented by cecal or colonic microflora; and

(3) selectively stimulate the growth and/or activity of those bacteria that contribute to colonic

and host health (Gibson and Roberfroid, 1995; Gibson et al., 2004; Roberfroid, 2007). A

prebiotic is an efficient way to significantly improve populations of gut microbiota, which has

been demonstrated by several nondigestible carbohydrates in both *in vitro* and *in vivo* models

(Gibson and Roberfroid, 1995). There are three established prebiotics: fructans,

galactooligosaccharides (GOS), and lactulose (Mussatto and Mancilha, 2007). A prebiotic may

affect the host either through microbes directly or their by-products. It selectively feeds one or a

limited number of microorganisms, causing a selective modification of the host's intestinal

microflora (Wang, 2008).

Main Prebiotics Fed to Dogs

The most common prebiotics studied have been fructans, which are now used in human

and companion animal nutrition products. Fructans are a class of fermentable carbohydrates that

are nondigestible by small intestinal enzymes (Hidaka et al., 1986; Roberfroid et al., 1993).

Because they are not digested by mammalian species, fructans pass undigested through the small

intestine and reach the large intestine. Fructooligosaccharides (FOS) are composed of sucrose

oligomers with additional fructose units. Fructooligosaccharides, including inulin, oligofructose

(OF), and short-chain fructooligosaccharides (scFOS), are examples of dietary constituents that

beneficially alter microbial populations in the gut and help prevent the invasion of pathogenic

bacteria. They have many functional and nutritional properties that may be useful in companion animal nutrition. Once reaching the large intestine, FOS serve as a substrate for some bacteria, but not all, promoting select bacteria to proliferate at the expense of others (Willard et al., 2000). Fructooligosaccharides are highly fermentable, decreasing fecal pH (Flickinger et al., 2003a; Propst et al., 2003) and increasing *Bifidobacterium* spp. and *Lactobacilli* spp. in dogs (Swanson et al., 2002a).

Inulin is a long-chain fructan derived from chicory root extract. During this process, chicory root (*Cichorium intybus)* undergoes direct hot water extraction, resulting in the collection of inulin OF (De Bruyn et al., 1992). The inulin extracted from chicory roots contains both FOS and other polysaccharides (Crittenden and Playne, 1996). Chicory inulin contains both $G_{py}F_n$ (α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-D-fructofuranoside) and $F_{py}F_n$ (β-D-fructopyranosyl-[α-D-fructofuranosyl]$_{n-1}$-D-fructofuranoside) compounds, with the number of fructose units varying from 2 to 70 (Roberfroid and Delzenne, 1998). Inulin is slowly fermented in the large intestine, beneficially altering the gut microflora.

Oligofructose is a medium-chain fructan, with a degree of polymerization of 3 to 10. Oligofructose may be a partial enzymatic hydrolysate of inulin or be synthesized and contain β-(2,1) fructose chains with terminal glucose units (Flickinger et al., 2003b). It is these β-(2,1) bonds that prevent inulin or OF from being hydrolytically digested in the upper intestinal tract of monogastric animals and, thus, allows them to be fermented in the large intestine for increased SCFA production by large intestinal bacteria (Fishbein et al, 1988; Flickinger et al., 2003b). Because OF typically has a degree of polymerization of less than 10, they are highly soluble in water and are rapidly fermented (Van Loo, 2007).

Short-chain fructooligosaccharides are primarily composed of short chains (~3-6 units) of fructose units bound by β-(2-1) linkages that are attached to a terminal glucose unit. While scFOS are naturally occurring in a variety of plants, such as onions, Jerusalem artichokes, asparagus, wheat, rye, and garlic (Clevenger et al., 1988), they can also be synthesized from sucrose. One method produces scFOS by the action of a fungal (*Aspergillus niger*) β-fructofuranosidase on sucrose, resulting in a mixture of: (1) fructose oligomers composed of 1-kestose, nystose, and 1-F-fructofuranosyl nystose; (2) sucrose; (3) glucose; and (4) fructose (Spiegel et al. 1994; Roberfroid and Slavin, 2000). This product is no different from the molecules found naturally in plants (Roberfroid and Slavin, 2000). Short-chain fructooligosaccharides are more rapidly fermented in the large bowel than inulin and OF. *In vitro* studies have shown that scFOS rapidly increased SCFA production and decreased pH as a result of fermentation (Sunvold et al., 1995; Flickinger et al., 2000; Smiricky-Tjardes et al., 2003).

Prebiotic Effects on Gut Microbial Populations

Given that inulin is a fructan, a group of carbohydrates known to possess prebiotic characteristics, it is expected to beneficially alter the gut microbial populations of the host. Inulin supplementation increases potential beneficial bacteria, such as *Bifidobacterium* spp. and *Lactobacillus* spp., and decreases potential pathogenic bacteria, such as *C. perfringens* and *E. coli*. Zentek et al. (2003) evaluated the effects of two high-protein extruded diets, with or without 3% dried whole chicory root (inulin content of 55%), on gut microbial populations of healthy adult beagles. Those researchers concluded that fecal concentrations of bifidobacteria were substantially increased with the inclusion of chicory root (log 9.7 colony forming units (cfu)/ g feces) when compared to the glucose control (log 9.4 cfu/g feces). Swanson et al.

(2002a) performed two experiments to test the effects of feeding scFOS (4 g/d) and

Lactobacillus acidophilus (1 x 10^9 cfu) separately or in combination on the fecal microbial

populations of healthy adult dogs. They concluded that after scFOS supplementation, dogs in

Experiment 1 tended to have lower *C. perfringens* concentrations (9.62 cfu $\log_{10}$/g fecal DM)

than dogs consuming the control diet containing sucrose (9.90 cfu $\log_{10}$/g fecal DM). In their

second experiment, Swanson et al. (2002a) concluded that dogs fed scFOS had greater total

aerobe and bifidobacteria concentrations (9.94 cfu $\log_{10}$/g fecal DM and 9.93 cfu $\log_{10}$/g fecal

DM, respectively) than dogs consuming the sucrose control (9.31 cfu $\log_{10}$/g fecal DM and 9.35

cfu $\log_{10}$/g fecal DM, respectively). Also in that experiment, dogs fed scFOS tended to have

greater fecal lactobacilli concentrations (9.79 cfu $\log_{10}$/g fecal DM) than the control fed dogs

(9.13 cfu $\log_{10}$/g fecal DM). Other studies have tested fructans in dogs and have reported similar

effects on gut microbes (Rao, 1999; Flickinger et al., 2003a).

<u>Prebiotic Effects on Fecal Fermentative End-Product Concentrations</u>

Fecal fermentative end-product concentrations are indicative of protein and carbohydrate

fermentation occurring in the large bowel. Carbohydrate fermentation primarily produces SCFA

in the large intestine and serves as an important energy source for colonocytes. In contrast,

increased phenol, indole, and BCFA production are an indication of protein fermentation

occurring in the large intestine. With the inclusion of dietary components such as inulin, SCFA

concentrations would be expected to increase. Vickers et al. (2001), who used dog fecal

inoculum in an *in vitro* fermentation procedure, tested four inulin products, FOS, a source of

mannanoligosaccharides (MOS; derived from YCW), soy fiber, beet pulp, and wood cellulose.

Of the four inulin products, two were commercially purified products of chicory root, one of

which was further processed to optimize solubility, and the two additional inulin extracts were

mixtures of oligo- and polysaccharides comprising fructose joined together by β (2-1) linkages. Inulin 1 and 2 had a DP of 9, inulin 3 had a DP > 12, and inulin 4 had a DP between 2 and 8. The researchers concluded that total mean production of SCFA [pooled for all durations (6, 12, and 24 h) of fermentation] was highest for fermentation of the 4 inulin products and FOS (3.1-3.6 mmol/g of OM) vs. cellulose (0.05 mmol/g OM) or beet pulp (1.47 mmol/g OM) (Vickers et al., 2001). Additionally, fermentation of the four inulin products and FOS produced higher mean acetate concentrations (1.9 to 2.4 mmol/g of OM) versus cellulose or beet pulp (mean acetate production of 0.02 and 1.08 mmol/g of OM, respectively).

Flickinger et al. (2003a), who tested the effects of OF (hydrolyzed inulin) supplementation in extruded diets fed to healthy adult beagles (Experiment 1), concluded that propionate concentrations were increased in feces of supplemented dogs. In Experiment 2, the same researchers tested three concentrations (1, 2, and 3 g/d) of scFOS supplementation administered orally to ileal cannulated, adult hound dogs. These researchers concluded that fecal total SCFA concentrations tended to be greater as dietary scFOS increased in those dogs, but researchers did not observe any changes in fecal pH with scFOS supplementation. Swanson et al. (2002a), who tested the effects of feeding scFOS and *Lactobacillus acidophilus* separately or in combination to healthy adult dogs, concluded that after scFOS supplementation, dogs had greater fecal concentrations of lactate (41.7 µmol/g DM) and tended to have greater fecal concentrations of butyrate (58.2 µmol/g DM) than those dogs fed the sucrose placebo (lactate = 2.7 µmol/g DM; butyrate = 40.8 µmol/g DM). Propst et al. (2003),who investigated the effects of varying concentrations of OF and inulin fed to healthy adult dogs, reported that dogs fed inulin tended to have a linear increase (P<0.10) in fecal SCFA concentrations.

