

**Evaluation of raw meat diets on macronutrient digestibility, fecal output, microbial presence, and  
general health status in domestic dogs**

by

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The student author and the program of study committee are solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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## TABLE OF CONTENTS

	Page
LIST OF FIGURES .....	v
LIST OF TABLES .....	vi
ACKNOWLEDGEMENTS .....	viii
ABSTRACT .....	x
CHAPTER 1 INTRODUCTION .....	1
Literature Cited .....	6
CHAPTER 2 LITERATURE REVIEW .....	8
History of Dog Domestication .....	8
Nutrition of Canids .....	9
Pet Food Trends and Effects of Processing on Nutrients .....	14
Advantages of RMBDs .....	17
Disadvantages of RMBDs .....	21
Assessing Nutritional Implications on Canine Health .....	29
Meat Sourcing for Carnivore Management .....	31
Conclusions .....	34
Literature Cited .....	35
CHAPTER 3 EVALUATION OF DIET COMPOSITION, APPARENT TOTAL TRACT MACRONUTRIENT AND ENERGY DIGESTIBILITY, FEED INTAKE, FECAL OUTPUT, AND MICROBIAL PRESENCE IN DOMESTIC DOGS FED COMMERCIAL RAW MEAT-BASED DIETS .....	48
Abstract .....	48
Introduction .....	49
Materials and Methods .....	51
Animals .....	51
Experimental Design .....	52
Experimental Diets .....	53
Diet and Fecal Chemical Analyses .....	53
Feed Intake and Fecal Output .....	54
Apparent Total Tract Digestibilities and Energy Calculations .....	54
Microbial Presence .....	55
Blood Panels .....	56
Statistical Analysis .....	57
Results .....	57
Diet and Fecal Chemical Analyses .....	57
Feed Intake, Fecal Output, and Fecal Scores .....	58
Apparent Total Tract Digestibilities and Energy Calculations .....	59
Microbial Presence .....	60
Blood Panels .....	60
Discussion .....	61
Diet Chemical Composition and Nutrient Digestibility .....	61
Meat Sourcing and Ingredient Selection for Carnivore Management .....	63

Economics of Energy .....	68
Microbial Presence .....	69
Blood Panels .....	70
Conclusions .....	72
Literature Cited .....	74
CHAPTER 4 EX VIVO EVALUATION OF INTESTINAL INTEGRITY AND GASTROINTESTINAL HISTOLOGY TO DETERMINE POTENTIAL GASTROINTESTINAL HEALTH IMPLICATIONS BETWEEN DOMESTIC DOGS FED COMMERCIAL EXTRUDED OR RAW MEAT-BASED DIETS .....	87
Abstract .....	87
Introduction .....	88
Materials and Methods .....	90
Animals .....	90
Experimental Design and Diets .....	90
Gastrointestinal Histology .....	91
Intestinal Transepithelial Electrical Resistance and Macromolecule Permeability .....	91
Statistical Analyses .....	93
Results .....	93
Diet Compositions .....	93
Gastrointestinal Histology .....	94
Intestinal Transepithelial Electrical Resistance and Macromolecule Permeability .....	94
Discussion .....	95
Diet Composition and Implications .....	95
Gastrointestinal Histology .....	99
Intestinal Transepithelial Electrical Resistance and Macromolecule Permeability .....	100
Conclusions .....	103
Literature Cited .....	104
CHAPTER 5 CONCLUSIONS .....	115
Literature Cited .....	120

## LIST OF FIGURES

	Page
Figure 3.1: Stool samples from domestic dogs ( <i>Canis lupus familiaris</i> ) fed commercially available horse-, pork-, and beef-based raw meat diets and associated fecal scores .....	86
Figure 4.1: Regional specific apparent permeability coefficients ( $P_{app}$ ) for domestic dogs ( <i>Canis lupus familiaris</i> ) fed extruded- versus RMBDs .....	114

