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NITROGEN METABOLISM, MACRONUTRIENT DIGESTIBILITY, AND FECAL
FERMENTATIVE END-PRODUCTS IN DOMESTIC CATS FED EXTRUDED,
RAW BEEF-BASED AND COOKED BEEF-BASED DIETS

BY

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THESIS

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ABSTRACT

The objective of this study was to determine differences in nitrogen (N) metabolism, nutrient digestibility, fecal and urine characteristics, and serum chemistry of domestic cats fed raw and cooked beef-based diets versus a high-protein extruded diet. Nine adult female domestic shorthair cats were utilized in a crossover design. Dietary treatments included an extruded diet [HP; ~57% crude protein (CP)], a raw beef-based diet (RB; ~53% CP), and a cooked beef-based diet (CB; ~52% CP). Cats were housed individually in metabolic cages and fed to maintain body weight. The study consisted of three 21-day periods: days 0-16 were used for diet adaptation; fecal and urine samples were collected on days 17-20; and blood samples were collected on day 21. Food intake was measured daily. During the collection phase, total feces and urine were collected. A fresh urine sample was also collected for urinalysis and acidified for N determination. In addition to total fecal collection, a fresh fecal sample was collected for determination of ammonia, short-chain fatty acid (SCFA), and branched-chain fatty acid (BCFA) concentrations. All feces were scored upon collection using a scale ranging from 1 (hard, dry pellets) to 5 (watery, liquid that can be poured). Blood was analyzed for serum chemistry. Total tract apparent dry matter (DM), organic matter (OM), CP, fat and gross energy (GE) digestibilities were higher ($P<0.05$) in cats fed the RB and CB versus cats fed HP. Nitrogen metabolism differed among treatments. Nitrogen intake and fecal N were lower ($P<0.05$) in cats fed the RB and CB versus cats fed HP, while urinary N was not different among groups. Differences were also noted in fecal fermentative end-product concentrations. Total fecal SCFA concentrations did not differ among dietary treatments; however, molar ratios of SCFA were modified by diet, with cats fed RB and CB having an increased ($P<0.05$) proportion of fecal propionate and decreased ($P<0.05$) proportion of fecal butyrate as compared to cats fed HP. Fecal concentrations of ammonia, isobutyrate, valerate, isovalerate, and total BCFA were higher ($P<0.05$) in cats fed HP compared to cats fed RB and CB. Our results suggest that cooking a raw

meat diet does not significantly decrease macronutrient digestibility or alter N metabolism, yet may minimize risk of microbial contamination. Given the increasing popularity of feeding raw diets and the metabolic differences noted in this experiment, further research focused on the adequacy and safety of raw beef-based diets in domestic cats is justified.

I dedicate this thesis to my parents, Tom and Kathy. I thank my siblings, Tommy and Christine,
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CHAPTER 1: LITERATURE REVIEW

NUTRIENT REQUIREMENTS OF DOMESTIC CATS

Felids are obligate carnivores, and evolutionary influence of a strictly carnivorous diet has resulted in specialized metabolic pathways and nutritional requirements. Within the past 4,000 – 11,000 years, domestic cats (*Felis catus*) were domesticated from the Near Eastern wild cat, *Felis sylvestris libyca* (Driscoll et al., 2009). In the wild, animal tissue provides all nutrients required by felids (Morris, 2002). Feral cats consume multiple small meals per day composed of small mammals with a lower body mass than the feral cats themselves. This behavior is reflected in domestic cats when fed ad libitum, as they will eat multiple small meals over the course of the day (Bradshaw et al., 1996; Bradshaw, 2006).

The digestive tracts of felids are composed of a simple stomach, short digestive tract and well developed canine and carnassiate teeth for tearing and gripping flesh. Thus, they are physically adapted to highly digestible animal prey diets (Kendall et al., 1982). Energy density and nutritional quality in carnivores' prey is relatively constant (Morris et al., 2006). Animal prey are compositionally high in protein, and low in carbohydrate, that is in contrast to many commercial diets that have a much higher carbohydrate concentration requiring adaptations pertaining to nutritional biochemistry. For example, the composition of white-footed mouse (*Peromyscus leucopus*) is 60% crude protein (CP) and 20% fat on a dry matter (DM) basis (Powers et al., 1989), while Hill et al. (2009) reported that 739 commercial extruded diets contained an average of 29% CP and 13% crude fat. Compared to omnivores, felids have evolutionarily lacked the need for rapid adaptation to a variety of diet types and are metabolically prepared for high metabolism of proteins and fat, with less emphasis on utilization of carbohydrates. As a result, felids have many unique requirements including high protein, taurine, and tyrosine requirements, and an obligate requirement for arginine.

Protein

The protein requirement of domestic cats (160 g CP/kg DM for diets containing 4000 kcal ME/kg; NRC, 2006) is 2-3 times higher than omnivores due to a high requirement for disposable protein/N (Rogers and Morris, 1979; Green et al., 2008). Protein metabolism is adaptive in omnivorous species (i.e., enzyme activity correlates with dietary protein concentration). When fed low protein diets, omnivorous species conserve N for re-utilization by decreasing the activity of aminotransferases (first step in amino acid catabolism) and urea cycle enzymes. Domestic cats are limited in their ability to adapt activity levels of these hepatic enzymes. When fed diets at, or greater than, their minimum protein requirement, protein oxidation is increased. Two possible mechanisms are increased liver size and mass action by increased substrate concentration. However, modification of enzyme activity is not believed to play a role. When fed diets below the minimum protein requirement, there is little or no adaptation in the activity of aminotranferases and the urea cycle enzymes; thus, cats are unable to adapt protein oxidation to conserve N (Rogers et al., 1977; Russell et al., 2002; Green et al., 2008).

Arginine

Because of the constitutively high levels of urea cycle enzymes in cats, during an overnight fast, urea cycle intermediates (arginine, citrulline, and ornithine) are depleted and the rate of ammonia removal is decreased and urea synthesis is limited. Upon refeeding, amino acids are deaminated as a source of energy, resulting in high production of ammonia. In the wild, cats ingest animal sources that provide sufficient arginine. Arginine has an anapleurotic effect on the urea cycle, and ammonia is incorporated into urea. However, when fasted cats are refed with an arginine-free diet, urea cycle intermediates remain low, and rapid onset of hyperammonemia occurs. This leads to emesis, lethargy, vocalization, frothing at the mouth, hypersalivation, ataxia, extended limbs, exposed claws, hypothermia, coma, and possibly death (Morris and

Rogers, 1978a, 1978b; Morris, 1985). Unlike most nutrient deficiencies that take days or weeks for symptoms appear, severe symptoms of arginine deficiency in the cat develop 1-4 hours after intake of the arginine-free diet (Morris and Rogers, 1978a, 1978b; Morris, 1985).

When arginine is deficient in non-carnivorous species, ornithine becomes an important source of urea cycle intermediates. In these species, ornithine is synthesized in the intestinal epithelium from glutamate by pyrroline-5-carboxylate (P5C) synthase and ornithine aminotransferase (OAT) and is subsequently converted to citrulline by ornithine carbamoyltransferase. In felids, the synthesis of ornithine and citrulline in the intestine is limited because activity levels of P5C synthase and OAT are very low when compared to rats (Morris, 1985; Rogers and Phang, 1985). Morris et al. (1979) fed arginine-free diets with either additional ornithine or citrulline to adult cats to determine the mechanism of arginine deficiency. The ornithine treatment prevented hyperammonemia, but did not support adequate synthesis of arginine for growth, while the citrulline treatment prevented hyperammonemia, and resulted in similar growth rates as compared to kittens fed a complete diet. The limited ability of the cat to endogenously synthesize citrulline has resulted in an obligate dietary requirement for arginine, and has enabled the cat to conserve N between meals (depletion of urea cycle intermediates limits urea production), while conserving the ability to respond rapidly to high ammonia loads after ingestion of a high protein meal.

Taurine

Taurine is a β -sulphonic amino acid that occurs as a free amino acid in tissues. Hayes and Carey (1975) determined that cats fed a casein diet (27% of calories as protein) had decreased blood (1 nmol/mL) and retinal (25 nmol/mg) taurine concentrations and developed symptoms of retinal degeneration in 3-12 months. Later, Pion et al. (1987) reported that low plasma taurine was associated with cardiomyopathy and symptoms could be reversed with supplemental taurine (0.5 g crystalline taurine twice per day). The dietary requirement of taurine varies with dietary

fiber, digestibility and quantity of protein, and type and composition of the diet (i.e., canned vs. extruded) (Anantharaman-Bar et al., 1994; Stratton-Phelps et al., 2002; Spitze et al., 2003). For example, the suggested NRC (2006) adequate intake level for diets containing 4.0 kcal ME/kg is 1,000 mg taurine/kg for commercial, dry, expanded diets, and 1,700 mg taurine/kg for commercial, canned diets.

Hepatic taurine synthesis from cysteine is limited in cats. Two enzymes in the taurine synthesis pathway, cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD), have low enzyme activities compared to the rat (De La Rosa and Stipanuk, 1985; Rentschler et al., 1986; Park et al., 1991). De La Rosa and Stipanuk (1985) reported that CDO and CSAD activities were 10% of that observed in rats. Instead, cysteine is primarily converted to pyruvate, which may be oxidized as a source of energy, or converted to glucose. Eighty percent of injected ¹⁴C labeled L-cysteine was recovered through the pyruvate oxidation pathway of cysteine metabolism in the cat compared to only 15% in the rat (De La Rosa and Stipanuk, 1985).

A major role of taurine is conjugation of bile acids. When fed taurine-deficient diets, body stores in the cat are depleted leading to decreased plasma taurine concentrations (Hayes and Carey, 1975) and an altered bile acid profile (Rabin et al., 1976). Hickman et al. (1992) determined that cats with low taurine status produced bile containing lower molar ratios of taurine conjugated bile salts (0.657 vs. 0.995), and higher molar ratios of free bile acids (0.31 vs. none detected) and glycocholate bile salts (0.03 vs. 0.005) compared to taurine replete cats. Work in other species suggest that production of bile acid-glycine conjugates in the cat is probably limited by N-acyl transferase, the enzyme that conjugates bile acids (Vessey, 1978; Morris, 2002). When taurine is present, N-acyl transferase has a low affinity for glycine and synthesizes mainly taurine conjugates (Vessey, 1978; Morris, 2002). Preference for taurine by this enzyme results in an obligatory loss of taurine from the body pool.

Tyrosine

Felids have a secondary nutrient requirement for aromatic amino acids, above that required for growth (8.5 g aromatic amino acids/kg diet; NRC, 1986) to maintain black coat color. Melanins are the pigments of hair and skin. Coat color is determined by the balance of eumelanin (black/brown) to pheomelanin (reddish brown) produced by melanocytes. Anderson et al. (2002) reported that cats needed greater than 18 g aromatic amino acids/kg diet to maintain black coat color.

Carbohydrates

Physiological adaptations of felids to the low carbohydrate concentrations of animal prey-based diets have resulted in the absence of or low levels of digestive enzymes and hepatic enzymes involved in glucose metabolism. Domestic cat taste perception predominantly responds to the presence of amino acids (i.e., meat), and unlike many other species, the cat is insensitive to the presence of sugars in the diet (Bradshaw et al., 1996). While they utilize cooked dietary starch efficiently (Morris et al., 1977; Kienzle, 1993b), domestic cats lack salivary amylase to begin starch digestion in the mouth and stomach, have low activities of intestinal and pancreatic amylase, and reduced activity of intestinal disaccharidases compared to omnivores (Kienzle, 1993a).