Swanson et al. (2002a) also concluded that dogs fed scFOS had lower fecal total phenol concentrations (1.22 µmol/g DM feces) than the control dogs (1.69 µmol/g DM feces), who were fed a sucrose placebo (Experiment 1). In Experiment 2, dogs fed scFOS also tended to have lower fecal indole concentrations (0.67 µmol/g DM feces) than the control dogs (1.11 µmol/g DM feces). Swanson et al. (2002b) tested the effects of feeding 1 g of scFOS, 1 g of MOS, or 1 g of scFOS + MOS to adult dogs. They concluded that fecal indole concentrations tended to decrease in dogs supplemented with scFOS, decreasing from 2.44 µmol/g fecal DM in the controls to 1.23 µmol/g fecal DM in the scFOS-fed dogs. Fecal total phenol and indole concentrations also were decreased in dogs fed scFOS supplementation in that study (3.03 µmol/g fecal DM in the control versus 1.50 µmol/g fecal DM in scFOS). Propst et al. (2003) evaluated the effects of three concentrations (0.3, 0.6, and 0.9% of the diet, as-fed basis) of OF and inulin on fecal protein catabolites in healthy adult dogs. They concluded that when dogs were fed OF, total branched-chain fatty acid (BCFA) concentrations were highest for the 0.3% treatment (52.5 µmol/g DM feces) and lowest for the 0.9% treatment (43.9 µmol/g DM feces), resulting in a quadratic trend. They also concluded that dogs supplemented with inulin tended to have lower fecal phenol concentrations [1.03 µmol/g DM feces (0.3%), 1.28 µmol/g DM feces (0.6%), and 1.29 µmol/g DM feces (0.9%)] vs. the control dogs (2.11 µmol/g DM feces). Dogs supplemented with 0.6 and 0.9% OF also tended to have lower total fecal phenol concentrations (2.20 µmol/g DM feces and 2.03 µmol/g DM feces, respectively) vs. the control dogs (3.03 µmol/g DM feces). Overall, these studies exhibit the general beneficial effects of prebiotic supplementation, including increased fecal SCFA concentrations, decreased fecal phenols and indoles, and decreased fecal pH.

Yeast Cell Wall Extract Supplementation

Yeast cell wall extracts (YCW) are moderately fermentable substrates containing a mixture of carbohydrates and proteins that have been shown to stimulate immune function and modify gut microbes *in vitro* or in healthy adult dogs (Hussein and Healy, 2001; Vickers et al., 2001; Swanson et al., 2002b; 2002c; Middelbos et al., 2007a; 2007b). Yeast cell wall fragments are derived from *Saccharomyces cerevisiae* var *boulardii* (Vickers et al., 2001). The fragments are obtained by centrifugation from a lysed yeast culture. The pellet containing the yeast cell wall fragments then is washed and spray dried. The YCW of *Saccharomyces cerevisiae* contains about 85-90% polysaccharide and 10-15% protein. The polysaccharide component is made up of a mixture of water-soluble mannans, alkali-soluble glucans, alkali-insoluble glucans, and small amounts of chitin (Nguyen et al., 1998). More specifically, YCW contains two alkali-insoluble glucans: predominantly (1-3)-β-D-linked glucan (Manners et al., 1973a) and highly branched (1-6)-β-D-linked glucan (Manners et al., 1973b). Most of the protein found in YCW is covalently linked to mannan (mannoprotein) (Nguyen et al., 1998). Because YCW is rich in mannans, it is believed to prevent adherence of bacteria expressing type-1 fimbriae to the intestinal wall (Ofek et al., 1977; Neeser et al., 1986). The potential effect on the intestinal immune system may make YCW preparations functional dietary ingredients in pet foods by improving intestinal health and resistance against intestinal upset (Middelbos et al., 2007b).

Yeast Cell Wall Extract Effects on Gut Microbial Populations

Yeast cell wall extracts are fermentable by canine intestinal microbes, leading to an increase in beneficial fecal bacteria concentrations, namely *Lactobacilli* spp. and *Bifidobacterium* spp. (Swanson et al. 2002c; Grieshop et al., 2004). Swanson et al. (2002c) evaluated the effects of 2 g of scFOS in combination with 1 g of MOS (scFOS + MOS) on the

immune function and microbial populations of adult ileal cannulated hound dogs. They concluded that scFOS + MOS supplementation increased ileal *Lactobacillus* spp. from 7.55 cfu $\log_{10}$/g ileal DM (control) to 8.66 cfu $\log_{10}$/g ileal DM (scFOS + MOS) and fecal *Lactobacillus* spp. from 8.24 cfu $\log_{10}$/g fecal DM (control) to 9.75 cfu $\log_{10}$/g fecal DM (scFOS + MOS). Fecal *Bifidobacterium* spp. concentrations also were greater in scFOS + MOS supplemented dogs (10.04 cfu $\log_{10}$/g fecal DM) vs. control dogs (9.42 cfu $\log_{10}$/g fecal DM). It is unclear whether these changes were due to scFOS, MOS, or the combination of the two substrates.

Grieshop et al. (2004) studied the effects of oligosaccharide treatments incorporated into extruded diets on gut microbial populations of healthy adult dogs. Oligosaccharide treatments included: no supplementation (control); 1% dietary chicory; 1% dietary MOS; or 1% chicory + 1% MOS. These researchers reported that greater concentrations of fecal bifidobacteria were measured in dogs fed the chicory-supplemented diet (10.5 cfu $\log_{10}$/g fecal DM) or MOS-supplemented diet (10.6 cfu $\log_{10}$/g fecal DM) vs. the control fed dogs (10.1 cfu $\log_{10}$/g fecal DM). Strickling et al., (1999) evaluated the effects of oligosaccharide addition to extruded diets on nutrient digestibility and fecal microbial populations of adult ileal cannulated dogs. The treatments were as follows: control; FOS (prepared from chicory root); MOS (derived from YCW); and xylooligosaccharide (XOS; composed of xylobiose and xylotriose). Those researchers reported that when supplemented with MOS, decreased fecal *C. perfringens* concentrations (4.48 cfu $\log_{10}$/g fecal DM) were observed in the dogs as compared to when fed the control (4.73 cfu $\log_{10}$/g fecal DM), FOS (4.74 cfu $\log_{10}$/g fecal DM), or XOS (5.16 cfu $\log_{10}$/g fecal DM) diets.

Middelbos et al. (2007b) evaluated the effects of YCW supplementation (0%, 0.05%, 0.25%, 0.45%, or 0.65% of diet) on nutrient digestibility, immune indices, and microbial

populations in adult ileal cannulated dogs. These researchers concluded that YCW

supplementation tended to increase fecal lactobacilli concentrations cubically, with the highest

counts observed with 0.05% and 0.45% YCW in the diet (11.6 cfu $\log_{10}$/g fecal DM and 11.8 cfu

$\log_{10}$/g fecal DM, respectively) vs. 0% of the diet (11.3 cfu $\log_{10}$/g fecal DM). Fecal

bifidobacteria concentrations were not statistically significant, but numerically increased at

0.45% of the diet (9.1 cfu $\log_{10}$/g fecal DM) versus 0% of the diet (8.7 cfu $\log_{10}$/g fecal DM).

Yeast Cell Wall Extract Effects on Fecal Fermentative End-Product Concentrations

Yeast cell wall extract may decrease the production of putrefactive compounds, such as

phenols and indoles, when fed in combination with fructans (Swanson et al., 2002b); however,

these effects of YCW have only been shown in extruded kibble diets. Swanson et al. (2002b)

measured the effects of supplemental scFOS and MOS on protein catabolites in the large bowel

of adult ileal cannulated dogs. The treatments included: control; 1 g scFOS; 1 g MOS; and 1 g

scFOS + 1 g MOS. These researchers reported that fecal indole concentrations decreased with

scFOS supplementation (1.23 μmol/g fecal DM) and scFOS + MOS supplementation (1.27

μmol/g fecal DM) as compared to the control dogs (2.44 μmol/g fecal DM). Total indole and

phenol concentration decreased with scFOS supplementation (1.50 μmol/g fecal DM) and scFOS

+ MOS supplementation (1.54 μmol/g fecal DM) as compared to the control dogs (3.03 μmol/g

fecal DM). Middelbos et al. (2007a) evaluated the use of fermentable oligosaccharides in

extruded diets fed to adult ileal cannulated hound dogs. The six treatments included: (1) control

(1.5% TDF); (2) control + 2.5% cellulose (poorly fermentable fiber); (3) control + 2.5% beet

pulp (moderately fermentable fiber); (4) control + 1.0% cellulose + 1.5% scFOS (CF); (5)

control + 1.0% cellulose + 1.2% scFOS + 0.3% YCW (CFY1); and (6) control + 1.0% cellulose

+ 0.9% scFOS + 0.6% YCW (CFY2). These researchers concluded that both CFY1 and CFY2

treatments resulted in greater fecal propionate concentrations (84 μmol/g of DM and 85 μmol/g of DM, respectively) as compared to the control and cellulose treatments (63 μmol/g DM and 49 μmol/g DM, respectively). As with other studies, these researchers question whether or not YCW produces these effects in the gut or if it is a result of the scFOS supplementation.