## LIST OF TABLES

	Page
Table 3.1: Ingredient composition of horse-, pork-, and beef-based raw meat diets fed to domestic dogs ( <i>Canis lupus familiaris</i> ) .....	78
Table 3.2: Chemical composition (DM basis) of horse-, pork-, and beef-based raw meat diets fed to domestic dogs ( <i>Canis lupus familiaris</i> ) .....	79
Table 3.3: Chemical composition (DM basis) of fecal samples collected from domestic dogs ( <i>Canis lupus familiaris</i> ) fed horse-, pork-, and beef-based raw meat diets .....	80
Table 3.4: Feed intake, fecal output, fecal scores, apparent total tract macronutrient and energy digestibilities, and energy calculations in domestic dogs ( <i>Canis lupus familiaris</i> ) (n=4) fed horse-, pork-, and beef-based raw meat diets .....	81
Table 3.5: <i>Salmonella spp.</i> and generic <i>E. coli</i> presence on diet (n=16), saliva (n=36), and fecal (n=36) samples obtained from domestic dogs ( <i>Canis lupus familiaris</i> ) fed commercially available horse-, pork-, and beef-based raw meat diets .....	82
Table 3.6: Serum metabolite and electrolyte concentrations of domestic dogs ( <i>Canis lupus familiaris</i> ) fed commercially available horse-, pork-, and beef-based raw meat diets.....	83
Table 3.7: Plasma complete blood count of domestic dogs ( <i>Canis lupus familiaris</i> ) fed commercially available horse-, pork-, and beef-based raw meat diets .....	84
Table 3.8: Cost analysis and total GE, DE, and estimated ME provided by commercially available horse-, pork-, and beef-based raw meat diets when fed to domestic dogs ( <i>Canis lupus familiaris</i> ) .....	85
Table 4.1: Ingredient composition of extruded- and RMBDs fed to domestic dogs ( <i>Canis lupus familiaris</i> ) .....	108
Table 4.2: Chemical composition of extruded- and RMBDs fed to domestic dogs ( <i>Canis lupus familiaris</i> ) .....	110
Table 4.3: Histopathology results for extruded-fed domestic dogs ( <i>Canis lupus familiaris</i> ) .....	111
Table 4.4: Intestinal transepithelial electrical resistance (TER, $\Omega\cdot\text{cm}^2$ ) and apparent permeability coefficients ( $P_{\text{app}}$ ) for domestic dogs ( <i>Canis lupus familiaris</i> ) fed extruded- versus RMBDs .....	113

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## ABSTRACT

Raw meat-based diets (RMBDs) are typically fed to exotic carnivores managed in zoological institutions and are gaining in popularity as dietary options for pet dogs. Current research themes of RMBDs for zoo carnivores have centered around utilization in exotic felid species with a paucity of data obtained from exotic canids. In addition, current research themes of RMBDs for domestic dogs have centered around microbial contamination. Conducting nutrition-related research in exotic canids is often limited because animals are group-housed, preventing accurate individual feed intake and fecal output collection. Due to functional and anatomical similarities between digestive systems of domestic dogs with their wild counterparts, dogs may serve as an experimental model for nutrition studies for certain exotic canid species.

The overall objectives of this research were to evaluate four commercially manufactured RMBDs formulated for zoological carnivores using domestic dogs as a model for exotic canids and to evaluate microbial risk to humans and canine health implications beyond digestibility. We hypothesize all RMBDs: 1.) evaluated would be highly digestible in domestic dogs, 2.) microbial risk to humans would be low, and 3.) there would be no adverse implications on canine health as a result of feeding RMBDs.



Overall, nutrients in RMBDs were highly digested by domestic dogs and diets did not result in clinical signs of gastrointestinal upset/distress. Further, RMBDs did not negatively influence general health status in dogs as measured by serum chemistry, electrolytes, complete blood count (CBC), and histology of gastrointestinal tract and associated tissues. Using chamber evaluation of intestinal integrity and barrier function indicated possible benefit of feeding RMBDs to dogs.