Disaccharide metabolism also has been affected by evolutionary adaptations in the feline liver. In non-ruminant mammals, enzymatic glucose phosphorylation in the liver by glucokinase is an important step for regulation of glucose uptake and its storage as glycogen. In cats, activity of hepatic glucokinase and glycogen synthetase is minimal and glucokinase is non-adaptive to blood glucose levels. In a comparative study across multiple species (including rabbit, guinea pig, dog, pig, possum, mouse, and rat), Ballard (1965) reported that cats have very low activity of glucokinase (<2 umol/g/hr vs. 75-394 umol/g/hr) and lower rates of glucose incorporation into glycogen (1.5 umol/g/2 hr vs. 11.5-50.7 umol/g/2 hr) compared to other non-ruminants.

However, no indication of diet was given, and dietary parameters could have affected the activity of these enzymes. Washizu et al. (1999) reported no activity or expression of glucokinase in feline liver. High intakes of sucrose or fructose result in fructosemia and fructosuria (Drochner & Müller-Schlösser, 1980; Kienzle 1994a). The mechanism for this response has not been examined.

Lipids

Fat is highly digestible for the cat. Kane et al. (1981a) reported an average fat digestibility of 98.0% when cats were fed diets containing 25% butter, lard, unbleached tallow, yellow grease, or chicken fat. The concentration and type of fat in a diet is important. High-fat diets are associated with increased palatability (NRC, 2006). However, using a two-choice preference test, Kane et al. (1981a) determined that cats preferred diets containing 25% yellow grease over diets containing 10% yellow grease ($P < 0.001$) and diets containing 50% yellow grease ($P < 0.02$). Hill et al. (2009) reported that 739 commercial extruded diets contained an average 13% crude fat. This is lower than values for wild prey such as the white-footed mouse (*Peromyscus leucopus*), which has been reported to be 20% fat on a dry matter (DM) basis (Powers et al., 1989).

Cats, like all mammals, require linoleic acid (ω -6; LA) in their diet. No recommendation has been made for α -linolenic acid (ω -3; LL). Linoleic acid and LL can be converted to the long chain polyunsaturated fatty acids by elongation and desaturation. Linoleic acid is converted to arachidonic acid (ω -6; AA) and LL is converted to eicosapentaenoic acid (ω -3; EPA) and docosahexaenoic acid (ω -3; DHA). Pawlosky et al. (1994) reported that cats have a low activity of Δ 6 desaturase, the first enzymatic step in the conversion of LA and LL to long chain polyunsaturated fatty acids. Due to this low activity, cats may have a conditional requirement for dietary AA, EPA and DHA (Morris, 2004). Macdonald et al. (1984) reported and Morris (2004) confirmed that in male cats, the conversion of LA to AA meets the requirement for reproduction,

while female cats require both LA and AA in the diet for full reproductive capacity. Additionally, LA and LL compete for $\Delta 6$ desaturase, and high LL relative to LA can lead to signs of essential fatty acid deficiency (Morris, 2004). No studies have been performed to determine the absolute requirements of long chain polyunsaturated fatty acids in the cat.

Water

Water needs can be met by drinking or as a component of food. When fed an all meat diet or canned diet, some research has suggested that cats do not need to drink additional water to survive (Kane et al., 1981b). Kane et al. (1981b) reported that while water intake (ml/g DM) from drinking was lower ($P < 0.01$) in cats fed a canned diet (23.4% DM; 0 ml/g DM) compared to cats fed a dry diet (92.3% DM; 1.8 ml/g DM), total water intake (ml/g DM; from drinking and food) was higher ($P < 0.01$) in cats fed canned (3.5 ml/g DM) compared to dry diets (1.9 ml/g DM). Even though cats may be able to survive without drinking water if fed a canned diet, it is not recommended, as differences in water intake have implications for urinary tract health. Higher total water intake may increase urine volume and decrease risk of urinary tract diseases in cats due to lower saturation of urine (NRC, 2006).

MEETING THE NUTRIENT REQUIREMENTS OF DOMESTIC CATS

Wild felines eating live prey and domestic cats fed nutritionally complete foods have little need to select between foods based on nutritional content. For the most part, a pet's diet is provided solely by the owner. Not only does a cat have little ability to dictate the food fed by the owner, but it also appears to have a limited ability to regulate intake based on nutrient content. Depending on the nutrient, cats fed a deficient diet may or may not develop aversion to that food source. Taurine deficient diets induce little aversion (Sturman et al, 1978), while cats rapidly learn to avoid diets deficient in arginine (Morris and Rogers, 1978a; 1978b). There is conflicting

evidence pertaining to the ability of cats to modify their feeding behavior based on energy density (Kanarek, 1975; Hirsch et al., 1978; Castronguay, 1981; Morris et al., 2006) and little evidence to suggest modification based on nutrient content of a meal (Bradshaw et al., 2000). Thus, a cat owner has the responsibility to provide the nutrients necessary for cellular repair and growth, and for health management.

There are a multitude of diet options for a pet owner to choose from, including commercially available extruded and canned diets that are more traditional, unconventional diets (e.g., vegetarian, natural, organic, and raw diets) that have recently increased in popularity, and homemade diets such as raw meat-based diets and home cooked meals. There are advantages and disadvantages associated with different diet types and the one chosen might not always meet the requirements of the animal. Methods to test nutritional adequacy of a pet food include: monitoring body weight, body condition, activity level, complete blood cell counts and serum chemistry profiles; measuring blood taurine concentration; observations of skin and hair color and texture; evaluation of lens and retina of the eye; and stool quality (Remillard, 2008). The diet itself must also be examined, with a focus on the nutrient content of the raw materials, special requirements of the animal, and the influence of processing methods on the bioavailability of the chemical components.

An important part of determining the nutritional adequacy of a diet is determining the nutrient composition. Proximate analysis, a set of methods including analyses for moisture, ash, crude protein, ether extract, crude fiber and nitrogen-free extract, or a slight variation of these methods (i.e., total dietary fiber instead of crude fiber) is commonly used to determine dietary chemical composition. Additionally, profiles of vitamins, minerals, amino acids, and fatty acids are of importance. Diet composition can then be compared to the nutrient recommendations of the cat provided by the National Research Council (NRC; 2006), the Association of American Feed Control Officials (AAFCO) Cat Food Nutrient Profiles (2009), or nutrient concentrations cited in scientific literature. Unfortunately, even with the large amount of feline nutrition

information available, there are still major strides to be made in determining the best diet for maintaining cat health. The recommendations provided by NRC and AAFCO still change periodically.

The determination of a diet's chemical composition does not ensure its nutritional adequacy, because it does not measure the bioavailability of nutrients. For this reason, feeding trials are the preferred method for determining the nutritional adequacy of pet foods. Feeding trials are advantageous because they may uncover unexpected safety issues that cannot be determined by chemical composition alone. As part of the Model Bill, AAFCO provides minimum testing protocols for determining nutritional adequacy during the life stages of adult maintenance, growth, and gestation/lactation.

Association of American Feed Control Official Regulations

The United States pet food industry is regulated by several agencies. Labels for complete and balanced commercial pet diets must contain a statement of nutritional adequacy, method of determination, and the life stage used to substantiate any claims. According to AAFCO (2009), there are three methods to substantiate nutritional adequacy claims. The AAFCO recommendations have no regulatory authority; however, most states have adopted the AAFCO models into their laws and regulations on pet foods and enforce them in this way (Dzanis, 2008).

The "formulation method" requires the diet nutrient composition be formulated to meet the AAFCO Cat Food Nutrient Profiles. The nutrient profile can be determined by calculation from ingredient profiles or by chemical analysis of the final product. The "feeding trial method" requires the manufacturer to perform an AAFCO-protocol feeding trial with the pet food as the sole food source. The feeding protocol outlines the minimum number of animals and length of the study to be used. The animals must be examined by a veterinarian at the beginning and the end of the study and common indicators of nutritional (in)adequacy are examined (e.g., body weight, blood count, blood taurine, etc.). The "family method" allows members of a product

family to claim adequacy if the lead member of the family has passed a food trial. Family members must be nutritionally similar to the lead product in processing type, metabolizable energy content (as determined by metabolizable energy feeding trial), and levels of crude protein, calcium, phosphorus, zinc, thiamin, potassium, and taurine for cat foods.

Traditional Commercial Extruded Diets

Commercial extruded diets are multi-component, nutritionally complete foods with well defined nutrient composition. Diets are available for a wide range of costs. They are convenient and consistent products with assurance of quality and nutritional balance on the label. Hill et al. (2009) compared analyzed values against those on the label for multiple commercial cat diet types including extruded, moist, and canned. Composition of dry diets were not different than their guaranteed analysis for DM, CP, crude fat, and crude fiber. For example, the difference in CP percentage of 739 extruded diets from the minimum CP value provided on their label was $1.6 \pm 2.0\%$.

Generally, extruded diets have high levels of vegetable source proteins, are relatively low in fat, and have low caloric density on a DM basis. Extrusion (i.e., heat, pressure, and moisture) increases availability of starch from plant components, increases nutrient digestibility, and sterilizes the food. Morris et al. (1977) reported that cooking wheat and maize starch increased diet digestibility 2-3% units.

High concentrations of carbohydrates in feline diets have been raised as a concern. Because cats are carnivores and have no absolute requirements for carbohydrates, concerns have been raised about the impact of high carbohydrate diets on diabetes mellitus and obesity. However, Slingerland et al. (2009) reported that while indoor confinement and lack of physical activity increased ($P < 0.05$) risk for diabetes mellitus, amount of dry food in the diet was not correlated ($P = 0.29$).

In a phone survey of 469 cat owners, 95.5% fed $\geq 75\%$ commercial food to their pet, while 2.7% fed $\geq 50\%$ noncommercial food (Michel et al., 2008). Often, cat owners feed noncommercial diets because of concerns about additives, preservatives and contaminants; distrust of pet food companies due to a misunderstanding/inability to understand pet food labels; or need to meet a medical condition. Michel et al. (2008) surveyed cat and dog owners by providing a statement and having respondents scale their attitude towards that statement from 1=strongly agree to 5=strongly disagree. Significant differences between noncommercial and commercial feeders on the processing and commercial foods were reported. Noncommercial feeders responded more negatively ($P<0.05$) towards statements on processing and cooking of pet foods, trust in manufacturers, levels of meat and additives in commercial foods, and the wholesomeness and nutritional adequacy of commercial pet foods.

Recent pet food recalls and widespread media coverage may increase owner mistrust of pet food companies. In the past five years, three large pet food recalls have included traditional diet types. In December 2005, Diamond Pet Foods recalled pet foods due to contamination of Aflatoxin. In April 2006, diets containing toxic levels of vitamin D were recalled by Royal Canin. And most recently in March 2007, Menu Foods, Inc. recalled foods due to contamination with melamine. The melamine contamination involved many manufacturers and diets. In two of these cases, nutritional adequacy of the diets was sound, but the inadvertent inclusion of toxins made the foods unsafe.

Unconventional and Homemade Diets

Popularity of raw foods and other unconventional diets has been increasing over the past decade [Center for Veterinary Medicine (CVM), 2004]) and a growing number of unconventional homemade diets (HMD) are promoted for dogs and cats, especially through internet sources. Food is a basic necessity of life, making the diet an easy way for owners to relate to their pets. In a survey of cat owners in Australia and the United States (Laflamme et al., 2008), 46% of cat

owners watch their cat eat, while 26% eat with their cat. Because food can affect human psychological well being, and has religious and ethical implications, a pet's diet can take on considerable importance for a pet owner. This attitude is reflected in the commercial market with vegetarian diets, raw meat diets, and diets that claim to be more "natural." Laflamme et al. (2008) reported that a majority of cat owners (54%) cite their veterinarian or veterinary staff as a primary source of information about pet nutrition, while approximately 16% reported the internet and other media. This implicates the importance of veterinarians to provide sound nutritional education and advice to their patients, and the role that the internet and other media plays in pet nutrition. While the internet provides a wealth of knowledge on HMD, much of it is unsubstantiated and could be confusing, misleading, and potentially harmful to a pet.