Thesis Objective

The objective of this thesis was to evaluate the feeding of raw meat-based diets to adult dogs. To our knowledge, there is no peer-reviewed research on this topic in dogs. Raw meat-based diets were expected to be highly palatable and digestible, but increase the bacterial load and alter fecal characteristics, bacterial balance, and fermentative end-product concentrations in the gut. Therefore, further objectives of this research were to evaluate the use of inulin and YCW supplementation in raw meat-based diets. Inulin and YCW have been previously studied in dogs and have been shown to beneficially alter the gut environment, but not when fed raw meat diets. We hypothesized that all diets would be highly digestible (DM digestibility > 85%; CP digestibility > 95%) and maintain N balance, with increased fecal SCFA concentrations, decreased fecal pH, and decreased fecal phenol and indole concentrations resulting from the inclusion of inulin or YCW. Beneficial changes in fecal microbial populations (decreased pathogenic bacteria and increased beneficial bacteria) also were expected with the inclusion of inulin or YCW to raw meat diets fed to healthy adult beagles. Another objective of this research was to evaluate the standardized amino acid digestibility by cecectomized roosters dosed with the raw meat-based diets.

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Chapter 3

Effects of Inulin or Yeast Cell Wall Extract on Nutrient Digestibility, Fecal Fermentative End-Product Concentrations, and Blood Metabolite Concentrations in Healthy Adult Dogs Fed Raw Diets, and on Standardized Amino Acid Digestibility by Cecectomized Roosters

Abstract

Raw diets are now commercially available for the canine, but few studies have been conducted testing their nutritional value. The objective of this experiment was to determine the effects of feeding poultry- and beef-based raw diets, with or without inulin or yeast cell wall extract (YCW), on total tract apparent macronutrient digestibility, blood cell populations, serum metabolite concentrations, and fecal fermentative end-product concentrations in healthy adult dogs. Six healthy, adult female beagles (5.5 ± 0.5 yr; 8.5 ± 0.5 kg) were randomly allotted to the following diets using a Latin square design: 1) Beef control; 2) Beef + 1.4% inulin (dry matter basis; DMB); 3) Beef + 1.4% YCW (DMB); 4) Chicken control; 5) Chicken + 1.4% inulin (DMB); and 6) Chicken + 1.4% YCW (DMB). Each period lasted 21 d (d 0-14 adaptation; d 15-20 total and fresh fecal and urine collection; d 21 fasted blood sample). Dogs were fed to maintain BW throughout the study. Food intake and refusals were measured daily. All dogs maintained desirable stool quality characteristics and produced low stool volume. All diets were highly digestible (total tract crude protein digestibility: 88.1-92.3%; fat digestibility: 96.7-97.8%). There were minor changes in fermentative end-product concentrations, but fecal short-chain fatty acid (SCFA) concentrations were increased ($P<0.05$) with inulin and YCW inclusion in dogs fed beef-based diets. Fecal spermine concentrations were increased ($P<0.05$) with inulin and YCW inclusion. In general, blood cell populations and metabolites were within the normal ranges for dogs. All diets maintained nitrogen balance. To evaluate the standardized amino acid digestibility of the six raw meat-based diets, a cecectomized rooster assay was conducted.

Twenty-four Single Comb White Leghorn cecectomized roosters were used in this study. Each rooster was crop-intubated and given an average of 24 g of each test diet. All excreta were collected and amino acid concentrations were measured in each sample. The results of the cecectomized rooster assay indicated that the standardized amino acid digestibility was high for all diets; however, differences in amino acid digestibility existed between protein sources. The beef control diet had the lowest total essential amino acid (TEAA), total non-essential amino acid (TNEAA), and total amino acid (TAA) digestibilities (90.2, 88.7, and 85.9%, respectively) and the chicken inulin diet had the highest TEAA, TNEAA, and TAA digestibilities (95.6, 95.2, and 92.2%, respectively). Our results agree with previous feline studies, demonstrating a high nutrient digestibility of raw diets in dogs. Inulin and YCW inclusion in raw meat-based diets had similar effects on large intestinal fermentation as extruded diets containing inulin and YCW. More research is needed to confirm our data and study such diets when fed long term.

Introduction

The use of unconventional diets, including raw meat diets, for pets continues to increase in popularity. Raw meat-based diets, as with other diets, have both potential benefits and risks. As seen previously in cats, raw meat-based diets often are fed because they do not contain preservatives, are highly digestible, and may improve stool quality or skin/coat quality (Michel, 2006; Kerr et al., 2010b; Vester et al., 2010a; 2010b). Conversely, raw meat-based diets have been shown to increase pathogen exposure, contain nutritional imbalances in some cases, and may be inconvenient for the pet owner to store or feed (Freeman and Michel, 2001; LeJeune and Hancock, 2001; Weese et al., 2005; Michel, 2006; 2008). To reduce health risks to both pet owners and their companion animals, the Center for Veterinary Medicine (CVM) has recommended specific guidelines to be followed by the manufacturer and consumer (CVM,

2004). However, no well-designed prospective study evaluating the feeding of raw diets to dogs has been reported.

Fructans are a group of fermentable carbohydrates that are classified as prebiotics. A prebiotic must (1) be resistant to gastric activity, enzymatic hydrolysis, and gastrointestinal absorption (non-digestible); (2) be fermented by cecal or colonic microflora; and (3) selectively stimulate the growth and/or activity of those bacteria that contribute to colonic and host health (Gibson and Roberfroid, 1995; Gibson et al., 2004; Roberfroid, 2007). Inulin is a long-chain fructan (10-60 units), which is derived from chicory root extract. It is not digested by mammalian enzymes and, therefore, reaches the colon to be fermented. It has been reported that inulin possesses the prebiotic properties listed above; however, nearly all research testing inulin supplementation in dogs has been in animals fed extruded kibble diets.

Yeast cell wall extracts (YCW) are moderately fermentable substrates containing a mixture of carbohydrates and proteins that have been shown to stimulate immune function in healthy adult dogs (Hussein and Healy, 2001; Vickers et al., 2001). Yeast cell wall is rich in mannans, which are believed to prevent adherence of bacteria expressing type-1 fimbriae to the intestinal wall (Ofek et al., 1977; Neeser et al., 1986). Additionally, YCW may decrease the production of putrefactive compounds, such as phenols and indoles, when fed in combination with fructans (Swanson et al., 2002b). The effects of YCW also have only been shown in dogs fed extruded kibble diets.

The use of fermentable substrates in high-protein, raw meat-based diets that are highly digestible may be beneficial to gut health by providing fecal bulk and/or positive fermentative profiles. Therefore, the objective of this study was to evaluate the effects of inulin or YCW on total tract apparent macronutrient digestibility, fecal characteristics, fecal fermentative end-

products, blood cell populations, and serum metabolite concentrations, in adult canines fed raw meat diets. We hypothesized that all diets would be highly digestible (DM digestibility >85%) and maintain nitrogen balance, with increased fecal short-chain fatty acid (SCFA) concentrations and decreased fecal phenol and indole concentrations resulting from the inclusion of inulin or YCW. Beneficial changes in fecal microbial populations (decreased pathogenic bacteria and increased beneficial bacteria) also were expected with the inclusion of inulin or YCW to raw meat diets fed to healthy adult beagles.

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 and diets—Six spayed female, healthy adult beagle dogs (5.5 ± 0.5 yr; 8.5 ± 0.5 kg) were used. An experiment using a 3 X 2 factorial in a Latin square design with 21-d periods was conducted. Each period consisted of a diet adaptation phase (d 0-14), total and fresh urine and fecal collection phase (d 15-20), and a day for collection of a fasted blood sample (d 21). During the first 12 days of the adaptation phase, dogs were housed individually in runs (1.0 m x 2.1 m x 1.8 m). Two days prior to and during collection days, dogs were house individually in stainless steel metabolic cages (0.9 m x 0.9 m x 0.8 m). Dogs were fed to maintain body weight (BW). Food was offered and intake was measured twice daily (8:00 and 17:00). Dogs were weighed and assessed for body condition score (BCS; 9 point scale) prior to the AM feeding on each Monday during adaptation and the first and last day of the collection periods.

Six diets were formulated to meet all nutrient needs of adult dogs according to the Association of American Feed Control Officials (AAFCO, 2009) and to contain approximately 30% crude protein (CP) and 45-50% fat. Dogs were randomly allotted to the following six test

diets using a Latin square design: 1) Beef control; 2) Beef + 1.4% inulin dry matter basis (DMB; Orafti HP, BENEO Group, Tienan, Belgium); 3) Beef + 1.4% YCW (DMB; Bio-Mos, Alltech Biotechnology, Nicholasville, KY); 4) Chicken control; 5) Chicken + 1.4% inulin (DMB); 6) Chicken + 1.4% YCW (DMB) (Tables 1 and 2). Inulin and YCW were added at the expense of the premix. Diets were mixed at Nature's Variety, Inc. (Lincoln, NE). Fresh water was offered ad libitum.

    Sample collection—Two days prior to and during the 5-day collection period, dogs were dosed twice daily with 0.5 g of chromic oxide (Cr_2O_3) contained within a gel capsule. Chromic oxide was used as a digestibility marker. During the 5-day collection phase, all fecal output was collected, including one fresh fecal sample from each dog. Although total tract macronutrient digestibility was based on the concentration of chromic oxide recovered, total feces excreted during the collection phase of each period were collected from the bottom of the cage, weighed, scored, and frozen at -20ºC until further analysis. The fecal samples were scored according to the following system: 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.