Our first aim was to evaluate diet composition, apparent total tract macronutrient and energy digestibility, feed intake, fecal output, and microbial presence of four commercially-manufactured RMBDs for zoological carnivores fed to domestic dogs. Diets varied in protein source including horse (Horse), pork (Pork) and two beef-based diets (Beef 1 and Beef 2). Our results indicated that diets were comparable in nutrient and energy composition and apparent total tract digestibility when fed to domestic dogs (n=4). Treatment nutrient concentrations ranged for dry matter (DM) (32.2 – 36.2%), organic matter (OM) (91.1 – 94.9%), crude protein (CP) (50.3 – 61.7%), fat (25.1 – 38.3%), and gross energy (GE) (5.8 – 6.4 kcal/g). Digestibility of nutrients and energy ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.2%, and 91.3 – 95.5% for DM, OM, CP, fat, and energy, respectively. Fecal chemical composition, specifically fat on a dry matter basis (DMB), differed markedly for dogs consuming one of the beef diets (Beef 2). Dogs fed Beef 2 had greater ( $P<0.05$ ) concentrations of fat in feces (21.5%) compared to 2.9, 6.1, and 6.3% for dogs fed Horse, Pork, and Beef 1, respectively. Despite the large fecal fat concentration, dogs fed Beef 2 diet had greater ( $P<0.05$ ) digestibility of DM (92.4%), OM (95.3%), CP (97.7%), and GE (95.5%) but lesser ( $P<0.05$ ) digestibility of fat (94.9%)

compared with all other diets evaluated. Additional digestibility differences were few. Feces were scored using the following scale: 1 = very hard, dry feces to 7 = watery diarrhea (Nestlé Purina). Fecal scores were lower ( $P<0.05$ ) when dogs were fed Horse (1.2) and Beef 2 (1.9) diets compared to Pork (2.7) and Beef 1 (3.1) diets. Detection of *Salmonella spp.* in diet and saliva samples was non-existent and only 5.6% ( $n=2/36$ ) of fecal samples were *Salmonella spp.* positive. Detection of generic *E. coli* was determined in 12.5% ( $n=2/16$ ) and 5.6% ( $n=2/36$ ) of diet and saliva samples, respectively.

Our second aim was to compare gastrointestinal histology, intestinal transepithelial electrical resistance (TER), and intestinal macromolecule permeability between domestic dogs fed commercial extruded- versus RMBDs. No differences were observed in gastrointestinal histology between dietary treatment groups. TER and macromolecule permeability data were highly variable and statistical analyses were not performed due to low sample size. Numerical increases in apparent permeability coefficient ( $P_{app}$ ) were observed in extruded-fed dogs indicating increased macromolecule permeability that is suggestive of decreased intestinal integrity and barrier function. These results indicate potential improvements in intestinal barrier function when dogs were fed RMBDs using a novel technique; however, further evaluation should be considered with a larger sample size.

This research demonstrates that RMBDs varying in protein source and ingredients can be effectively utilized by domestic dogs and potentially exotic canids. While these experiments evaluated four commercial products manufactured for exotic carnivores in domestic dogs,

further research should evaluate direct comparisons between digestive efficiencies of domestic dogs compared to various exotic canid species. Our research also indicates that human exposure to pathogens associated with feeding RMBDs to dogs is possible but risk appears low based on presence of measured microbes. Additionally, this research indicates value in the use of gastrointestinal histology and Ussing chamber evaluation of intestinal integrity and barrier function as novel approaches for determining health effects beyond nutrient digestibility of various diets in domestic dogs.