In the past, HMD have been commonly fed in response to a suspected food allergy or to working dogs, including sled dogs and racing greyhounds (Freeman and Michel, 2001; Verlinden et al., 2006). Roudebush (1992) reported that 86% of veterinarians in North America prescribed HMD for cats with suspected food allergy. Due to the nature of allergy testing, 92% of HMD prescribed are nutritionally inadequate and need to be balanced with essential vitamins and minerals if they are to be fed for a prolonged period of time (Roudebush, 1992; Verlinden et al., 2006). Veterinarians choose HMD to meet a pet's specific needs, compose a diet based on nutritional history, and allow owner involvement in the nutritional therapy and control of ingredients (Verlinden et al., 2006).

Advantages of choosing a HMD in a food allergy situation are to a large extent the advantages (specificity and control) that a pet owner will find appealing. Pet owners might turn to HMD because of concerns about additives, preservatives and contaminants; distrust of pet food companies due to a misunderstanding/inability to understand pet food labels; or need to meet a medical condition (Freeman and Michel, 2001; Michel et al., 2008). Homemade diets allow the owner to select the ingredients and their quality, prepare diets without the use of added preservatives, and tailor to the needs of the individual animal. Another reason many owners

desire to feed HMD is the ability to provide their animals with variety from day to day with nutrient balance met over time. Many guidelines for this type of diet are available, including Dr. Pitcairn's Complete Guide to Natural Health for Dogs and Cats (1995) and the BARF (Bones and Raw Food) diet by Dr. Billinghurst (1993). These guidelines are not accompanied with research data, and are based on anecdotal evidence and opinion only.

Pet owners also should take into consideration the risks of a HMD. Homemade diets are often expensive to sustain, preparation is time consuming, and nutritional adequacy is often not determined. Additionally, they can be inconvenient to provide when a pet is boarded, hospitalized, or accompanies its owner during travel. A majority of the disadvantages to a HMD are dependent on the owner determining for themselves if they have the time and resources to devote to a HMD – disadvantages of high cost and time commitment must be weighed against the advantages.

The development of nutritionally inadequate HMD is common, problems are not always foreseeable, and can have detrimental effects (Niza et al., 2003; Polizpoulou et al., 2005). Even the most well intentioned owner can inadvertently prepare a diet that is nutritionally inadequate. The most common problems that arise with HMD revolve around maintaining a nutritionally adequate diet from formulation to feeding, including unbalanced supplementation of vitamins and minerals, and changes in recipe (Remillard, 2008). Additional problems arise for HMD when considering the source of protein used in raw meat diets [inverse calcium (Ca) to phosphorus (P) ratio with most cuts of muscle meat and bacterial contamination], and cooked meat diets (inconsistent nutrient profiles due to cooking method, time, etc.).

Imbalances in macro- and micronutrients from improper diet formulation are common with HMD. Pet owners could potentially provide their pets with too much energy, either by miscalculating the amount of food necessary for their pet, or due to the high nutrient variability of ingredients. Excess energy over time can lead to obesity if not balanced with additional physical activity. Low quality or incomplete proteins also can lead to imbalances. Providing inadequate

amounts of an essential amino acid or N can result in decreased protein synthesis affecting every body system (Steiff and Bauer, 2001).

Major ingredients for HMD are rarely balanced for minerals and vitamins. Streiff et al. (2002) chemically analyzed the composition of 35 HMD for domestic dogs, and compared the data to the AAFCO recommendations at that time. Energy, fat, and protein were above AAFCO recommendations in those diets, while Ca, Ca:P ratio, and vitamins A and E were lower than AAFCO recommendations. Problems can arise when owners fail to understand the importance of properly balancing the diet and when supplements are inconvenient and/or expensive. The proper nutrient supplements can be located in stores, or ordered from the production company.

However, some owners may incorrectly use over-the-counter vitamin and mineral supplements that are not intended for balancing the pet's nutritional intake, potentially resulting in deficiencies of some nutrients while providing excesses of others. Errors in supplementation also can be introduced when feeding or dosing instructions are not provided or are confusing to the owner (Remillard, 2008). Errors in supplementation may lead to nutrient excesses or deficiencies. For example, under-supplementation of Ca can result in loss of bone mineral, and bone pain (Krook et al., 1963), while over-supplementation results in an increased requirement for magnesium, depressed food intake and growth (Howard et al., 1998).

Nutritional adequacy of the diet can be improved if owners use recipes that are formulated to meet all of the nutrient recommendations of the pet and/or use of formulation software to develop balanced diets. Greater assurance could be obtained by testing the final product via chemical analyses to determine the chemical composition, but it still does not measure the bioavailability of nutrients. Very few HMD recipes have been tested by feeding trials (Freeman and Michel, 2001; Streiff et al., 2002). Additionally, the current protocols set by AAFCO may not be appropriate for the ideology behind some HMD trends. Diets that provided high variability in ingredients day to day with balanced nutrients overtime would not meet the criteria of being the sole food source for an AAFCO feeding trial.

Another problem related to nutritional adequacy is that even if a recipe is balanced to provide nutrient levels that meet or exceed the nutrient requirements of the cat, owners tend to deviate from recipes over time. Inappropriate substitutions can be due to an owner's preference, affordability of ingredients, convenience of obtaining ingredients, and mimicry of trends seen in human nutrition. Alterations to balanced recipes should be minimized. If a change to a recipe is made, the formulation must be rebalanced to ensure that it will still meet the needs of the animal.

For some owners, nontraditional, commercially prepared diets such as Bravo! Balance® or Nature's Variety, Inc. raw diets for cats, may be a better choice than HMD. These diets are likely to be less expensive and more convenient, but provide the owner with less control and ingredient variability than a HMD. A major benefit is that like traditional diets, commercially available nontraditional diets usually provide a tested formulation that is known to meet the basic nutritional needs of the animal (Freeman and Michel, 2001).

Raw Meat Diets

The raw meat diet (RMD) is one that has increased in popularity recently. Historically, raw meat has been used in diets for sled dogs and racing greyhounds (Chengappa et al., 1993; Cantor et al., 1997; Hill, 1998; Morley et al., 2006), and more recently, use of RMD for show animals and pets has increased (Freeman and Michel, 2001). There are three major types of raw food diets: commercially available complete RMD, homemade complete RMD, and combination diets (Freeman and Michel, 2001). Commercially available RMD usually provide tested formulations that are known to meet the basic nutritional needs of the animal and do not require additional supplements. As with other unconventional diets, commercial RMD are likely to be less expensive and more convenient than HMD, but with less owner control and ingredient variability. Homemade RMD are nutritionally complete if based on balanced recipes. Recipes can be obtained from books, articles and the internet; however, the owner often has no knowledge of the validity of these diets. For example, while the internet provides a wealth of knowledge on

HMD, much of it is unsubstantiated and could be confusing, misleading, and potentially harmful to a pet. Some popular examples for homemade RMD are the BARF diet (Billinghurst, 1993), the Ultimate diet (Schultze, 1998), and the Volhard diet (Volhard and Brown, 1995). Like most popular HMD, these guidelines are not accompanied with research data, and are based on anecdotal evidence and opinion only. Combination diets are raw meat combined with a commercially available grain and supplement mix (Freeman and Michel, 2001).

Much of the rationale for feeding raw meat is based on the cat's evolutionary history as a carnivore. Additionally, many people who feed RMD believe that heat processing may decrease some of the nutritional benefits in the food, including heat labile nutrients such as thiamin, and potentially destroying functional proteases found in the raw meat (Freeman and Michel, 2001; Berschneider, 2002). Owners who feed RMD anecdotally claim that they improve coat color and quality, increase physical activity levels, improve behavior, improve health and immune function, and reduce incidence of allergies, arthritis, pancreatitis, and parasites (Freeman and Michel, 2001).

Little research has been done on RMD for pets, and most has focused on diets for domestic dogs (Freeman and Michel, 2001; Berschneider, 2002). The benefits of RMD have not been substantiated by well-designed research trials, and there are many potential risks to feeding raw meat diets, including health problems that arise from inclusion of feeding raw bones, potential for nutritional inadequacy, and bacterial contamination present in most raw meats. Research regarding the risks and disadvantages of RMD also is lacking. Further research is needed to highlight the advantages and disadvantages of RMD. Such data would provide owners with enough evidence to allow for educated decisions.

The inclusion of bones in RMD is another potential risk due to the medical complications that can arise after ingestion. No research pertaining to the incidence of complications due to feeding raw bones has been performed. However, there are reports of intestinal obstruction, gastrointestinal perforation, gastroenteritis, and fractured teeth in animals eating raw bones as a

component of RMD (Freeman and Michel, 2001). The CVM (2004) recommends feeding bone only in the ground form to decrease risk of dental and gastrointestinal trauma.

Nutritional inadequacy can arise when RMD are not balanced for Ca and P, resulting in a low Ca:P ratio. A Ca:P ratio of 1:1-2:1 is recommended (AAFCO, 2009). Many meat, grain and vegetable sources used in RMD are high in P and low in Ca. Additionally, it is common for owners to believe that a cat's diet should consist of mainly meat (Remillard, 2008). Calcium is important for the structure of bones and teeth and cellular signaling. Plasma Ca concentrations are carefully regulated. Imbalanced Ca:P can result in abnormal bone metabolism and skeletal problems, including osteomalacia and rickets (Steff and Bauer, 2001). When formulating a HMD, vitamin and mineral supplements meant to balance a RMD are necessary.

The use of sulfur dioxide to preserve fresh meat that is used as pet food can increase risk of thiamin deficiency with RMD, because it inactivates thiamin. This risk is high in countries such as Australia that do not require the use of sulfites to be marked on the label. The Australian veterinary practice has reported multiple cases of thiamin deficiency due to feeding of unmarked sulfite-treated meat (Studdert and Labuc, 1991; Steel, 1997; Singh et al., 2005).

Raw meat diets brought into the home introduce significant risk of pathogenic bacterial infection of the owners and pets. Meat producing animals carry many potentially pathogenic microorganisms including *Salmonella*, *Campylobacter* spp. and pathogenic strains of *Escherichia coli*. The greatest risk of disease with RMD comes from direct contact with raw meat itself. There is also a large risk for humans that handle the bowls and other surfaces that come into contact with it. Very few studies have examined human illness derived from pets (Morse et al., 1976; Sato et al., 2000), but contact with animals is known to increase risk of infection (Fone and Barker, 1994; Wall et al, 1994). Thirty percent of known food-borne illness in humans is due to pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli*. Households with at-risk persons / pets should be cautious about feeding RMD, including but not limited to households

with persons or pets with immune suppressive infections and drug treatments, and households with pregnant, elderly, or young persons (Remillard, 2008).

Bacterial Contamination of Raw Meat Diets

Meat-producing animals carry many potentially pathogenic microorganisms, including the zoonotic bacteria, *Salmonella* and *Campylobacter* spp. and pathogenic strains of *Escherichia coli*. These can be transmitted to pets and humans via direct contact or from the consumption of contaminated food or milk (Fone and Barker, 1994; Wall et al, 1994). There are three major sources of animal tissues for RMD: meat from human-food processing facilities; meat from animals that have died from processes other than slaughter; and meat originally intended for human consumption, but deemed no longer suitable (CVM, 2004).

Raw meat from animals that have died by means other than slaughter, and meat no longer suitable for human consumption are not subjected to rigorous inspection and pose an increased risk of contamination (CVM, 2004). However, all raw meat poses a risk of being contaminated with pathogens. The United States Department of Agriculture (USDA) determines meat grades based on acceptability for human consumption after proper cooking, not fed raw. White et al. (2001) recovered *Salmonella* isolates from 20% of retail ground meat. The frequency of bacterial contamination of red meat products at retail was lower than that seen in poultry. Thirty five percent of ground chicken, and 24% of ground turkey samples were contaminated, while only 6% of beef samples were contaminated (White et al., 2001). Red meat animals undergo a slower slaughter than poultry resulting in decreased contamination from spillage of gut contents. Additionally, red meat animals are chilled for an extended time before entering the food chain. On dry surfaces like that produced during freezing, *Campylobacter* species survive poorly and survival is decreased for some types of *Salmonella* (Humphrey and Jorgensen, 2006). Georgsson et al. (2005) reported a 97-100% decrease (1.57-2.87 log₁₀ colony forming unit/1000 g broiler) in *Campylobacter* spp. after freezing by spray chilling and frozen storage (-20°C) for 31 days.