    A fresh fecal sample was collected within 15 min of defecation on d 1 of the 5-d collection phase. Fresh fecal samples were prepared immediately to minimize loss of volatile components. Samples were weighed and pH determined using a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA). Fresh fecal dry matter (DM) was determined. An aliquot of feces was mixed with 5 ml 2N hydrochloric acid (HCl) for ammonia, SCFA, and branched-chain fatty acid (BCFA) determinations and stored at -20ºC until analyzed.

Total urine output was collected from d 15-20 and volume recorded. A fresh urine sample (non-acidified) also was collected for complete urinalysis, including specific gravity (SG), measured by the University of Illinois Veterinary Medicine Diagnostics Laboratory (Urbana, IL) on a Leica TS METER refractometer (Leica Microsystems, Inc., Buffalo, NY). Urine samples were collected in vessels containing 5 ml 2N HCl for immediate acidification upon urination to prevent loss of N. The acidified urine samples then were subsampled and stored at 4ºC until analysis.

On the final day of the period (d 21), 6 ml of blood was collected via jugular puncture for blood cell count and serum metabolite measurements. Samples were immediately transferred to appropriate vacutainer tubes (2 ml of blood into # 367841 BD Vacutainer® Plus; 4 ml of blood into # 367974 BD Vacutainer® Plus; BD, Franklin Lakes, NJ) for sampling and transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory (Urbana, IL) using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics) for analysis.

Also on the final day of every collection period (d 21), two evaluators scored hair and skin condition based on the following scoring system: Hair condition score scale: 1 = dull, coarse, dry; 2 = poorly reflective, nonsoft; 3 = medium reflective, medium soft; 4 = highly reflective, very soft; 5 = greasy; Skin condition score scale: 1 = dry; 2 = slightly dry; 3 = normal; 4 = slightly greasy; 5 = greasy as described by Rees et al. (2001). The skin and hair was evaluated in both the shoulder region, specifically between the shoulder blades, and at the base of the tail. The evaluators were blinded to which treatment the dogs were consuming.

Cecectomized Rooster Assay—A cecectomized rooster assay was conducted as described by Sibbald (1979) to evaluate standardized amino acid digestibility of the six raw meat-based diets. Twenty-four, Single Comb White Leghorn roosters at approximately one year of age were

used in this study. At age 25 wk of age, all roosters underwent a cecectomy under general anesthesia following the methods of Parsons (1985). Animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Roosters were allowed to recover for 8 wk following surgery before the experiment. Roosters were individually housed in raised wire cages in an environmentally controlled room with 16 h light: 8 h dark cycle. Roosters had ad libitum access to food and water before beginning the experiment.

Roosters were fasted for 24 h before being dosed with the test diets. Each rooster was crop-intubated and given an average of 24 g of each test diet. Four roosters were fed each diet. After crop intubation, roosters were again fasted and all excreta were collected on a plastic tray under the cage for 48 h. Excreta were freeze-dried, weighed, and ground through a 0.25 mm screen. Amino acid concentrations were measured in each sample. Endogenous excretion of amino acids was measured using three roosters that were fasted during the test period. Standardized amino acid digestibility was calculated using the method described by Sibbald (1979).

Chemical analyses—Diet samples were subsampled, freeze dried (DuraTop[TM] Digital Programmer Bulk Tray Dryer, FTSSystems[TM]), and ground through a 2-mm screen in a Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ). Diet and fecal samples were analyzed according to procedures by the Association of Official Analytical Chemists (AOAC) for DM, organic matter (OM), and ash (AOAC, 2006; methods 934.01, 942.05). Diet, fecal, and urine CP content was calculated from Leco total N values (AOAC, 2006; method 992.15). Total lipid content (acid hydrolyzed fat) of the diets and feces was determined according to the methods of the American Association of Cereal Chemists (AACC, 1983) and Budde (1952). Gross energy (GE) of diet, fecal, and urine samples was measured using an oxygen bomb calorimeter (model

1261, Parr Instruments, Moline, IL). Dietary fiber concentrations [total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF)] were determined according to Prosky et al. (1992). All six raw meat diets and rooster excreta samples were sent to the University of Missouri Experiment Station Chemical Laboratories for complete amino acid profile analysis (AOAC, 2006; method 982.30E) and mineral analysis, including calcium (Ca), phosphorus (P), zinc (Zn), iron (Fe), mercury (Hg), and magnesium (Mg).

Chromium (Cr) concentrations in fecal samples were analyzed according to Williams et al. (1962) using atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT). Short-chain fatty acid and BCFA concentrations were determined by gas chromatography according to Erwin et al. (1961) using Hewlett-Packard 5890A series II gas chromatograph (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). Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003). Ammonia concentrations were determined according to the method of Chaney and Marbach (1962). Biogenic amine concentrations were measured by HPLC according to methods described by Flickinger et al. (2003).

Calculations—Dry matter recovery was calculated by dividing Cr intake (mg/d) by Cr concentrations in fecal samples (mg of Cr/g of feces). Total tract apparent macronutrient digestibility values were calculated as nutrient intake (g/d) minus fecal output (g/d), then divided by nutrient intake (g/d) multiplied by 100.

Digestible energy (DE) was determined by subtracting the GE of the feces from the GE of the food consumed. Metabolizable energy (ME) was determined by subtracting the GE of the feces and urine from the GE of the food consumed.

A comparison of all 6 treatments was planned. However, due to differences in food intake between the two protein sources (i.e., greater intake by dogs fed beef-based diets), it was determined to be inappropriate to compare data between dogs fed beef-and chicken-based diets. Therefore, all statistical analyses were limited to the effects of inulin or YCW within a protein source. Therefore, the statistical analyses were conducted as a repeated measures cross-over design within protein source. Statistical evaluation was completed using the Proc Mixed and Proc Glimmix (Proc Glimmix used for fecal scores and skin/coat condition scores) procedures of SAS (version 9.2, SAS Inst. Inc., Cary, NC). Dog was utilized as the experimental unit for all data. $P<0.05$ was considered significant and $P<0.10$ a trend.

For the cecectomized rooster assay, statistics were conducted using Proc Mixed in SAS. Data were analyzed as a 3 x 2 factorial and evaluated for differences between protein sources, between fiber sources, and a protein*fiber interaction. $P<0.05$ was considered significant and $P<0.10$ a trend.

Results

Dietary ingredient and chemical composition data are presented in Tables 3.1 and 3.2. All six diets were not of similar composition, as was intended. The chicken-based diets were similar to our targeted composition, containing an average of 31.65% CP and 50.92% fat. However, the beef-based diets contained lower protein (an average of 25.08% CP) and higher fat (63.65% fat) than expected. Total dietary fiber composition also varied among treatments, with the inulin treatments containing the lowest TDF value. Calculated ME was similar among treatments within a protein source, but differed between protein sources (average for beef-based diets = 6.87; average for chicken-based diets = 5.96), as was expected. All essential and

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nonessential amino acid, Ca, and P concentrations were within the recommended ranges for adult dogs.

Food intake (g/d) was not different among treatments within a protein source, but dogs consumed more (P<0.05) of the beef-based diets than the chicken-based diets (Table 3.3). Fecal output (g/d) on a DMB and fecal output (as is)/food intake (DMB) tended to be greater (P=0.07 and P=0.06, respectively) in dogs fed the beef + YCW diet versus those fed the beef control or beef + inulin diets. In dogs fed beef-based diets, DM, OM, CP, and energy digestibilities were greater (P<0.05) with the inclusion of inulin, but lower (P<0.05) with the inclusion of YCW as compared to when dogs consumed the control diet. In dogs fed beef-based diets, fecal scores were lower (P<0.05; harder stools) in dogs fed beef control or beef + inulin diets. Although fecal output and nutrient digestibility was not different due to inulin or YCW in dogs fed chicken-based diets, both ingredients decreased (P<0.05) fecal pH.

In dogs fed beef-based diets, fecal total SCFA and acetate concentrations were greater (P<0.05) with the inclusion of inulin or YCW (Table 3.4). Fecal propionate tended to be greater (P=0.11) with the inclusion of inulin to the beef-based diets. In dogs fed chicken-based diets, fecal indole was lower (P<0.05) in dogs fed inulin or YCW, while fecal total indoles and phenols were lower (P<0.05) only with the inclusion of inulin. Fecal spermine concentrations were greater (P<0.05) with the inclusion of inulin or YCW in dogs fed either protein source. All other fecal fermentative end-products were not affected by inulin or YCW inclusion.

Except for serum alanine aminotransferase (ALT) in one dog, all mean blood cell populations and serum metabolite concentrations were within the normal range for healthy adult dogs throughout the experiment (Tables 3.5 and 3.6; Merck, 2005). In dogs fed beef-based diets, circulating eosinophils tended to be greater (P=0.11) with the inclusion of inulin. Percentage of

eosinophils of total white blood cells was greater (P<0.05) with the inclusion of inulin in dogs fed the beef-based diets. In dogs fed the chicken-based diets, blood platelets (10^3/µL) were lower (P<0.05) with the inclusion of YCW. In dogs fed beef-based diets, blood glucose tended to be greater (P=0.06) with the inclusion of YCW. In dogs fed beef-based diets, corticosteroid-alkaline phosphatase (c-Alk Phos) tended to be greater (P=0.07) with the inclusion of inulin. In dogs fed beef-based diets, blood cholesterol was greater (P<0.05) with the inclusion of inulin or YCW. However, blood cholesterol was lower (P<0.05) with the inclusion of YCW in dogs fed the chicken-based diets.