## **CHAPTER 1**

### **INTRODUCTION**

Raw meat-based diets (RMBDs) are typically fed to exotic carnivores maintained in zoological institutions either exclusively or in combination with other feed ingredients including extruded and canned products. Studies have documented high digestibility of RMBDs to exotic and domestic carnivores (Kendall et al., 1982; Hendricks et al., 1999; Vester et al., 2008; Vester et al., 2010a, 2010b; Kerr et al., 2012; Kerr et al., 2013; Hamper et al., 2015; Iske et al., 2016). However, the majority of recent research relating to exotic carnivores fed RMBDs has been conducted with felid species. Similar studies evaluating digestibility differences, fecal characteristics, and health status using canid species are few.

Domestic dogs share many functional and anatomical similarities of the gastrointestinal tract with exotic canids (Bosch et al., 2015). As a result, nutrition research conducted in domestic dogs has potential to provide valuable insight for nutrition management of exotic canids. Nutrition research in exotic canids is difficult to conduct and expensive because housing of exotic canids is typically in social groups or packs making individual sample collection difficult (Vester-Boler et al., 2009). Utilizing domestic dogs as an experimental model for nutrition studies can help reduce research costs, allow for more controlled experimental conditions, increase number of animals available to use in studies, and provide valuable information to animal managers for diet formulation or selection. However, it is important to note that

experiments directly comparing digestibility of varying diet types between domestic dogs and exotic canids have not yet been performed.

In recent years, feeding RMBDs has increased in popularity with owners of companion animals (Michel, 2006). This practice is subject to controversy as undocumented health claims and potential human and animal health concerns are common (Freeman et al., 2013). In companion animals, published RMBD research, almost exclusively, have focused on microbial contamination (Lenz et al., 2009). Although microbial contamination of RMBDs does not typically cause clinical illness in healthy pets, associated microbial shedding in feces of pets fed raw diets along with the handling of RMBDs have potential human health implications (Carter and Quinn, 2000; Lenz et al., 2009). Because companion animals typically share households with humans, dogs and cats consuming RMBDs may serve as vectors for microbial pathogens (Carter and Quinn, 2000). Additionally, publications regarding RMBDs fed to companion animals include case studies of improperly formulated RMBDs that lack nutrients meeting animal requirements (Kawaguchi et al., 1993; Niza et al., 2003; Polizopoulou et al., 2005; Taylor et al., 2009; Zeugswetter et al., 2013).

Data related to health status of companion animals fed properly formulated RMBDs are lacking. Further, short-term feeding studies evaluating RMBDs may not be long enough to identify changes in health status of animals. As a result, research should objectively evaluate health effects associated with varying diet types. Due to ethical considerations, obtaining tissues for histological evaluation or other analyses often is not feasible with companion

animals. Therefore, most nutrition research relies on changes in serum biochemical values or complete blood count as one of few and often sole indicators of health status in animals. Additional research methods to evaluate health status need evaluation to determine long-term health effects associated with diet type, particularly RMBDs.

**Research Objective:**

Our overall research objectives were to evaluate commercially manufactured RMBDs formulated for zoological carnivores in domestic dogs as a model for exotic canids and to evaluate human and animal health implications of feeding RMBDs to domestic dogs. For purposes of this thesis, health is defined as the absence of any disease or impairment.

**Specific Aim 1: Evaluate diet composition, apparent total tract macronutrient and energy digestibility, feed intake, fecal output, and microbial presence in domestic dogs fed RMBDs commercially manufactured for zoological carnivores.**

A total of four intact male domestic dogs were fed four different commercial RMBDs manufactured for zoological carnivores varying in protein source and ingredients. Feed intake and fecal output were recorded and analyzed to determine apparent total tract macronutrient and energy digestibilities for each dietary treatment. Fecal scores were recorded daily. Saliva, fecal, and dietary samples were obtained and analyzed for presence of *Salmonella spp.* and *Escherichia coli*.