Campylobacter spp. count decreased further after 73 days, but it does not completely eliminate the pathogenic bacteria.

The greatest risk of infection with RMD comes from the direct contact with raw meat itself. However, the presence of *Salmonella* or *Campylobacter* on food does not necessarily mean that infection will result. The presence of bacterial pathogens in RMD is well documented (Joffe and Schlesinger, 2002; Weese et al., 2005; Harrison et al., 2006; Strohmeyer et al., 2006). Routine surveillance of feed and feed ingredients by the FDA CVM between 2001 and 2004 found 72% of animal-origin feeds were contaminated with *Salmonella* (Ekelman, 2007). In an evaluation of commercial RMD for felines and canines in 2005, 64% were contaminated with *Escherichia coli* and 20% were contaminated with *Salmonella* spp. (Weese et al., 2005).

The number of reported cases of food-borne illness in pets is believed to be underreported (CVM, 2004), and there has been an increase in reports related to raw meat diets and bacterial contamination of animals in recent years (Joffe and Schleglsinger, 2002; Stiver et al., 2003; Morley et al., 2006;). Stiver et al. (2003) examined two cases of salmonellosis in cats. The cats in that study were fed diets containing uncooked beef. The *Salmonella* strains identified by plating were identical to isolates collected from the raw beef used in the diet. Although salmonellosis is considered uncommon in felines, estimates may be lower than the actual incidence. Some clinical signs of salmonellosis include gastroenteritis, weight loss, and anorexia. However, it was reported that 1-18% of cats may be in a state of asymptotic salmonellosis (Stiver et al., 2003).

Although the data available to quantify the risk to human and animal health is sparse, the FDA believes that raw meat as food for animals is a significant health risk when brought into the home (CVM, 2004). The groups at greatest risk for infection and death are the very young, elderly, pregnant women, and the immuno-compromised. In 1996, collectively these groups represented 20% of the United States population, and were expected to increase as a proportion of the population significantly by 2000 (Gerba, 1996). Thirty percent of known food-borne illness

in humans is due to pathogenic bacteria such as *Salmonella* spp. and *E. coli*, and 72% of the deaths due to food-borne illness are caused by bacteria. Furthermore, human cases of food-borne illness from food are underreported and contamination from animal feeds is often not considered in determining the source of infection (Mead et al., 1999).

The contribution that contact with infected pets makes to the *Salmonella*, *Campylobacter*, and *E. coli* diseases has not been accurately assessed. However, human contact with infected farm animals has been shown to increase risk of infection. For example, in a retrospective examination of initial questionnaires completed by patients with *Salmonella typhimurium* DT104, Wall et al. (1994) reported that infection was significantly ($p=0.0001$) associated with contact with ill farm animals (odds ratio = 4.78). Similarly, Fone and Barker (1994) reported that a farming community, Herefordshire, had higher rates of *Salmonella typhimurium* DT104 infection as compared to all of Wales and England.

In 2004, the CVM released a guidance document for industry regarding the use of raw meat and poultry for companion and captive exotic carnivores. Because of the increased health risks posed by bringing raw meat into the home, the FDA does not support the use of raw meat foods for feeding domestic pets. However, because mishandling raw meat/poultry foods can increase risk of illness, they provided this guidance to decrease risk of disease. The CVM recommends that raw meat and poultry products for animal consumption bear “Handling and Guidelines for Safe Use.” These guidelines include: 1) Keep frozen until ready to use; 2) Thaw in refrigerator or microwave; 3) Keep raw meat and poultry separate from other foods; 4) Wash working surfaces, utensils, hands and any other items that touch or contact raw meat or poultry with hot soapy water; and 5) Refrigerate leftovers immediately or discard. The guidance also supports the use of meat that is passed for human consumption by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS).

Cooked Meat Diets

Cooked meat diets (CMD) are an alternative option to feeding RMD. Throughout their guidance for RMD, the CVM (2004) maintains that adequate heat treatment is the most effective and efficient means of reducing risk of food-borne illness. Nutritive content and sensory qualities of foods may be altered by household cooking techniques. Making broad generalizations for cooked meat, however, is difficult given the large range of possible cooking techniques and differences in execution (i.e., oil used, length of time, etc). Additionally, retention is different for each nutrient and varies by meat type.

Although heat treatment is the most effective means of reducing risk from food-borne pathogens, the effectiveness of killing microbes in meat is affected by cooking method, length of time, and bacterial pathogen of interest (Angelotti et al., 1961; Murphy et al., 2004). Microwave cooking is considered the least effective cooking method for destroying microorganisms because of the shorter time and lower temperatures at which food is cooked. The problem can be resolved by wrapping meat in aluminum foil after microwaving to allow the temperature of the inner portions to elevate to that of the external surface temperature. Convection heating is considered a better heating method than microwaving because the meat is heated slowly to a higher internal temperature (Hollywood et al., 1991).

Cooking may also increase the digestibility of certain ingredients/nutrients. This has been well documented with starches (Morris et al., 1977; Kienzle, 1994b). Cooking beef between 50 and 60°C, denatures collagen and causes softening and solubilization of the connective tissue sheaths surrounding the muscle fibers. These changes increase the access to the tissue by proteolytic enzymes and gastric acids, increasing digestibility of the meat. Thus, the cost and time of gastric and intestinal digestion is decreased, thereby increasing the net energy gain. Another benefit of cooking is an increase in tenderness, resulting in easier chewing (i.e., requiring less time and effort; Boback et al., 2007). Increased digestibility and utilization could be beneficial when feeding cats.

However, cooking meat causes loss of water, fat, minerals, and vitamins (Berry and Leddy, 1984; Kimura et al., 1990a; Kimura and Itokawa, 1990b; Love and Prusa, 1992; Riccio et al., 2006) that may negate its benefits. Cooking losses can vary due to cooking method and interactions with other dietary components (Berry and Leddy, 1984). For example, the cooking method affects moisture losses and resulting fat percentage. Microwaving ground beef patties with 19% fat resulted in increased ($P < 0.05$) fat percentage (3% unit), when other cooking methods (charbroiling, convection cooking, frying, broiling, and roasting) had no change or decreased fat percentage (1-2% units; Berry and Leddy, 1984). Although there is a change in fat concentration with cooking, little difference in lipid composition before and after cooking beef is reported. Microwave cooking, for example, results in a slight decrease of LA, LL, and DHA, while eight other fatty acids including oleic acid and EPA show no change (Escharte et al., 2003). A greater change in fatty acid profile is observed when beef is fried with the resulting lipid composition reflecting the oil in which it was fried (Anderson et al., 1976). Losses of water, fat, minerals, and vitamins change the overall composition of the meat, and could potentially decrease the nutritional adequacy of a diet.

Cooking also affects sensory factors for humans, which include aspects of tenderness and juiciness (Yang et al., 1994). Although these factors are based on human preferences, these and other sensory factors may play a large role in palatability for the cat; however, the effect of specific sensory factors due to cooking methods on palatability in the cat has not been tested.

INDICATORS OF NUTRITIONAL ADEQUACY IN DOMESTIC CATS

Digestibility

Nutrient digestibility of diets can differ due to the nature of raw materials, source and type of nutrient, or differences among processing methods. Thus, feeds with similar chemical composition can vary widely in nutrient digestibility. In recent literature, apparent total tract

macronutrient digestibilities of diets fed to cats were highly variable and ranged from 75-92% DM, 74-94% CP, and 82-99% fat. (Hesta et al., 2001; Fekete et al., 2004, 2005; de-Oliveira et al., 2008; Prola et al., 2009). Kane et al. (1981a) reported that cats tolerated 8 different fat sources at 10, 25, and 50% diet on an as-is basis. Average fat digestibility ranged from 90-99%, and differences in source and amount of fat did not affect DM or CP digestibilities. Inclusion of raw starches and carbohydrates also can affect digestibility. Morris et al. (1977) reported that cooking increased starch digestibility of coarsely ground wheat starch from 93% to 96% and coarsely ground maize starch from 80% to 88%. Kienzle (1994b) reported a 14% increase in protein digestibility when maize in the diet was cooked (77%) vs. uncooked (88%).

Vester et al. (2009a) reported that raw beef and horse meat diets were highly digestible (DM: 89-90%; CP: 94-96%; fat: 95-97%) in domestic cats, however, no recent studies have examined the differences in digestibility between raw and extruded diets. Kendall et al. (1982) measured apparent total tract digestibility in cats fed a fresh mince diet (DM: 33.6%; CP: 53.9%) and an experimental dry cat food (DM: 92.1%; CP 22.4%). The fresh mince diet had higher apparent total tract digestibility of all nutrients measured, including DM (94.6% vs. 67.5%), CP (95.7% vs. 76.6%), fat (95.7% vs. 56.2%) and GE (95.0% vs. 72.4%). However, digestibility of the extruded diet fell below those reported in recent literature, and with advances in pet food formulation and manufacturing, digestibility of extruded diets today may be more similar to raw diets.

Nitrogen Metabolism

Nitrogen balance/metabolism studies are important for monitoring nutritional quality of a diet. A N balance study is conducted by measuring N intake, and excretion including urine and feces. Excretion of N is subtracted from the N intake and used as an indicator of the amounts of N absorbed (i.e., N intake – fecal N) and N retained (i.e., N intake – fecal N – urinary N). Positive N retention or balance is an indication of N accretion or growth, while negative N

balance is an indication of N loss. Minimal changes in BW and N balance are indicators that cats are in a reasonably steady metabolic state. With varying protein intakes above protein requirement, N balance in the cat is maintained by adaptive mechanisms, including changes in ureagenesis (Russell et al., 2000), and protein oxidation (Russell et al., 2002; Green et al., 2008). Protein turnover, however, is non-adaptive (Russell et al., 2003). Russell et al. (2000) reported a 340% increase in ureagenesis (19.0 vs. 65.4 mmol N/kg BW/day) when protein was increased from 20% to 70% of dietary energy. In a subsequent study, Russell et al. (2003) used ¹⁵N stable isotopic measurements to test the effects of a 50% decrease in protein as a percentage of energy, reporting a decrease in both protein synthesis and breakdown. The decreased protein breakdown matched the decreased protein synthesis (i.e., protein turnover remained the same), resulting in maintenance of N metabolism.

Cats have been reported to have N balance values, expressed as g N retained/kg BW/d, from 0.08 to 0.29 when fed to maintain BW (Funaba et al., 2001; 2005; Riond et al., 2003; Green et al., 2008). Errors in N balance technique lead to apparent positive balance at high protein intakes. The two main sources of experimental error are failure to measure all uneaten food (N intake), and failure to collect all N losses. Underestimation of N loss occurs with incomplete collection of urine and feces, major routes of N loss in cats, and because minor losses of N from skin, hair, claws, saliva, etc. are not accounted for in the technique. Overestimation of N intake due to failure to measure uneaten food is exacerbated with increasing dietary protein concentrations.

Fermentation

Fiber has long been considered to provide health benefits to the colon of humans, swine, rats, etc. Because of its carnivorous origins and relatively small colon (~20% of digestive tract length) and lack of cecum, fermentation of dietary fibers in domestic cats has been historically under-researched. In the late 20th century, attention to dietary fiber for companion animals

increased, and fiber has become a common component in dog and cat foods. In the cat, inclusion of dietary fiber is known to alter gut morphology. Fiber inclusion increases the colonic weight and mucosal cell activity in cats, including enhanced mucosal tissue energetics and SCFA absorption (Bueno et al., 2000a, 2000b). Bueno et al. (2000a) reported an increase ($P < 0.05$) in colonic weight in cats fed diets with cellulose (38% increase; 12.8 g colonic weight/kg BW) or pectin/gum arabic (26% increase; 10.8 g colonic weight/kg BW) fiber sources compared to cats fed a non-fiber treatment (9.3 g colonic weight/kg BW). These effects on colonic weight and increased mucosal activity may be due to tactile response from distention or abrasion of gut surface, or by chemical response to the fermentative end-products of microbial breakdown of fiber.