Urinalysis was normal in dogs fed all diets. Urine SG was not different among treatments; mean SG of dogs fed beef-based and chicken-based diets was 1.0503 ± 0.004 and 1.0465 ± 0.003, respectively. Nitrogen balance did not vary among treatments; mean N balance of the beef-based diets was 1.061 and mean N balance of the chicken-based diets was 0.810. In dogs fed beef-based diets, skin condition score in the tail region was lower (P<0.05) with the inclusion of inulin (control: 3.1; inulin: 2.8; YCW: 2.9). All other skin and coat scores were not affected by diet (data not shown).

Cecectomized Rooster Amino Acid Digestibility—Standardized amino acid digestibility coefficients are presented in Table 3.7. Arginine, histidine, isoleucine, leucine, methionine, threonine, valine, alanine, aspartic acid, and total amino acid digestibilities differed between protein sources, all being greater (P<0.05) in chicken-based diets. Phenylalanine, glutamic acid, proline, tyrosine, and total non-essential amino acid digestibility coefficients tended to be greater (P≤0.10) in chicken- versus beef-based diets. Fiber source affected the standardized amino acid digestibility coefficients for histidine and lysine, with YCW diets having greater (P<0.05) standardized amino acid digestibility than control diets.

Discussion

      Due to the growing trend of pet owners choosing to feed raw meat-based diets to their

pets, more research is needed in this area. Areas of further study include: diet composition and

palatability, stool characteristics, nutrient digestibility, fecal fermentative end-products, and fecal

microbial populations. It is known that there are many compositional differences among animal-

based protein sources fed to dogs and cats (Murray et al., 1997; Dust et. al, 2005; Faber et al.,

2010; Kerr et al., 2010a). For instance, Murray et al. (1997) studied the chemical composition

and nutrient digestibilities of various animal products used in dog food, reporting that the CP

percentage ranged from 30.4 to 67.6 and the fat percentage ranged from 11.6 to 50.7 among

protein sources.

      Given these inconsistencies in raw materials, homemade and commercial diets must be

carefully formulated and checked regularly to verify composition. Variability of the diets is

dependent on the source and quality of the animal ingredients used (e.g., skeletal muscle, organ

meats, offal, etc). The diets used in the current study were compositionally different between

protein sources, with the largest differences observed in CP, fat, and TDF, but were similar

within a protein source. All diets were targeted to contain about 45-50% fat and 30% CP. It

appeared that the beef-based products used to manufacture our diets were more variable or of

different quality than the beef-products listed in our formulation program. The chicken-based

products were not as variable as the beef-based products and, thus, the chicken-based diets were

closer to the intended composition than the beef-based diets. While we were pleased to see that

the chicken-based diets were close to our targeted diet composition, the large difference in

dietary fat in the beef-based diets is concerning. Metabolizable energy (ME) values were similar

within a protein source, but differed between the two protein sources. This was expected given

that the beef-based diets contained a much higher fat percentage than that of the chicken-based diets.

Dietary fiber concentrations are often quite low in raw meat-based diets (Kerr et al., 2010a; Vester et al., 2010a). Although large amounts are not necessary, its inclusion is important to minimize constipation with such diets. In the current study, TDF values were lowest for the treatments containing inulin for both beef- and chicken-based diets. However, overall, the chicken-based diets contained higher TDF values than the beef-based diets. Inulin is a water soluble oligosaccharide and, thus, is not able to be quantified using the TDF methodology. Portions of YCW also may be unaccounted for using this assay. Other differences in dietary fiber may be due to collagen and/or connective tissue concentration variations among the diets. Whole carcass chicken tends to contain more animal fiber (i.e., connective tissue) than other protein sources (Dust et al., 2005; Otten et al., 2006).

Macro-and microminerals are often difficult to balance in raw meat diets, with Ca and P requiring extra attention. It is recommended that a Ca:P ratio of 1:1 to 2:1 be fed to adult dogs (AAFCO, 2009). Most skeletal muscle meats, however, contain 20-30 times higher P than Ca concentrations. Because of this, ground bone was included in the beef-based diets and a chicken source containing bone was used in the chicken-based diets. For the 6 treatments evaluated in this study, the Ca:P ratios were within AAFCO recommendations for adult dogs; however, there were some differences among treatments—beef-based diets had a lower Ca:P ratio (1.1:1 to 1.3:1) than the chicken-based diets (1.8:1 to 1.9:1).

Variability in the composition of raw meat diets can lead to differences in food intake in order for the animal to meet its dietary energy requirement. In the current study, food intake data were reflective of the 5-d collection phase. Because all dogs were fed to maintain BW

throughout the duration of the study, differences in food intake were not due to dog preference for one particular diet. Given our previous experience feeding raw diets to cats (Kerr et al., 2010b; Vester et al., 2010a), we were not surprised to see that fecal output (as is; g/d) in the current study was about half of that from dogs that were fed a kibble diet in previous studies (Diez et al., 1998; Flickinger et al., 2003; Rodriguez et al., 2007). Those studies also used beagle dogs with a BW of 11.3-13.4 kg, 12.0 ± 1.3 kg, and 14.4 ± 0.6 kg, respectively.

Fermentable substrates, such as inulin and YCW, were tested herein due to interesting results obtained as regards fecal quality, nutrient digestibilities, fermentative end-product concentrations, and fecal microbial populations observed in previous literature from our lab evaluating cats fed raw diets (Kerr et al., 2010b; Vester et al., 2010a). Vester et al. (2010a) reported that when adding a nonfermentable fiber source to the diets, low fecal scores were observed in domestic cats (1.2/5; hard feces). They also concluded that when a more fermentable fiber source was included in the diets, ideal fecal scores were observed in exotic cats (3.4/5; normal feces), which may indicate that a fermentable fiber source needs to be included in such diets. Kerr et al. (2010b) arrived at a similar conclusion when a moderately fermentable fiber source was included in a raw beef diet fed to domestic cats (2.9/5; normal feces). In this study, dogs fed all treatments produced desirable fecal scores throughout the duration of the study. Dogs fed the beef + YCW diet produced softer stools than the beef control and beef + inulin diets. Overall, dogs fed the beef-based diets had softer stools (higher fecal scores) than dogs fed the chicken-based diets, but all were of acceptable quality.

Total tract apparent CP and fat digestibilities by the dogs in the current study were similar to those obtained in previous raw meat studies in cats (CP digestibility = 92.9 to 93.9; fat digestibility = 93.9 to 95.5) performed in our laboratory (Vester et al., 2008; Kerr et al., 2010b;

Vester et al., 2010a; 2010b). Crude protein digestibility is commonly affected to the greatest

extent due to variability and source of protein used in such diets. Total tract CP digestibility can

also be affected and misleading due to microbial metabolism of CP in the hindgut. Of the

macronutrient digestibilities in the current study, CP digestibility was the most variable. Given

the differences in food intake, it is not appropriate to compare CP digestibility between protein

sources. However, it was interesting that CP digestibility was numerically greater when dogs

consumed beef-based diets compared with chicken-based diets. Differences exist when

comparing the total tract CP digestibility data from dogs in the current study and our data

obtained in roosters, which demonstrated increased CP and amino acid digestibilities of the

chicken-based diets. The primary reason for this difference is likely microbial fermentation,

which occurs at a much greater extent in the colon of the dog as compared to the cecectomized

rooster. Thus, we believe the data from the precision-fed rooster assay provides a better estimate

of ileal CP digestibility in dogs fed raw diets.

 Amino acid digestibility can be affected by many factors including the presence of

connective tissue and processing temperature (Kies, 1981; Friedman, 1996; Parsons, 2002).

Because raw diets were used and all diets were gently freeze-dried before analysis, the

processing factor was minimized in our study. The cecectomized rooster assay results indicate

that the standardized amino acid digestibility was high for all diets. Even though the chicken-

based diets contained higher total dietary fiber and had numerically lower total tract CP

digestibility in dogs, most of the amino acid digestibility coefficients for these diets in the rooster

assay were higher than that of the beef-based diets.

 Crude protein digestibility was different between diets fed to exotic felids, being greater

in animals fed a horse-based diet versus a beef-based diet (Vester et al., 2010a). Vester et al.

(2010a) discussed the difficulty of distinguishing whether digestibility differences are due to actual differences in CP digestibility among protein sources or due to variation in the fiber sources included in the diets. In that study, fiber sources differed between the two diets; the horse-diet contained a nonfermentable fiber source (cellulose) and the beef-based diet contained a moderately fermentable fiber source (beet pulp). They concluded that composition and fermentability of dietary fiber sources may have affected microbial metabolism and fecal protein concentration and, thus, total tract CP digestibility. Therefore, the importance of controlling fiber sources and concentration was stressed. In the current study, dogs fed the beef-based diets had higher total tract apparent macronutrient digestibility than dogs fed the chicken-based diets. Higher concentrations of fiber in a diet may decrease the digestibility of other nutrients due to increased rate of passage through the small intestine. Additionally, fiber may appear artificially high in diets due to collagen. Collagen analyzes as dietary fiber in the diet, but in the body, much of it may be either broken down in digestion or fermented in the large bowel (fermented as protein; Cummings and Macfarlane, 1991). Because the fiber sources and amounts utilized in this study were tightly controlled, differences in total tract apparent macronutrient digestibility were likely not due to fiber. The different outcomes of the dog and rooster assays are interesting and demonstrate the need to determine ileal compared with total tract digestibility.