**Specific Aim 2: Application of novel technology to compare gastrointestinal histology, intestinal transepithelial electrical resistance, and intestinal macromolecule permeability between domestic dogs fed commercial extruded- versus RMBDs.**

Two intact female domestic dogs were fed a rotation of two different commercial extruded diets (extruded-fed group) and two intact male domestic dogs were fed a rotation of four different RMBDs (RMBD-fed group). Following a seven-month feeding period, all four dogs were humanely euthanized and tissue samples of the gastrointestinal tract and associated tissues were obtained for histological evaluation. Additionally, intestinal tissue samples were used for Ussing chamber evaluation of intestinal transepithelial electrical resistance and macromolecule permeability as a novel approach to understanding the potential influence of RMBDs on intestinal integrity and barrier function for dogs.

**Research Implications:**

This study provides valuable and novel insights into possible canine health implications, beyond digestibility, of feeding RMBDs to domestic dogs that have previously been unevaluated in the scientific literature. Moreover, evaluation of bacterial presence in diets, saliva, and feces of dogs fed RMBDs provides further understanding of potential pathogen transfer to humans in close contact with animals fed these types of diets. Data obtained from gastrointestinal histology and Ussing chamber evaluation of intestinal membrane integrity provides a regional specific evaluation of intestinal health effects of differing diet types. Data presented in this thesis demonstrate dogs tolerate a wide range of raw meat diets varying in protein and ingredient sources. After seven months on RMBDs, dogs maintained body condition, fecal

scores and nutrient digestibility. Additionally, data from this research can be directly used by managers of exotic canids when making diet purchasing decisions that impact institutional budgets and nutrition management as minor differences in nutrient and energy digestibility may impact annual budgets.



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## CHAPTER 2

### LITERATURE REVIEW

#### History of Dog Domestication:

According to the 2015-2016 APPA (American Pet Products Association) National Pet Owners Survey, 54.4 million households in the U.S. own a dog. Dogs have been an important part of human society since their domestication began approximately 30,000 years ago (Fan et al., 2015). As dogs evolved from wolves to their current form, their roles in society also changed. As a result, dog nutritional physiology evolved.

Dogs were the first species domesticated and domestication began when humans were almost exclusively nomadic hunter-gatherers (Clutton-Brock, 1999). Wolves (*Canis lupus*) proved valuable in hunting and guarding for nomadic people (Müller, 2002). Currently, it remains unclear how dogs originally were domesticated from wolves. A population of less-fearful wolves likely scavenged kills from nomadic camps (Lindsay, 2000). One thought is that wolf pups were captured for use in hunting and guarding (Axelsson et al., 2013). Puppies that showed more tameness into adulthood were likely bred to other wolves showing similar tameness (Grandin and Dessing, 1998). Another suggestion is that human preferences for paedomorphic characteristics may have contributed to selection and subsequent domestication of tamer wolves (Waller et al., 2013). Nutrition and feeding of early dogs most likely involved assisted foraging. Additional food provided to early dogs likely consisted primarily of scraps, bone, and other edible sources that were unwanted by humans (McNamara, 2006).

During the Neolithic Revolution, approximately 10,000 years ago, humans began to shift from a nomadic hunter-gatherer society to settled plant and animal agriculture (Boessnek, 1985; Driscoll et al., 2009a). During this time, domestication of several other species began including sheep, pigs, cattle, and goats (Driscoll et al., 2009a). These animals, like dogs, followed a dominance hierarchy, had a relatively flexible diet, and could live and breed in confinement (Clutton-Brock, 1999). Humans exploited these mechanisms to begin domestication primarily through artificial selection (Driscoll et al., 2009a). As dogs assumed roles in herding and livestock guarding, this time period marked the end of wolf admixture in dog genetics (Freedman et al., 2014). With the rise of agriculture in the Fertile Crescent, dogs consumed plant and animal refuse, offering a new dietary niche (Bosch et al., 2015), one likely higher in starch (Axelsson et al., 2013).