Microbial populations of the cat are capable of degrading highly fermentable fibers (e.g., citrus pectin, guar gum, locus bean gum), but less capable of fermenting others (e.g., solka floc). In vitro organic matter disappearance (OMD) often used as an indirect measure of fermentability, is widely variable among substrates when using feline fecal inocula (Sunvold, 1995a; 1995b). Sunvold (1995b) reported in vitro OMD from 1% with Solka Floc to 84% with citrus pectin. Results from the in vitro fermentation OMD technique, using feces as the inoculum source was highly correlated ($R^2 \geq .90$; $P < 0.05$) with in vivo total dietary fiber (TDF) digestibility (Sunvold, 1995c). For example, fermentability of beet pulp using feline inoculum in vitro OMD calculations was estimated to be 35%, while in vivo data from cats fed diets with beet pulp as the primary fiber source had 38% apparent total tract TDF digestibility.

Dietary fiber type and amount can affect microbial populations (Terada et al., 1993; Sunvold et al., 1995a; Bueno et al., 2000b). Terada et al. (1993) reported increased fecal *Bifidobacteria* and decreased *C. perfringens* numbers in cats supplemented with lactosucrose (50 mg/kg/d for two wk), a non-digestible oligosaccharide. Bueno et al. (2000b) reported alterations in fecal aerobic and anaerobic bacterial counts, and colonic flux of electrolytes and SCFA in cats fed differing fiber types (e.g., non-fiber, cellulose, beet pulp, and pectin/gum arabic). These data

support the notion that altered intestinal microflora and fermentative activity occur with changes in amount and types of fermentable dietary fiber in felines. Changes in fermentative activity can be due to many factors, including increased microbial enzymatic activity, increased microbe numbers, altered microbial population or a combination of these factors.

Dietary fiber also may play a role the digestibility of other dietary constituents as well. Total tract dietary fiber digestibility can range from 6-51% in cats (Sunvold et al., 1995b). Highly fermentable, viscous fibers may also interfere with the absorption of other nutrients in cats. Sunvold (1995b) reported lower ($P < 0.05$) DM (61.3%) and N (59.0%) digestibility in cats fed a diet containing a rapidly fermentable fiber blend (35% citrus pectin, 30% locust bean gum, 20% carob bean gum, and 15% guar gum) as compared to cats fed a non-fiber treatment (DM: 88.0%; N: 86.7%).

Meat-based diets may have additional materials not normally considered as fiber that analyze as TDF in the lab. Protein-based polysaccharides found naturally in animal meat protein products are not susceptible to cleavage by endogenous digestive enzymes, but may act as fiber and are available for fermentation (Banta et al., 1979). The physiological effects of these components have not been examined in cats.

DOMESTIC CAT AS A MODEL FOR SMALL CAPTIVE EXOTIC FELIDS

Of the 36 extant non-domestic felid species, 16 are endangered/threatened (US Fish and Wildlife Services, 2009). They are a diverse group of species, exhibiting a wide range of body weights (2.5 to >250 kg), behaviors, and dietary habits. Felids are euphagous, primarily feeding on one to a few species of prey for a majority of their meals, but opportunistically eat 20-30 prey species (Lindburg, 1988). Small exotic felids include 27 species that are <20 kg, such as bobcats (*Lynx rufus*), African wildcats, and sand cats (Mellen, 1997). Prey species depend largely on body size, regional availability, and opportunity. For example, Radloff and Du Toit (2004)

examined >4000 kills reported for lions, leopards, cheetahs, and African wild dogs. There was a significant ($r^2 = 0.0.86$, $P=0.002$) relationship between mean prey mass and predator mass, and while, minimum prey mass was not related, maximum prey mass was related to predator mass ($r^2 = 0.71$, $P=0.017$) Smaller felids typically eat rodents, other small mammals, and birds.

The nutrient requirements of captive exotic felids have not been determined. Nutrient requirements of domestic cats are the primary resource when formulating diets for captive exotics. To our knowledge, there is only one peer-reviewed article comparing nutrient digestibility in domestic cats to large exotic felids fed the same diet (Vester et al., 2009a); however, none have compared them to small exotic felids. Vester et al. (2009a) evaluated the effects of species (domestic cats, cheetahs, jaguars, and Malayan and Amur tigers) and diet (horsemeat- and beef-based diets) on apparent macronutrient digestibility and fecal characteristics. Few interactions of diet and species were reported, indicating that all species responded in a similar manner to dietary modification, and the domestic cat appears to be an appropriate model for these responses.

Observations of wild felids are also utilized for diet formulation, including feeding habits, scat analysis, and composition of prey. However, composition of prey species is rarely determined, and observations of feeding habits and scat analysis can be of limited use without determination of prey composition. Digestibility trials in captive exotic species, when possible, are also important references. However, peer-reviewed literature on digestibility efficiencies in felids are lacking and primarily focus on large felids (Morris et al., 1974; Barbiers et al., 1982; Wynne, 1989).

The ability of zoological parks to obtain digestibility data can be limited by the number of animals and species available for trials, and housing conditions. Natural exhibits and group housing situations decrease the ability to accurately measure food intake and fecal output. Additionally, a major source of raw meat for such diets includes excess connective and other tissues after slaughter that are highly variable and high in fat. The resulting diets are also highly

variable in nutrient composition. For example, reported dietary DM, CP, and fat for exotic species ranges from 29-40%, 38-84%, and 8-38%, respectively (Barbiers et al., 1982; Hackenburger, 1983; Wynne, 1989; Crissey, 1997; Edwards, 2001, 2007; Betchert, 2002). Reported values for apparent digestibility are also highly variable [DM: 66-89%, CP: 73-96%, fat: 73-99% (Barbiers, 1982; Wynne, 1989; Crissey, 1997; Edwards, 2001; Vester et al., 2009a; 2009b)

Raw meat increases risk of bacterial contamination in the zoo setting (Clyde et al. 1997; Crissey et al., 2001). Irradiation has been examined as a possibility (Crissey et al., 2001); however, because of high cost this is not a viable option for most zoos. Kibble may be an alternative option for small exotic felids. Few studies have examined raw meat diet and extruded diets in small captive exotics (Crissey et al., 1997; Vester et al., 2009b)

Crissey et al. (1997) reported numerical differences in apparent total tract digestibility between sand cats fed a chicken and soy-based extruded diet (DM: 94%, CP: 40.2%) and a raw horsemeat-based diet (DM: 32%, CP: 57.2%). Dry matter (84%), CP (92%) and GE (90%) digestibilities of the raw meat-based diet were higher ($P < 0.05$) than that of the extruded diet (DM: 73%, CP: 78%, GE: 77%). Because of a confounded study design, however, statistical analysis was not possible for this study.

Vester et al. (2009b) compared apparent total tract digestibility and N metabolism in African wildcats fed a high-protein extruded diet (DM: 94%, CP: 52.9%, fat: 23.5%) and raw beef-based diet (DM: 38.2%, CP: 44.9%, fat: 36.9%). Apparent total tract DM, OM, fat, and GE digestibilities were numerically higher in African wildcats fed the raw beef-based diet as compared to the extruded diet, but not significantly different. Apparent total tract CP digestibility was higher ($P < 0.05$) in African wildcats fed raw beef-based diets (91.7% vs. 84.1%). Nitrogen intake (g/d) and fecal output (g/d) were higher ($P < 0.05$) in African wildcats fed the extruded diet. Nitrogen balance in cats fed both dietary treatments were positive (0.8 and 2.0 g/d). Few

alterations of blood metabolites were reported. It appears that a high-protein kibble diet is an adequate replacement for meat.

Details regarding nutrient metabolism by small exotic felids are still unclear, and further research is necessary. Domestic cats are an important resource for basic nutritional requirements used in diet formulation; however, this relationship has not been evaluated. Further research comparing domestic cats to small exotic felids is warranted.

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CHAPTER 2: NITROGEN METABOLISM, MACRONUTRIENT DIGESTIBILITY, AND FECAL FERMENTATIVE END-PRODUCTS OF DOMESTIC CATS FED EXTRUDED, RAW BEEF-BASED AND COOKED BEEF-BASED DIETS

ABSTRACT

The objective of this study was to determine differences in nitrogen (N) metabolism, nutrient digestibility, fecal and urine characteristics, and serum chemistry of domestic cats fed raw and cooked beef-based diets vs. a high-protein extruded diet. Nine adult female domestic shorthair cats were utilized in a crossover design. Dietary treatments included an extruded diet [HP; ~57% crude protein (CP)], a raw beef-based diet (RB; ~52% CP), and a cooked beef-based diet (CB; ~52% CP). Cats were housed individually in metabolic cages and fed to maintain BW. The study consisted of three 21-day periods: days 0-16 were used for diet adaptation; fecal and urine samples were collected on days 17-20; and blood samples were collected on day 21. Food intake was measured daily. During the collection phase, total feces and urine were collected. A fresh urine sample was also collected for urinalysis and acidified for N determination. In addition to total fecal collection, a fresh fecal sample was collected for determination of ammonia, short-chain fatty acid (SCFA), and branched-chain fatty acid (BCFA) concentrations. All feces were scored upon collection using a scale ranging from 1 (hard, dry pellets) to 5 (watery, liquid that can be poured). Blood was analyzed for serum chemistry. Total tract apparent dry matter (DM), organic matter (OM), CP, fat and gross energy (GE) digestibilities were higher ($P < 0.05$) in cats fed the RB and CB vs. cats fed HP. Nitrogen metabolism differed among treatments. Nitrogen intake and fecal N were lower ($P < 0.05$) in cats fed the RB and CB vs. cats fed HP, while urinary N was not different among groups. Differences were also noted in fecal fermentative end-product concentrations. Total fecal SCFA concentrations did not differ among dietary treatments;

however, molar ratios of SCFA were modified by diet, with cats fed RB and CB having an increased ($P<0.05$) proportion of fecal propionate and decreased ($P<0.05$) proportion of fecal butyrate as compared to cats fed HP. Fecal concentrations of ammonia, isobutyrate, valerate, isovalerate, and total BCFA were higher ($P<0.05$) in cats fed HP compared to cats fed RB and CB. Our results suggest that cooking a raw meat diet does not significantly decrease macronutrient digestibility or alter N metabolism, yet may minimize risk of microbial contamination. Given the increasing popularity of feeding raw diets and the metabolic differences noted in this experiment, further research focused on the adequacy and safety of raw beef-based diets in domestic cats is justified.

INTRODUCTION

Felids are strict carnivores and evolutionary influence of a strictly carnivorous diet has resulted in specialized metabolic pathways and nutritional requirements. The digestive tracts of felids are composed of a simple stomach, short digestive tract and well developed canine and carnassiate teeth for tearing and gripping flesh. Thus, they are physically adapted to highly digestible animal prey diets (Kendall et al., 1982). Animal prey are compositionally high in protein, and low in carbohydrate that is in contrast to many commercial diets that have a much higher carbohydrate concentration.

Compared to omnivores, felids have evolutionarily lacked the need for rapid adaptation to a variety of diet types, and are metabolically prepared for high metabolism of proteins and fat, with less emphasis on utilization of carbohydrates. Unique requirements of domestic cats include high requirement for protein and taurine, need for preformed vitamins A and D, and an obligate requirement for arginine.

The primary role of a felid diet is to provide a mixture of ingredients that will meet these unique metabolic requirements. Traditionally, consumers have fed commercially prepared, nutritionally-complete extruded and canned diets; however, there is an increasing trend for the

feeding of unconventional diets (e.g., vegetarian, natural, organic, and raw diets). The raw meat diet is one type that has increased in popularity recently (CVM, 2004). Historically, raw meat has been used in diets for sled dogs and racing greyhounds (Chengappa et al., 1993; Cantor et al., 1997; Hill, 1998; Morley et al., 2006), and more recently, use of raw meat diets for show animals and pets has increased (Freeman and Michel, 2001).