Fecal fermentative end-product concentrations are indicative of protein and carbohydrate fermentation occurring in the large bowel. Carbohydrate fermentation primarily produces SCFA in the large intestine and serves as an important energy source for colonocytes. In contrast, increased phenol, indole, and BCFA production are an indication of protein fermentation occurring in the large intestine. Vickers et al. (2001), who used dog fecal inoculum in an *in vitro* fermentation procedure, tested 4 inulin products, fructooligosaccharide (FOS), a source of

mannanoligosaccharides (derived from YCW), soy fiber, beet pulp, and wood cellulose. They

concluded that total mean production of SCFA (pooled for all durations of fermentation) was

highest for fermentation of the 4 inulin products and FOS (3.1-3.6 mmol/g of OM) compared

with cellulose (0.05 mmol/g OM) or beet pulp (1.47 mmol/g OM) (Vickers et al., 2001).

Additionally, fermentation of the 4 inulin products and FOS produced higher mean acetate

concentrations (1.9 to 2.4 mmol/g of OM) compared with cellulose or beet pulp (mean acetate

production of 0.02 and 1.08 mmol/g of OM, respectively).

Increased SCFA concentrations were expected in those diets with added inulin or YCW,

which we observed in the beef-based diets, but not in the chicken-based diets. Flickinger et al.

(2003), who tested the effects of fructan supplementation in extruded diets fed to healthy adult

beagles, concluded that fecal propionate concentrations were increased in feces of dogs fed an

average of 0.6% oligofructose (OF; hydrolyzed inulin). Additionally, fecal total SCFA tended to

be greater in those dogs. In the current study, fecal acetate and total SCFA concentrations were

greater and propionate concentrations tended to be greater with the inclusion of inulin or YCW

in dogs fed beef-based diets. Similar numerical changes occurred in dogs fed chicken-based

diets. Flickinger et al. (2003) did not observe any changes in fecal pH with short-chain

fructooligosaccharide (scFOS) supplementation when all scFOS-supplemented dogs were

compared to the control dogs. This may have been due to the dietary level at which they were

fed (1, 2, or 3 g scFOS/d). In the current study, fecal pH was decreased by adding 1.4% inulin or

YCW to raw meat-based diets, further supporting the fermentable nature of these ingredients.

However, the premix, which was included at a minimum of 10.15% (as is) in all 6 diets, included

various fruits and vegetables that contain fiber. It is unclear how much the premix may have

masked the effects of the added inulin/YCW, or if an interaction between the premix and

inulin/YCW was occurring. Swanson et al. (2001) evaluated the fermentability extent of substrate disappearance during fermentation, and gas production of fruit and vegetable fibers compared to dietary fiber standards for use in premium dog foods. The standards included psyllium husk, citrus pectin, and Solka Floc. The fruit and vegetable fiber sources included apple pomace, carrot pomace, flaxseed, fruit blend (a mixture of peach, almond, nectarine, and plum), grape pomace, pea hulls, ground pistachio, and tomato pomace. Substrates were fermented *in vitro* for 4, 12, or 24 h with the fecal microflora obtained from 3 healthy, adult dogs. They determined that TDF of the test substrates were 79.3, 55.2, 32.6, 65.3, 54.7, 69.7, 85.9, and 56.9% DMB, respectively. After 24 h of fermentation, the apple, carrot, and tomato pomaces seemed to be moderately fermented. These pomaces are similar to the fruits and vegetables that were included in our premix. The effects of inulin may have been greater if the basal diet contained no fiber or a nonfermentable fiber, such as cellulose. Researchers have observed lower SCFA concentrations when a nonfermentable fiber source (cellulose) was added to the horse-based diets fed to cats (Vester et al., 2010a).

In the current study, fecal phenol and indole concentrations were decreased with inulin in dogs fed the chicken-based diets. Similar numerical changes occurred in dogs fed beef-based diets containing YCW; however, the differences were not statistically significant. The diets containing added inulin or YCW likely had more carbohydrate available for microbial fermentation, allowing those substrates to be fermented instead of only protein. With the inclusion of fermentable substrates, such as inulin, the concentrations of the harmful protein-containing end-products can be reduced and may be useful in raw meat diets. Swanson et al. (2002a), who tested the effects of feeding scFOS and *Lactobacillus acidophilus* separately or in combination to healthy adult dogs, concluded that dogs fed scFOS had lower fecal total phenol

concentrations than the control dogs, who were fed a sucrose placebo. In that study, dogs fed scFOS tended to have lower fecal indole concentrations than the control dogs. Swanson et al. (2002b), tested the effect of feeding scFOS, MOS, or scFOS + MOS to adult dogs. They concluded that fecal indole concentrations tended to decrease in dogs supplemented with scFOS, decreasing from 2.44 µmol/g fecal DM in the controls to 1.23 µmol/g fecal DM in the scFOS-fed dogs. Fecal total phenol and indole concentrations were also decreased in dogs fed scFOS supplementation in that study (3.03 µmol/g fecal DM in the control compared with 1.50 µmol/g fecal DM in scFOS).

In general, blood cell and metabolite data were within normal ranges for all dogs and were not greatly changed due to diet. In the current study, we did not measure cellular function or immunity because healthy adult dogs were studied, but eosinophils tended to increase with the inclusion of inulin in dogs fed the beef-based diets. When eosinophil data were expressed as a percentage of total white blood cells, there was an increase with the inclusion of inulin in the beef-based diets. Middelbos et al. (2007) tested the effects of various concentrations of YCW supplementation on immune indices in adult dogs. In that study, eosinophil concentrations did not change with YCW supplementation. Kelly (2000) concluded that increased eosinophils may be indicative of a response to a potential food allergy because eosinophils are involved in the intestinal inflammatory response. The dogs in the current study did not exhibit any outward signs indicating the presence of any food allergies. The biological significance of the change in eosinophils in the current study is not known, but may be of interest in future studies.

Elevation of blood ALT, a cytosolic enzyme, can be indicative of liver dysfunction or toxic insult (Duncan et al., 1994; Merck, 2005). In the current study, ALT concentrations were elevated in one dog when fed the beef + YCW diet. The ALT elevation was observed only

during one period in this dog, who exhibited no other adverse health effects. Alanine aminotransferase concentrations also were higher when African wildcats consumed a commercial raw meat diet compared with a kibble diet (Vester et al., 2010b). While elevated ALT concentrations alone in one dog in one period may simply be an anomaly, ALT concentrations should be closely monitored when feeding raw meat diets to companion animals and may be a topic of study in future experiments.

Improved skin and coat condition are commonly attributed to raw feeding. However, this has not been tested in raw feeding studies to date. In this study, two blinded evaluators scored the dogs' skin and coat condition every period, but did not detect differences due to diet. Several potential reasons exist for no observed change in skin and coat. First, the skin and coat condition scores utilized in this study were subjective. It is possible that a more objective means of evaluating improvement in skin and coat condition may be more accurate and reliable. Additionally, feeding for a longer duration of time may be needed to show any significant improvement in coat quality. For instance, Rees et al. (2001) tested the effects of dietary flax seed and sunflower seed supplementation on canine skin and coat condition in healthy adult dogs and fed each diet for a period of approximately 84 days. They concluded that there was a numerical improvement in hair coat and skin condition scores with the flax seed and sunflower seed supplementation; however, the improvement occurred soon after supplementation but was not sustained for the entire 84 days, indicating that some adaptation to the diets may have occurred. In the current study, the dogs were only fed each diet for a period of 21 days. Lastly, the current study utilized healthy dogs. Studying the use of such diets in dogs with a skin condition, such as atopic dermatitis, may provide a model in which improved skin and/or coat condition may be tested.

In conclusion, results from these experiments agree with previous raw feeding studies in cats, demonstrating a high nutrient digestibility of raw diets. Inulin and YCW inclusion in raw meat-based diets had similar effects on large intestinal fermentation as extruded diets containing inulin and YCW. More research is needed to confirm our data and/or study the use of such diets over the long term.