Raw meat diets are a source of contamination for potentially pathogenic microorganisms, including *Salmonella*, *Campylobacter* spp. and pathogenic strains of *E. coli* to the pet and handler. The greatest risk of disease with raw meat diets comes from direct contact with raw meat itself. There is also a large risk for humans that handle the bowls and other surfaces that come into contact with it. Few studies have examined human illness derived from pets (Morse et al., 1976; Sato et al., 2000); however, the presence of bacterial pathogens in raw meat diets has been well documented (Joffe and Schlesinger, 2002; Weese et al., 2005; Harrison et al., 2006; Strohmeyer et al., 2006). It is estimated that 1-18% of cats may be in a state of asymptomatic salmonellosis (Stiver et al., 2003), and the number of reported cases of food-borne illness in pets is believed to be underreported (CVM, 2004).

In 2004, the CVM released a guidance document for industry regarding the use of raw meat and poultry for companion and captive exotic carnivores. Because of the increased health risks posed by bringing raw meat into the home, the FDA does not support the use of raw meat foods for feeding domestic pets. Throughout their guidance for raw meat diets, the CVM (2004) maintains that adequate heat treatment is the most effective and efficient means of reducing risk of food-borne illness. However, the effectiveness of killing microbes in meat is affected by cooking method, length of time, and bacterial pathogen of interest (Angelotti et al., 1961; Murphy et al., 2004).

The nutritional adequacy of raw and cooked meat diets for cats has not been adequately studied. Vester et al. (2009a) reported that raw beef and horsemeat diets were highly digestible [dry matter (DM): 89-90%; crude protein (CP): 94-96%; fat: 95-97%] in domestic cats.

However, no recent studies have examined macronutrient digestibility or N metabolism between raw and extruded diets in domestic cats. Although evidence for reduced digestibility of extruded vs. raw diets exists in the literature for sand cats (Crissey et al., 1997), continued advances in pet food formulation and manufacturing have resulted in high quality extruded diets that may have greater digestibility.

Also of interest is the use of the domestic cat as a model for small captive exotic felids. The ability of zoological parks to obtain digestibility data can be limited by the number of animals and species available for trials, and housing conditions. Small exotic felids include 27 species that are <20 kg and includes bobcats (*Lynx rufus*), African wildcats, and sand cats (Mellen, 1997). Because nutrient requirements of captive exotic felids have not been determined, those of domestic cats are the primary resource when formulating diets. To our knowledge, there is only one peer-reviewed article comparing nutrient digestibility in domestic cats to large exotic felids fed the same diet (Vester et al., 2009b); however, none have compared domestic cats to small exotic felids. Extruded diets may be an alternative option for small exotic felids, but few studies have examined them against raw meat diets in small captive exotics (Crissey et al., 1997; Vester et al., 2010)

Vester et al. (2010) compared apparent total tract macronutrient digestibility and N metabolism in African wildcats fed a high protein extruded diet (DM: 94%; CP: 52.9%; fat: 23.5%) and a raw beef-based diet (DM: 38.2%; CP: 44.9%; fat: 36.9%). Apparent total tract DM, OM, fat, and GE digestibilities were numerically higher in African wildcats fed the raw beef-based diet as compared to extruded diet, but not significantly different. Apparent total tract CP digestibility was higher ($P<0.05$) in African wildcats fed the raw beef-based diets (91.7% vs. 84.1%). Nitrogen intake (g/d) and fecal output (g/d) were higher in African wildcats fed the extruded diet. Nitrogen balance in cats fed both dietary treatments were positive (0.8 and 2.0 g/d). Few alterations of blood metabolites were reported.

Details regarding nutrient metabolism by small exotic felids are still unclear, and further research is necessary. Domestic cats are an important resource for basic nutritional requirements used in diet formulation; however, this relationship has not been evaluated. The objective of this study was to compare apparent total tract nutrient digestibility, fecal characteristics, N balance, and blood metabolite concentrations between a high-protein extruded diet, and raw and cooked meat-based diets fed to domestic cats. We hypothesized that all diets would have similar total tract apparent nutrient digestibilities, N retention, and fecal characteristics; therefore, raw and cooked meat diets may be a suitable replacements for high-protein extruded diets.

MATERIALS AND METHODS

Experimental Design and Animals

Nine healthy, intact adult female domestic shorthair cats (*Felis catus*; mean age = 1.51 ± 0.03 y; mean BW = 3.12 ± 0.19 kg) were utilized in a crossover design consisting of three, 21-d periods. Each period included a 16-d adaptation phase, followed consecutively by a fecal and urine collection phase (d 17-20) and blood collection (d 21). Cats were housed individually in stainless steel cages (0.61 m x 0.61 m x 0.61 m) at the University of Illinois in a temperature- (21°C) and light-controlled (14 h light:10 h dark) room. Water was provided ad libitum. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC) prior to animal experimentation.

Diets

Cats were randomly allotted to one of three dietary treatments (Table 2.1) at the beginning of the experiment: 1) a dry extruded diet [HP; 57% CP, 17% fat; Natura Manufacturing, Inc., Fremont, NE]; 2) a raw beef-based diet (RB; 53% CP, 21% fat; Nebraska Brand® Special Beef Feline, Nebraska Packing, Inc., North Platte, NE) or 3) a raw beef-based

diet (Nebraska Brand® Special Beef Feline, Nebraska Packing, Inc., North Platte, NE) that had been cooked prior to feeding (CB; 52% CP, 18% fat).

The raw beef-based diet used for treatments 2 and 3 was stored frozen until 1-3 d before feeding, when it was thawed in a refrigerator. On the day of feeding, the raw beef-based diet for treatment 3 was cooked in a microwave 45-60s to an internal temperature of at least 160°F (71 °C), which adheres to the safe food handling procedures recommended for ground beef by the USDA (2002), and then cooled to room temperature. To minimize microbial growth of the cooked and raw beef-based diets, cats on these treatments were fed twice daily. The extruded diet was stored in a cool dry place until feeding.

Cats were fed to maintain BW, and food offered and refused was measured daily. Food refusals of beef-based diets were dried at 105°C to allow measurement of DM intake. All diets were formulated to meet or exceed the nutrient requirements of domestic cats (NRC, 2006).

Sample Collection

Diet sub-samples were collected and stored at -20°C. Sub-samples were composited for each diet, lyophilized in a Dura-Dry MP microprocessor-controlled freeze-dryer (FTS Systems, Stone Ridge, NY), and ground with dry ice through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ).

During the collection phase (d 17-20), total fecal and urinary output were collected. To ensure complete collection, cats were acclimated to a multi-tier litter box. A freshly voided urine sample was obtained during the collection phase for complete urinalysis. The remaining urine was acidified immediately after urination with 10 mL of 2N HCl to prevent loss of N. Acidified urine of individual cats was composited by period and stored at -20°C until further analysis.

A fresh fecal sample (within 15 min of defecation) was obtained during the collection phase. The fresh fecal sample was weighed and aliquots were obtained. A 3-4 g aliquot was immediately mixed with 5 mL 2N HCl to minimize loss of volatile components. All fresh fecal

aliquots were stored at -20°C until further analysis. Total fecal output for each period was collected, composited, dried at 55°C, and ground through a 2-mm screen in a Wiley Mill (intermediate, Thomas Scientific, Swedesboro, NJ).

On the final day of each period (d 21), 4 mL of blood was collected by jugular venipuncture. Prior to collection, cats were fasted overnight. Samples were immediately transferred to glass BD vacutainer® SST™ tubes (BD, Franklin Lakes, NJ) and stored on ice. All tubes were centrifuged within 1 h of collection at 1100-1300 x g for 15 min at 4°C. The supernatant was collected and stored at -80°C.

Chemical Analyses

Diets and feces were analyzed for DM and organic matter (OM) according to AOAC (2000); fat concentration by acid hydrolysis according to AACC (1983) followed by ether extraction according to Budde (1952); and gross energy (GE) by bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL). Dietary and fecal CP, and urinary N were determined according to AOAC (2000) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, MI). Diet samples were analyzed for total dietary fiber (TDF) according to Prosky et al. (1992). Prior to the TDF procedure, high fat (>15%) and very high fat (>30%) samples were incubated overnight, in 15 or 30 mL of 2:1 chloroform:methanol, respectively, and then filtered through 8 layers of dacron. Because the diets were high in protein, water bath times were increased to 1 h, and amounts of Termamyl solution 120L (0.2 mL) and protease P-5380 (0.5 mL) were greater than the standard assay.

Upon collection, all fecal samples were scored using the following scale: 1 = hard, dry pellets; 2 = dry, well formed stools; 3 = soft, moist, formed stool; 4 = soft, unformed stool; and 5 = watery, liquid that can be poured. Fresh fecal pH was determined immediately upon collection using an Accumet 1001 pH meter (Fischer Scientific, Inc, Pittsburg, PA) equipped with a MI-410 micro-combination pH electrode probe (Microelectrodes, Inc., Londonderry, NH).

Fresh fecal concentrations of ammonia, short chain fatty acids (SCFA; acetate, propionate, butyrate) and branched chain fatty acids (BCFA; isovalerate, valerate, isobutyrate), were determined from the acidified aliquot. Ammonia concentration was determined according to Chaney and Marbach (1962). Short chain fatty acid and BCFA concentrations were determined as described by Faber et al. (2009).

Serum metabolite concentrations were determined using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA) by the University of Illinois Veterinary Diagnostic Laboratory.

Statistical Analysis

All data were analyzed using the Mixed Models procedure of SAS® (SAS Institute, Cary, NC). The fixed effect of dietary treatment was tested. Cat and period were considered random effects. Fecal score data were compared using the GLIMMIX procedure of SAS. A $P < 0.05$ was considered statistically significant and a $P < 0.10$ was considered to be a trend. Reported pooled standard error of the means (SEM) were determined according to the Mixed Models procedure of SAS.

RESULTS

Food intake (g DM/d and kcal/d) was higher ($P < 0.05$) in cats fed HP compared to cats fed RB and CB, and in cats fed RB compared to CB (Table 2.2). Fecal output and fecal output (g as-is)/intake (g DM) were higher ($P < 0.05$) in cats fed HP compared to those fed RB and CB. Urine specific gravity and pH did not differ between dietary treatments.

Apparent total tract DM, OM, CP, fat, and energy digestibilities were greater ($P < 0.05$) when cats consumed RB and CB compared to cats fed HP (Table 2.3). Dry matter digestibility tended to be higher ($P < 0.10$) in cats fed RB compared to cats fed CB. Cats had higher ($P < 0.05$) consumption and fecal excretion of N when fed the HP compared to cats fed RB and CB.

Urinary N excretion did not differ among dietary treatments. Nitrogen balance was higher ($P<0.05$) in cats fed HP compared to cats fed CB.

Fecal DM did not differ among dietary treatments (Table 2.4). Fecal scores and ammonia concentrations for cats fed HP were higher ($P<0.05$) compared to cats fed RB and CB. Fecal propionate concentrations in cats fed CB were higher ($P<0.05$) compared to cats fed HP, and tended to be higher ($P<0.10$) in cats fed CB compared to cats fed RB. Fecal butyrate concentrations in cats fed CB and RB were lower ($P<0.05$) compared to cats fed HP. Total fecal SCFA concentrations did not differ among dietary treatments; however, molar ratios of SCFA were modified by diet, with cats fed RB and CB having an increased ($P<0.05$) proportion of fecal propionate and decreased ($P<0.05$) proportion of fecal butyrate as compared to cats fed HP (Figure 2.1). Fecal concentrations of isobutyrate, valerate, isovalerate, and total BCFA were higher ($P<0.05$) in cats fed HP compared to cats fed RB and CB.