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Tables

Table 3.1. Ingredient composition of raw chicken-and beef-based diets with and without the inclusion of inulin or yeast cell wall (YCW)

| | Diet | | | | | |
| | Beef | | | Chicken | | |
Ingredient	Control	Inulin	YCW	Control	Inulin	YCW
	-------------------------% (as is)-------------------------					
Chicken (with bone)	--	--	--	49.81	49.81	49.81
Chicken fat	--	--	--	5.64	5.64	5.64
Chicken meat	--	--	--	13.16	13.16	13.16
Chicken heart	--	--	--	10.34	10.34	10.34
Chicken liver	--	--	--	10.34	10.34	10.34
Beef	47.46	47.46	47.46	--	--	--
Beef liver	10.34	10.34	10.34	--	--	--
Ground beef bone	6.86	6.86	6.86	--	--	--
Beef heart	11.28	11.28	11.28	--	--	--
Added Water	11.94	11.94	11.94	--	--	--
Dicalcium phosphate	1.41	1.41	1.41	--	--	--
Premix[1]	10.71	10.15	10.15	10.71	10.15	10.15
YCW[2]	--	--	0.56	--	--	0.56
Inulin[3]	--	0.56	--	--	0.56	--

[1]Premix includes: apple (15.2%), carrot (15.2%), butternut squash (15.2%), chicken egg (11.4%), salmon oil (10.9%), broccoli (8.7%), spinach (8.7%), dried kelp (6.5%), alfalfa sprouts (2.2%), taurine (2.2%), apple cider vinegar (1.1%), parsley (1.1%), blueberry (1.1%), mixed tocopherols (0.5%).
[2]Bio-Mos, Alltech Biotechnology, Nicholasville, KY.
[3]Orafti HP, BENEO Group, Tienan, Belgium.

Table 3.2. Chemical composition of raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW)

Item	Beef			Chicken		
	Control	Inulin	YCW	Control	Inulin	YCW
Dry matter, %	41.43	41.78	42.15	32.61	33.03	32.73
---% DM basis---						
Organic matter	93.70	94.52	93.52	91.41	92.60	91.64
Crude protein	24.99	25.83	24.43	32.00	31.47	31.49
Acid hydrolyzed fat	63.86	64.13	62.97	51.10	51.35	50.30
Total dietary fiber	3.45	1.01	3.14	4.55	3.53	4.53
Insoluble	3.13	0.98	2.17	3.33	1.64	2.41
Soluble	0.31	0.03	0.97	1.22	1.89	2.12
Gross energy, kcal/g	7.46	7.60	7.56	6.79	6.92	6.88
ME_{AAFCO}[1], kcal/g	6.57	6.67	6.54	5.59	5.94	5.86
ME_C[2], kcal/g	6.79	6.96	6.85	5.88	6.03	5.96
Amino Acids (AA), %						
Essential						
Arginine	1.65	1.54	1.46	2.02	1.94	1.98
Histidine	0.64	0.63	0.60	0.81	0.76	0.77
Isoleucine	1.06	1.04	1.00	1.38	1.30	1.32
Leucine	2.00	1.94	1.87	2.40	2.27	2.30
Lysine	1.94	1.90	1.81	2.40	2.32	2.33
Methionine	0.54	0.52	0.48	0.69	0.67	0.67
Phenylalanine	1.09	1.09	1.03	1.30	1.17	1.19
Threonine	0.99	0.89	0.90	1.24	1.20	1.23
Tryptophan	0.27	0.27	0.27	0.32	0.32	0.32
Valine	1.33	1.30	1.27	1.60	1.50	1.56
Nonessential						
Alanine	1.63	1.54	1.45	1.84	1.77	1.84
Aspartic acid	2.13	2.04	1.93	2.67	2.56	2.59
Cysteine	0.33	0.29	0.27	0.36	0.37	0.36
Glutamic acid	3.08	3.06	2.75	3.85	3.63	3.66
Glycine	2.11	1.89	1.74	2.05	2.01	2.15
Hydroxylysine	0.14	0.09	0.12	0.14	0.10	0.11
Hydroxyproline	0.62	0.47	0.45	0.51	0.53	0.59
Ornithine	0.04	0.05	0.05	0.03	0.03	0.03
Proline	1.43	1.33	1.27	1.49	1.41	1.52
Serine	0.93	0.76	0.78	1.07	1.05	1.08
Taurine	0.13	0.13	0.12	0.21	0.21	0.21
Tyrosine	0.76	0.72	0.76	1.04	0.97	0.96
TEAA[3]	11.51	11.12	10.69	14.16	13.45	13.67

Table 3.2 (cont.)

TNEAA[4]	13.33	12.37	11.69	15.26	14.64	15.10
TAA[5]	24.84	23.49	22.38	29.42	28.09	28.77
Minerals						
Calcium	1.12	1.07	1.00	2.05	1.68	2.10
Phosphorus	0.87	0.88	0.86	1.11	0.92	1.18
Iron	0.08	0.06	0.07	0.06	0.05	0.06
Magnesium	0.07	0.05	0.07	0.11	0.10	0.12
Zinc	0.007	0.007	0.007	0.005	0.005	0.005

[1]ME_{AAFCO} = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.
[2]ME_C = GE intake (kcal/d) - fecal GE (kcal/d) - urinary GE (kcal/d)/ DM intake (g/d).
[3]TEAA = total essential AA.
[4]TNEAA = total nonessential AA.
[5]TAA = total AA.

Table 3.3. Food intake, fecal characteristics, and total tract apparent macronutrient digestibility in adult dogs fed raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW)

Item	Beef				Chicken			
	Control	Inulin	YCW	SEM†	Control	Inulin	YCW	SEM†
Food intake								
g DM/d	98.9	95.6	103.5	4.91	77.6	83.3	74.3	4.99
g OM/d	92.7	90.3	96.8	4.59	71.0	77.1	68.1	4.62
g CP/d	24.7	24.7	28.3	1.20	24.8	26.2	23.4	1.57
g fat/d	63.2	61.3	65.2	3.11	39.7	42.8	37.4	2.57
kcal/d	737.5	725.9	782.0	36.62	527.5	576.0	511.1	34.47
Fecal output, g/d (as is)	28.2	24.7	37.6	3.83	38.0	40.0	37.5	3.29
Fecal output, g/d (DMB)	12.3^x	11.1^x	17.1^y	1.46	16.0	15.5	14.6	1.88
Fecal output (as is)/food intake (DMB)	0.29^x	0.25^x	0.36^y	0.02	0.53	0.48	0.51	0.03
Digestibility								
Dry matter (DM), %	87.36^b	89.30^c	86.26^a	0.34	77.64	80.14	78.95	0.70
Organic matter (OM), %	93.28^b	94.26^c	91.74^a	0.22	88.75	89.84	88.52	0.40
Crude protein (CP), %	91.84^b	92.25^c	89.95^a	0.29	88.59	88.38	88.10	0.46
Acid hydrolyzed fat (AHF), %	97.48	97.81	97.34	0.13	96.68	97.63	97.04	0.29
Energy, %	94.92^b	95.66^c	93.99^a	0.19	91.78	92.73	91.83	0.37
Fecal Scores[1]	2.27^a	2.34^a	2.63^b	0.21	1.81	1.76	1.83	0.16
Fecal DM %	43.48	43.23	37.30	2.31	43.68	38.61	40.65	2.09
Fecal pH	6.78	6.55	6.63	0.24	6.65^b	6.20^a	6.16^a	0.11

[a,b,c]Means within a protein source not sharing a common superscript differ (P<0.05) due to fiber source.
[x,y]Means within a protein source not sharing a common superscript differ (P≤0.10) due to fiber source.
[1]Fecal score scale: 1= hard, dry pellets; 2= dry, well formed stool; 3=soft, moist, formed stool; 4= soft, unformed stool; 5= watery, liquid that can be poured.
[†]Pooled SEM.

Table 3.4. Fecal short-chain fatty acid (SCFA), branched-chain fatty acid (BCFA), ammonia, phenol, indole, and biogenic amine concentrations of adult dogs fed raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW)

Item	Beef				Chicken			
	Control	Inulin	YCW	SEM[†]	Control	Inulin	YCW	SEM[†]
	------------------umol/g--------------				------------------umol/g--------------			
Short-chain fatty acids								
Acetate	142.6[a]	205.3[b]	189.1[b]	13.32	150.2	220.3	220.2	21.29
Propionate	45.0	83.0	69.2	10.12	54.1	94.9	79.3	14.92
Butyrate	37.6	42.9	53.8	5.64	32.8	39.3	69.7	12.19
Total SCFA[1]	225.2[a]	331.1[b]	312.1[b]	23.88	237.1	354.5	369.2	42.34
Branched-chain fatty acids								
Valerate	1.25	1.01	1.19	0.18	0.92	0.72	1.09	0.20
Isovalerate	9.85	10.25	9.21	1.97	7.67	7.50	9.33	1.23
Isobutyrate	6.50	6.47	6.01	1.28	5.06	4.49	5.57	0.73
Total BCFA[1]	17.60	17.73	16.42	3.20	13.65	12.71	15.99	2.13
Ammonia	125.90	131.10	128.20	12.63	105.14	140.16	108.97	21.94
Phenols and indoles								
Phenol	0.43	0.29	0.17	0.20	0.34	0.11	0.15	0.10
Indole	1.56	1.04	0.89	0.21	0.97[b]	0.37[a]	0.59[a]	0.09
Total phenols and indoles	1.99	1.32	1.06	0.39	1.32[b]	0.48[a]	0.74[ab]	0.18
Biogenic amines								
Tryptamine	0.32	0.32	0.31	0.03	0.28	0.21	0.36	0.09
Putrescine	2.84	1.79	2.52	0.61	2.18	1.50	1.46	0.33
Cadaverine	0.58	0.33	0.84	0.15	0.45	0.46	0.33	0.21
Tyramine	1.03	0.26	0.93	0.50	0.57	0.25	0.45	0.15
Spermidine	0.89	0.99	1.17	0.11	1.25	1.28	1.53	0.20
Spermine	0.97[a]	2.70[c]	1.73[b]	0.17	1.24[a]	2.21[b]	1.82[b]	0.14
Total biogenic amines	6.62	6.40	7.49	1.13	5.96	5.90	5.95	1.02

[a,b]Means within a protein source not sharing a common superscript differ (P<0.05) due to fiber source.
[1]Total SCFA = acetate + propionate + butyrate; total BCFA = valerate + isovalerate + isobutyrate.