Dietary treatment affected ($P<0.05$) food-restricted serum concentrations of creatinine and triglycerides (Table 2.5). Serum creatinine concentration was higher ($P<0.05$) in cats fed RB and CB compared to cats fed HP. Serum triglyceride concentration was higher in cats fed CB compared to cats fed HP. All other serum metabolites did not differ between dietary treatments.

DISCUSSION

Feeding commercially prepared, raw meat-based diets to captive exotic felids is common in zoological parks, and the use of raw meat diets in the home for domestic cats is growing. However, there are few peer-reviewed trials that have examined the digestibility of raw meat-based diets in exotic (Crissey et al., 2001; Vester et al., 2008; 2009a; 2010) and domestic felids (Vester et al., 2009a). Moreover, exposure to raw meat increases the risk of bacterial contamination and illness to humans and animals. Feeding a commercially available, nutritionally complete extruded diet or cooking raw meat-based diets are two ways to decrease risk of bacterial contamination. A comparison of these three diet types has not been performed

in domestic cats; however, Vester et al. (2010) compared a commercially available extruded diet and a raw beef-based diet in African wildcats. This study was designed to determine the nutritional quality of raw and cooked beef-based diets for domestic cats.

Digestibility

All diets tested in this experiment were highly digestible. Diet influenced apparent total tract macronutrient digestibilities, which may have been due to differences in ingredient composition, macronutrient composition, or processing procedures of the diets. Apparent total tract macronutrient digestibility values in cats fed HP were within ranges reported in recent literature (Fekete et al., 2004, 2005; de-Oliveira et al., 2008; Prola et al., 2009). Beef-based diets, RB and CB, tested in this study had similar macronutrient digestibilities. The authors are unaware of any experiments that have determined the digestibility of a cooked meat-based diet in domestic cats. Vester et al. (2009a) fed domestic cats raw-beef diets of ingredient composition similar to those fed in this study; however, macronutrient composition differed. Dietary CP in that study was 5-6% units higher and fat was 6-8% units higher than that of the RB fed in this study. Apparent total tract DM, CP, fat, and GE digestibilities were similar to those observed in that study; however, OM digestibility in this study was 5% units lower than in that study. This difference could be due to differences in macronutrient composition (i.e., CP, fat). Percentage of TDF could also have influenced the results, however, Vester et al. (2009a) did not report fiber values.

The differences in digestibility observed between cats fed HP and cats fed RB were similar to previous studies in African wildcats (Vester et al., 2010) and sand cats (*Felis margarita*; Crissey et al., 1997). In Vester et al. (2010), diets fed to African wildcats had an identical ingredient composition to those fed in the current study: high protein extruded diet (DM: 94%, CP: 52.9%, fat: 23.5%) and raw beef-based diet (DM: 38.2%, CP: 44.9%, fat: 36.9%). Apparent total tract DM, OM, fat, and GE digestibilities were numerically higher in African

wildcats fed the raw beef-based diet as compared to extruded diet, but not significantly different. Apparent total tract CP digestibility was higher ($P<0.05$) in African wildcats fed raw beef-based diets (91.7% vs. 84.1%). Similarly, we observed a 12% unit decrease in CP digestibility in cats fed HP compared to cats fed RB; however, we also noted decreased ($P<0.05$) DM (9% unit decrease), OM (7% unit), fat (4.2% unit), and GE (6.8% unit) apparent total tract digestibilities in cats fed HP. This discrepancy could be due to a larger sample size used in our study ($n=9$ vs. $n=4$). Despite the statistical differences, our apparent total tract DM, OM, fat, and GE digestibilities in domestic cats fed RB were nearly identical to those reported by Vester et al. (2010), while digestibility values in domestic cats fed HP were 3-4% units lower than those reported for African wildcats. This suggests that our HP diet may have been less digestible than the extruded diet fed by Vester et al. (2010), which was the same formulation.

Crissey et al. (1997) measured numerical differences in apparent total tract digestibility between sand cats fed a chicken and soy-based extruded diet (DM 94%, CP 40.2%) and a raw horse-meat based diet (DM 32%, CP 57.2%). Dry matter, CP and GE digestibilities were 11% units, 14% units, and 13% units higher, respectively, in sand cats fed the raw-meat based diet than sand cats fed the extruded diet. In the current study, smaller digestibility differences were observed when comparing cats fed raw and extruded diets. Apparent total tract DM, CP and GE digestibilities of each diet type from the current study were higher than those reported in sand cats, and the magnitude of the difference was greater in extruded diets (5% units higher) than raw-meat diets (3% units higher). Differences observed between studies could also be due to differences in ingredient and macronutrient composition of diets tested.

Food Intake and Nitrogen Balance

Although dietary treatment influenced food intake (g/d DM and kcal/d), and all cats had positive N balance, BW was maintained throughout the experiment. These results were likely due to the differences noted in GE digestibility. For example, although energy intake was higher

in cats fed HP than cats fed RB and CB, the GE digestibility was 6% units lower. Positive N balance, with BW maintenance has been reported in other experiments (Funaba et al., 2001; 2002; Green et al., 2008; Vester et al., 2010). In N balance experiments, it is assumed that missing food has been eaten, and all urine and feces were collected, resulting in overestimates of N intake and underestimates of N excretion. Our results are similar to those reported in previous experiments.

Nitrogen balance also differed among treatments. In cats fed HP, N balance was 1.2 g/d, while in cats fed CB, N balance was close to zero. Although cats fed CB had 11% units higher ($P < 0.05$) absorption as a percent of N intake than cats fed HP, N intake (g/d) was only 67% of that in cats fed HP, and retention as a percentage of N intake was numerically 21% unit less than that of cats fed HP.

Nitrogen balance data for RB and HP were lower than values (0.8 and 2.0 g/d) reported by Vester et al. (2010) in African wildcats fed diets similar in ingredient composition and type. Percentages of N absorbed (RB: 88%; HP: 97%) and N retained (RB: 38%; HP: 28.9) were higher than those in the current study, while excretion of N as % of N intake (RB: 44% urine N, 12% fecal N as % of N intake; HP: 61.5% urine N, 3.0% fecal N as % of N intake) was lower. Most differences in N metabolism [N intake (g/d), fecal N (g/d; % N intake), N absorption (% N intake)] between African wildcats fed RB and those fed HP were similar to those observed in the current study. In both studies, there was a numerical increase in urinary N (% N intake) in cats fed RB compared to cats fed HP; however, in the current study the difference reached statistical significance. Again, this difference may be due to differences in variability or the larger sample size used in our study.

Blood Metabolites

Serum creatinine and triglyceride concentrations were altered by diet, but within reference ranges. Serum albumin concentrations were higher than feline reference values (Merck

Veterinary Manual, 2005). Serum albumin is a major determinant of osmotic pressure in the blood. Concentrations are affected by dietary and metabolic influences. Hypoalbuminemia, in conjunction with other abnormal values, can be used in many diagnoses including malnutrition and liver damage. Increased serum albumin have been associated with intake of high-protein diets and dehydration (Mutlu et al., 2006). The high protein content of diets fed in the current experiment is likely the cause of high serum albumin. Serum albumin concentrations were similar to those reported by Vester et al. (2009b) in kittens fed a high-protein extruded diet (53% CP). In that study, albumin concentrations were increased from 36 mg/dL in cats fed a high-carbohydrate (34% CP) extruded diet to 40 mg/dL in cats fed the high-protein extruded diet. Cats had free access to water at all times; however, water intake was not measured and could have influenced albumin levels.

Vester et al. (2010) reported alterations in serum chemistry not observed in the current experiment. Serum alanine aminotransferase (ALT) and bicarbonate concentrations were increased in African wildcats when fed the RB vs. HP diet, while serum creatinine and triglyceride concentrations were not changed in that study. Both ALT and bicarbonate concentrations, however, were within normal ranges reported for either domestic cats or African wildcats.

Urine

Urinary pH did not differ between diets, however, urine was more alkaline than normally recommended for domestic cats (6.3-6.6, MERCK Veterinary Manual, 2005). Vester et al. (2010) also reported an alkaline urinary pH (6.7-7.8) in African wildcats fed similar diets, but was lower than urine of cats in the current study. Urine specific gravity was not influenced by diet in either study, and values were similar.

Fecal Characteristics and Fermentative End-Products

The higher fecal output (g/d, as-is and DM) in cats fed HP can be explained by the higher intake (g/d DM) and lower digestibility of the diet. Higher TDF in the HP diet may have also played a role. Although fecal DM percentage was not different between dietary treatments, fecal score was altered by diet. All fecal scores were close to the ideal score (3 out of 5), but cats fed HP had higher fecal scores (looser stools) as compared to cats fed RB and CB.

Fecal SCFA and BCFA concentrations were similar to values for domestic cats reported in the literature (Hesta et al., 2001; Vester et al., 2009a). Fecal ammonia, propionate, butyrate, isobutyrate, valerate, isovalerate and total BCFA concentrations and SCFA ratio were altered by diet. Variations in dietary ingredient and macronutrient composition can influence microbial fermentation, often resulting in modified fecal fermentative end-product concentration.

Fecal SCFA concentrations are an indication of carbohydrate fermentation, and have been associated with health benefits, including increased gut morphology (e.g., villus height) and function. Although fecal concentrations of acetate and total SCFA did not differ, cats fed CB had the highest numerical concentrations, with cats fed HP being intermediate. High variability may have masked any dietary effects on acetate and total SCFA concentrations. Feces of cats fed HP had a higher ratio of butyrate, and lower ratio of propionate compared to cats fed RB and CB diets. This indicates that carbohydrate metabolism in the hindgut may have been modified by diet. However, because high amounts of SCFA are absorbed, fecal concentrations are difficult to interpret. Increases in SCFA may be due to increased carbohydrate reaching the large intestine, decreased absorption of SCFA, or both. The inclusion of chicory root, a source of inulin, in the HP diet may have contributed to these results. Hesta et al. (2001) reported a decrease ($P < 0.05$) in ratio of fecal acetate:propionate when cats were fed diets containing 3% or 6% inulin.

Ammonia and BCFA are putrefactive compounds produced during colonic fermentation of endogenous and undigested amino acids. High levels of these putrefactive compounds can be toxic and are some of the components responsible for the malodor of feces. Fecal ammonia and

BCFA concentrations were increased in cats fed HP, resulting in an increased contribution to malodor from these components. This is often due to a higher dietary percentage and the lower digestibility of CP, and was likely the reason for differences noted herein.

Fecal scores in cats in the current study were higher and closer to the ideal score than those reported by Vester et al. (2010) in African wildcats fed similar diets. Fecal ammonia concentration was similar for cats in the current study and African wildcats reported by Vester et al. (2010) fed HP, but were 64% less in our cats vs. African wildcats fed RB. An increase in butyrate proportion of fecal SCFA, similar to that in this experiment, was observed in African wildcats fed HP compared to those fed RB (Vester et al., 2010). However, Vester et al. reported no other dietary related differences in fecal SCFA and BCFA concentrations.

Conclusions

Although the beef-based diets, RB and CB, were more digestible than HP, all diets were highly digestible in this experiment. All cats had positive N balance and maintained BW throughout the study. Because cats fed CB had N balance close to zero, and N balance tends to be overestimated, the ability of cats to maintain BW long term on CB diets should be determined. Few differences in serum metabolites were detected when cats were fed HP compared with RB and CB. Urine parameters did not differ between diets. All fecal scores were close to ideal (3), but cats fed HP had higher scores (looser stools) compared to cats fed RB and CB. Similarities in fecal SCFA concentrations indicate that carbohydrate fermentation was similar for all diets. Fecal putrefactive compounds, namely ammonia and BCFA, were increased in cats fed HP. These compounds are indicators of increased protein fermentation, and could have negative effects on the health of the gastrointestinal tract. Fecal BCFA were similar to values reported in the literature for healthy cats (Hesta et al., 2001; Vester et al., 2009a).

Given the increasing popularity of feeding raw diets, and the metabolic differences of cats fed raw vs extruded diets in this experiment, further research focused on the adequacy and

safety of raw beef-based diets in domestic cats is justified. Therefore, it appears that the cooked beef-based diet tested herein is an adequate diet choice for domestic cats. Because cooking may minimize risk of microbial contamination, further evaluation of raw vs. cooked meat-based diets for domestic cats is warranted.

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FIGURES AND TABLES

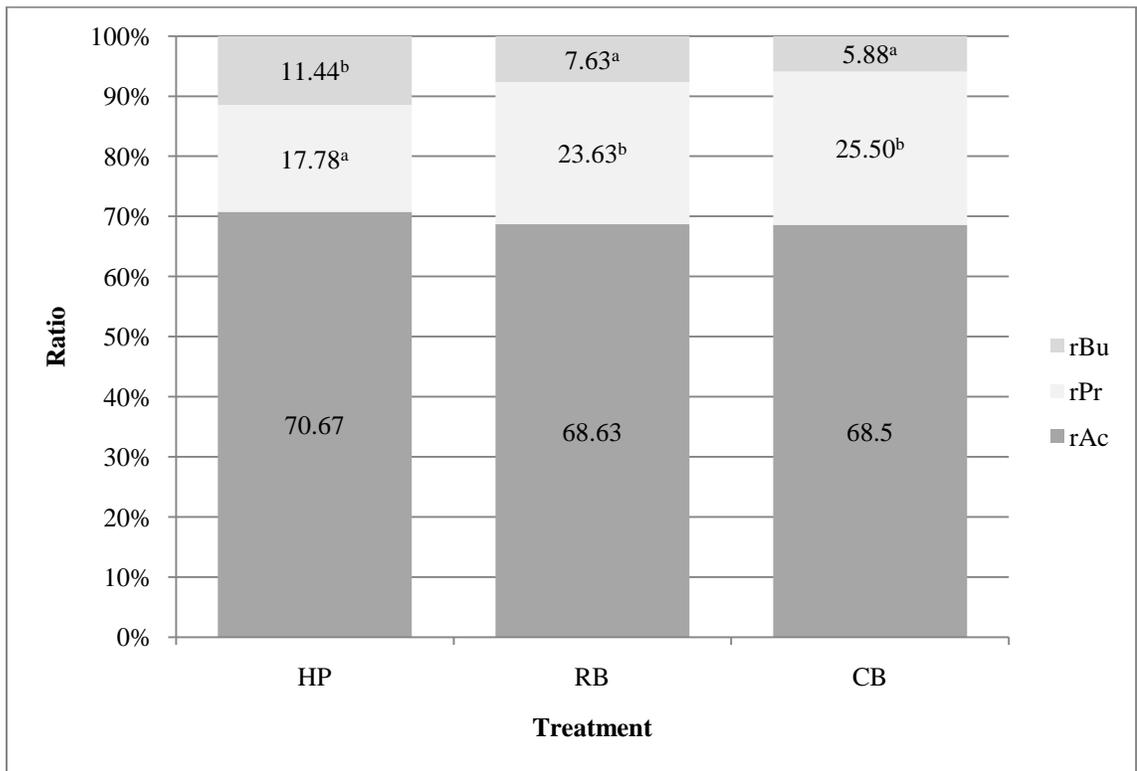


Figure 2.1 Molar ratios of fecal short-chain fatty acids (acetate, propionate, and butyrate) of domestic cats (n=9) fed a high-protein extruded (HP), raw beef-based (RB), or cooked beef-based (CB) diet.

^{a,b} Means within a row lacking a common superscript letter are different ($P < 0.05$).

Table 2.1 Chemical and ingredient composition of the high-protein extruded (HP), raw beef-based (RB), and cooked beef-based (CB) diets fed to domestic cats (n=9)

Item	HP	RB	CB
Dry matter (DM), %	94.3	29.3	29.2
	-----% of DM-----		
Organic matter	89.9	92.2	92.1
Crude protein	57.0	52.5	52.0
Acid hydrolyzed fat	17.4	20.5	18.3
Total dietary fiber	9.0	7.1	7.5
Gross energy, kcal/g	5.6	6.0	6.0
Calculated ME ¹ , kcal/g	3.7	4.0	3.9
Extruded diet ingredients:	Chicken meal, potato product, chicken fat, dried egg, herring meal, beet pulp, natural flavors, herring oil, premium cat vitamin premix, salt, premium cat mineral mix, potassium chloride, dried chicory root, dried natural antioxidant, DL-methionine.		
Beef-based diet ingredients:	Beef, meat by-products, fish meal, soybean meal, dried beet pulp, calcium carbonate, dried egg, brewers dried yeast, Nebraska Brand feline vitamin premix, salt, Nebraska Brand trace element premix.		

¹ME = metabolizable energy; calculated using modified Atwater factors (8.5 kcal ME/g for fat and 3.5 kcal ME/g for protein and nitrogen-free extract).

Table 2.2 Food intake, fecal output, and urine characteristics of domestic cats (n=9) fed a high-protein extruded (HP), raw beef-based (RB), or cooked beef-based (CB) diet

Item	HP	RB	CB	SEM ¹
Food Intake, g/d DM	56.6 ^c	49.5 ^b	42.1 ^a	2.83
Caloric intake, kcal/d	315.4 ^c	295.9 ^b	253.4 ^a	16.43
Fecal output, g/d as-is	36.1 ^b	17.6 ^a	17.4 ^a	3.34
Fecal output, g/d DM	13.0 ^b	6.7 ^a	7.2 ^a	0.60
Fecal output (g as-is)/intake (g, DM)	0.6 ^b	0.4 ^a	0.5 ^a	0.04
Urine Volume, mL/d	53.4	54.5	59.5	7.16
Urine specific gravity	1.064	1.065	1.067	0.004
Urine pH	7.8	7.8	7.9	0.48

¹ SEM = Standard error of the mean.

^{a,b,c} Means within a row lacking a common superscript letter are different (P < 0.05).

Table 2.3 Apparent total tract nutrient digestibility and nitrogen metabolism of domestic cats (n=9) fed a high-protein extruded (HP), raw beef-based (RB), or cooked beef-based (CB) diet

Item	HP	RB	CB	SEM ¹
Apparent digestibility (%)				
Dry matter	78.2 ^a	86.7 ^{b‡}	83.8 ^{b†}	1.69
Organic matter	83.9 ^a	90.5 ^b	88.5 ^b	1.31
Crude protein	81.6 ^a	93.3 ^b	92.9 ^b	1.23
Fat	91.3 ^a	95.5 ^b	95.3 ^b	0.42
Energy	84.7 ^a	91.5 ^b	89.8 ^b	1.14
Nitrogen (g/d)				
Intake	5.2 ^b	4.1 ^{a‡}	3.5 ^{a†}	0.24
Digestible Intake	4.2 ^b	3.5 ^a	3.2 ^a	0.27
Urine	2.6	2.5	2.9	0.34
Feces	1.0 ^b	0.3 ^a	0.3 ^a	0.03
Percentage of nitrogen intake				
Urine	54.7 ^a	72.2 ^{ab}	87.0 ^b	9.89
Feces	18.5 ^b	6.7 ^a	7.1 ^a	1.23
Absorbed	81.5 ^a	93.3 ^b	92.9 ^b	1.23
Retained	24.5	18.78	3.6	10.53
Nitrogen balance (g/d)	1.2 ^b	0.68 ^{ab}	0.001 ^a	0.38
Percent of digestible N Intake				
Urine	69.2	79.4 [†]	96.2 [‡]	11.71
Feces	23.2 ^b	7.1 ^a	7.6 ^a	2.86

¹SEM = standard error of the mean.

^{a,b} Means within a row lacking a common superscript letter are different (P < 0.05).

^{†,‡} Means within a row lacking a common superscript letter tend to be different (P < 0.10).

Table 2.4 Stool quality and ammonia, short-chain fatty acid (SCFA), and branched-chain fatty acid (BCFA) concentrations of domestic cats (n=9) fed a high-protein extruded (HP), raw beef-based (RB), or cooked beef-based (CB) diet

Item	HP	RB	CB	SEM ¹
Fecal DM, %	38.9	38.5	41.1	2.80
Fecal score ²	3.3 ^b	2.9 ^a	2.8 ^a	0.16
	--- μ mol/g DM of feces---			
Ammonia	190.4 ^b	69.4 ^a	72.0 ^a	17.92
Acetate	214.6	178.2	275.3	48.89
Propionate	50.9 ^a	65.3 ^{ab†}	102.7 ^{b‡}	16.61
Butyrate	38.2 ^b	21.2 ^a	25.5 ^a	3.23
Total SCFA ³	305.1	266.3	404.7	66.88
Isobutyrate	10.1 ^b	4.9 ^a	5.1 ^a	0.87
Valerate	18.3 ^b	6.0 ^a	5.3 ^a	1.89
Isovalerate	15.3 ^b	6.7 ^a	6.4 ^a	1.33
Total BCFA ⁴	43.7 ^b	17.6 ^a	16.8 ^a	3.45

¹SEM = standard error of the mean.

²Fecal scores based on the following scale: 1= hard, dry pellets; 2= dry, well formed stools; 3= soft, moist, formed stool; 4= soft, unformed stool; and 5= watery, liquid that can be poured.

³Total SCFA=acetate + propionate + butyrate.

⁴Total BCFA=isobutyrate + valerate + isovalerate.

^{a,b} Means within a row lacking a common superscript letter are different ($P < 0.05$).

^{†,‡} Means within a row lacking a common superscript letter tend to be different ($P < 0.10$).

Table 2.5 Food-restricted blood metabolite concentrations of domestic cats (n=9) fed a high-protein extruded (HP), raw beef-based (RB), or cooked beef-based (CB) diet

Item	HP	RB	CB	SEM ¹	Reference Range ²
Urea nitrogen, mg/dL	29.9	27.4	28.7	1.34	15.4-31.2
Total protein, g/dL	7.0	7.2	7.2	0.19	5.7-8.0
Albumin, g/dL	4.0	4.0	4.1	0.16	2.4-3.7
Calcium, mg/dL	10.7	11.0	10.9	0.17	7.9-10.9
Phosphorus, mg/dL	4.7	5.0	5.2	0.18	4.0-7.3
Sodium, mmol/L	151.8	153.2	152.2	0.66	140.3-153.9
Potassium, mmol/L	4.3	4.5	4.5	0.12	3.8-5.3
Chloride, mmol/L	117.5	116.8	115.5	0.80	107.5-129.6
Glucose, mg/dl	72.6	80.4	81.6	5.97	60.8-124.2
ALT ³ , U/L	57.0	67.8	70.1	6.75	8.3-52.5
Cholesterol, mg/dL	154.9	176.7	165.3	13.01	71.3-161.2
Bicarbonate, mmol/L	17.5	18.4	17.4	0.90	16.4-22.0
Creatinine, mg/dL	1.2 ^a	1.5 ^b	1.5 ^b	0.09	0.5-1.9
NEFA, mEq/L	0.5	0.5	0.5	0.07	NA
Triglycerides, mg/dL	26.7 ^a	32.4 ^{ab}	37.3 ^b	1.93	8.9-71.2 ⁴

¹SEM = standard error of the mean; NA = None available.

²MERCK Veterinary Manual (2005).

³ALT = Alanine aminotransferase.

⁴Kluger et al. (2008).

^{a,b} Means within a row lacking a common superscript letter are different (P < 0.05).

AUTOBIOGRAPHY

Katherine Rose Kerr was born on October 14, 1983 in La Mesa, CA to Thomas F. and Kathleen J. Kerr. Katherine received a joint Bachelor of Science in Zoology and Biological Sciences from Colorado State University in 2006.

In the future, Katherine plans to continue her education and receive her doctorate in Nutritional Sciences at the University of Illinois. Katherine hopes to continue her to investigations into the nutritional requirements of captive exotic animals.