[†]Pooled SEM.

Table 3.5. Blood cell populations and characteristics of adult dogs fed raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW)

Item[1]	Beef				Chicken			
	Control	Inulin	YCW	SEM[†]	Control	Inulin	YCW	SEM[†]
WBC (10^3/uL):	4.93	4.84	4.39	0.38	5.24	4.98	4.31	0.33
Neutrophils	3.27	3.05	3.03	0.31	3.49	3.17	2.70	0.25
Lymphocytes	1.35	1.41	1.10	0.15	1.42	1.37	1.26	0.10
Monocytes	0.25	0.19	0.15	0.06	0.20	0.31	0.20	0.05
Eosinophils	0.06[x]	0.19[y]	0.12[xy]	0.02	0.13	0.12	0.14	0.03
% Neutrophils	64.86	63.21	68.83	1.49	64.67	62.67	62.83	0.80
% Lymphocytes	29.05	28.55	25.33	2.44	29.33	28.33	29.17	1.48
% Monocytes	5.12	4.12	3.50	1.01	3.67	6.50	4.83	0.82
% Eosinophils	0.99[a]	4.10[b]	2.37[ab]	0.35	2.33	2.50	3.17	0.70
RBC (M/uL):	7.02	7.16	7.10	0.27	7.14	7.19	7.13	0.12
Hgb (g/dL)	16.16	16.49	16.35	0.63	16.42	16.52	16.42	0.22
Hct (%)	50.22	51.10	50.72	1.79	51.22	51.35	50.97	0.81
Mcv (fL)	71.75	71.41	71.62	0.25	71.77	71.37	71.47	0.24
Mch (pg)	22.96	23.03	23.07	0.17	23.00	22.98	23.02	0.10
Mchc (g/dL)	32.13	32.26	32.20	0.24	32.05	32.20	32.20	0.16
Rdw (%)	16.91	17.45	16.50	0.34	16.27	16.13	16.38	0.27
Plt (10^3/uL)	363.93	382.57	376.17	30.93	389.00[b]	394.67[b]	313.83[a]	19.26
Mpv (fL)	8.40	7.80	8.35	0.23	8.89	9.20	9.71	0.20

[a,b]Means within a protein source not sharing a common superscript differ (P<0.05) due to fiber source.
[x,y]Means within a protein source not sharing a common superscript differ (P≤0.10) due to fiber source.
[1]Hgb = hemoglobin; hct = hematocrit; mcv = mean corpuscular volume (average size of RBC); mch = mean corpuscular hemoglobin (average amount of oxygen-containing Hb in RBC); mchc = mean corpuscular hemoglobin concentration (average concentration of Hb in RBC); rdw = red cell distribution width (variation in size of RBC); plt = platelets; mpv = mean platelet volume (mean size of platelets--new platelets are larger).
[†]Pooled SEM.

Table 3.6. Serum metabolites of adult dogs fed raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW)

	Beef				Chicken			
Item	Control	Inulin	YCW	SEM†	Control	Inulin	YCW	SEM†
Creatinine (mg/dl)	0.45	0.42	0.45	0.02	0.43	0.43	0.47	0.02
Urea Nitrogen (mg/dl)	11.30	10.78	11.68	0.84	11.75	11.43	10.67	0.48
Total Protein (g/dl)	5.77	5.82	5.77	0.08	5.82	5.78	5.75	0.04
Albumin (g/dl)	3.73	3.75	3.75	0.05	3.82	3.80	3.87	0.03
Calcium (mg/dl)	10.28^{ab}	10.38^b	10.20^a	0.03	10.28	10.30	10.27	0.06
Phosphorus (mg/dl)	3.33	3.18	2.90	0.15	3.23	3.53	3.20	0.16
Sodium (mmol/L)	148.67	148.00	148.00	0.43	148.50	147.67	148.00	0.51
Potassium (mmol/L)	4.45^b	4.40^b	4.22^a	0.05	4.38	4.27	4.28	0.11
Chloride (mmol/L)	114.67	113.67	113.67	0.54	113.83	113.00	113.17	0.26
Glucose (mg/dl)	88.33^x	88.17^x	97.17^y	2.27	88.50	86.83	88.17	3.22
Alk Phos (U/l)	18.83	33.50	24.17	6.60	18.67	20.83	17.17	1.25
C-Alk Phos (U/l)	5.83^{xy}	8.17^y	5.17^x	0.73	6.00	6.67	5.00	0.57
ALT (U/l)	23.83	81.50	112.00	44.31	25.00	27.00	24.67	2.31
GGT (U/l)	2.67	6.17	3.33	1.21	2.17	3.50	3.33	0.57
Total Bilirubin (mg/dl)	0.08	0.08	0.08	0.00	0.10	0.10	0.10	0.00
Cholesterol (mg/dl)	183.83^a	200.33^b	199.33^b	2.71	197.67^b	192.50^b	184.17^a	1.53
A/G Ratio	1.87	1.83	1.88	0.02	1.92^a	1.98^a	2.12^b	0.03
NA/K Ratio	33.33^x	33.67^x	35.50^y	0.55	34.00	34.83	35.00	1.18
Lipemic Index	5.67	3.33	5.67	1.31	3.67	2.83	6.17	1.58
Hemolytic index	36.50	47.33	46.83	12.07	39.00	32.33	45.83	7.78
Anion Gap	15.57	16.63	16.58	0.56	16.43	15.92	16.03	0.41
Triglycerides (mg/dl)	43.83	46.33	44.83	3.51	36.83	37.50	37.67	1.59
Bicarbonate (mmol/l)	22.88	22.10	21.97	0.57	22.62	23.02	23.08	0.83

^{a,b}Means within a protein source not sharing a common superscript differ (P<0.05) due to fiber source.
^{x,y}Means within a protein source not sharing a common superscript differ (P≤0.10) due to fiber source.
†Pooled SEM.

Table 3.7. Standardized digestibility (%) of amino acids in canine raw meat diets determined using the precision-fed cecectomized rooster assay[1]

Amino acids	Beef			Chicken			SEM[†]	P value		
	Control	Inulin	YCW	Control	Inulin	YCW		Protein	Fiber	Protein*Fiber
Essential										
Arginine	92.29	94.08	94.45	94.54	97.09	95.68	1.026	0.02	0.12	0.69
Histidine	83.92[a]	86.42[ab]	89.28[b]	87.75[a]	91.58[ab]	89.38[b]	1.313	0.01	0.03	0.16
Isoleucine	90.27	92.42	92.50	93.90	95.73	94.28	1.199	0.01	0.27	0.72
Leucine	92.07	94.27	94.35	95.11	96.92	95.40	1.153	0.03	0.24	0.67
Lysine	88.08[a]	90.08[ab]	92.66[b]	87.77[a]	94.37[ab]	92.24[b]	1.829	0.44	0.04	0.36
Methionine	92.26	94.66	94.26	95.19	96.48	95.39	0.915	0.02	0.16	0.62
Phenylalanine	90.83	92.98	93.36	93.63	95.66	94.06	1.270	0.06	0.26	0.66
Threonine	87.95	88.75	89.88	93.06	94.28	92.50	2.124	0.02	0.89	0.76
Tryptophan	95.41	96.12	100.23	100.38	99.06	73.97	10.189	0.47	0.50	0.26
Valine	88.91	91.12	91.34	92.92	94.59	93.25	1.471	0.02	0.42	0.76
Non-Essential										
Alanine	90.88	92.04	91.88	92.76	95.63	93.46	1.271	0.04	0.30	0.70
Aspartic acid	89.68	91.52	91.32	93.07	94.85	92.96	1.294	0.02	0.39	0.75
Cysteine	83.00	87.97	89.13	90.03	94.85	89.83	3.506	0.11	0.39	0.60
Glutamic acid	90.84	93.39	93.71	93.60	95.90	93.75	1.236	0.10	0.17	0.49
Proline	89.66	90.69	90.94	91.79	95.24	92.80	1.982	0.10	0.54	0.76
Serine	89.26	90.97	90.39	92.58	95.74	92.33	2.423	0.11	0.57	0.84
Tyrosine	87.26	90.06	91.64	90.77	93.85	91.77	1.761	0.10	0.21	0.52
TEAA[2]	90.20	92.09	93.23	93.43	95.57	91.61	1.512	0.19	0.41	0.19
TNEAA[3]	88.65	90.95	91.29	92.08	95.15	92.41	1.857	0.07	0.37	0.69
TAA[4]	85.93	86.64	87.90	90.58	92.15	88.73	1.760	0.02	0.77	0.39

[a,b]Means within a protein source not sharing a common superscript differ (P<0.05) due to fiber source.
[1]Data are means of four roosters.
[2]TEAA = Total essential amino acids.

[3]TNEAA = Total non-essential amino acids.
[4]TAA = Total amino acids.
[†]Pooled SEM.