### **Nutrition of Canids:**

Domestic dogs (*Canis lupus familiaris*) and the gray wolf (*Canis lupus*) are both members of the family *Canidae* within the order of *Carnivora* (Wilson and Reeder, 2005). As carnivores, dogs possess simple stomachs, short digestive tracts, and canine flesh tearing teeth (Kendall et al., 1982; Peterson and Cuicci, 2003; NRC, 2006). Most carnivores consume a predominately meat-based diet, although some species' dietary and feeding habits range from strictly carnivorous (e.g., felids, polar bears), to omnivorous (e.g., most canids and ursids), to strictly herbivorous (i.e., Panda bears) in nature. Canids are a diverse family consisting of 34-38 different species characterized as terrestrial, mostly nocturnal, predators and scavengers

(Clutton-Brock, 1998). Most canids hunt in packs (e.g., wolves, coyotes, etc.) but others are solitary hunters (e.g., foxes) (Clutton-Brock, 1998). However, dogs are the only fully domesticated canid (Clutton-Brock, 1998).

Dietary diversity exists between species of exotic canids. African Wild Dogs (*Lycaon pictus*) are exclusively carnivorous in nature (Pribyl and Crissey, 1999) compared with Maned Wolves (*Chrysocyon brachyrurus*) that consume diets consisting of approximately 50% animal material and 50% plant material (Phipps and Edwards, 2009). Most canids have broad feeding habits consisting of large and small prey, fruits, and other plant matter (NRC, 2006). Intake is dictated primarily on availability of feedstuffs and social hierarchy within the pack, with pack leaders getting preference to kills and scavenged finds (Zimen, 1976; Mech, 1981; Peterson and Ciucci, 2003). Wolves have been the most studied of exotic canids, especially from a nutrition and feeding standpoint. Wolves can be considered true carnivores in nature as non-prey, plant matter makes up only a very small percentage (0.1 – 3.0%) of their overall diet (Bosch et al., 2015). Wolves primarily rely on large ungulates as their main source of food but, as opportunistic predators, can survive on any prey they are able to catch (Peterson and Ciucci, 2003). Dietary intake of the gray wolf is highly varied based on geographical location including some documentation of animals hunting domestic species or scavenging garbage (Peterson and Ciucci, 2003).

Wolves feeding on large prey will preferentially consume internal organs (e.g., heart, liver, lungs, spleen, kidneys, etc.) after tearing into the carcass (Peterson and Ciucci, 2003;

Stahler et al., 2006). The rumen of ungulates is usually punctured during this process but vegetation in the rumen and remaining gastrointestinal tract is largely ignored while the stomach and intestinal walls are consumed (Peterson and Ciucci, 2003). Large muscle masses of leg are typically consumed next and provide the bulk of the wolves' diet (Peterson and Ciucci, 2003). Consumption of bones, blood, hide, fur, brain, and other portions of the carcass is necessary for wolves to fully meet their nutritional requirements (e.g., vitamins, minerals, essential fatty acids, etc.) and help regulate digesta passage rate (Peterson and Ciucci, 2003). Researchers have estimated that approximately 65.0 – 75.0% of large prey carcass weight is consumed by a pack of wolves (Peterson, 1977; Jędrzejewski et al., 2002).

Considering nutrient intakes, Bosch et al. (2015) reported average nutrient intake of wild wolves consisted of 38.6% dry matter (DM), 67.2% crude protein (CP), 24.9% fat, 6.4% ash, 1.4% carbohydrates, a Ca:P ratio of 1.1, and an energy density of 5.0 kcal/g DM. Further, in thermoneutral environments, wolves can obtain their maintenance water requirements from prey and through production of metabolic water alone but they rely on free-water intake for cooling purposes in warm climates (Peterson and Ciucci, 2003). Fat stores are used for thermogenesis in cold climates as with other species (Kreeger, 2003).

Although exact durations are unknown, both wolves and dogs can adapt to long periods of fasting (Peterson and Ciucci, 2003; Bosch et al., 2015), utilizing ketone bodies generated from fat stores (de Bruijne and van den Brom, 1986). Additionally, they can quickly recover lost weight (Kreeger et al., 1997; Bosch et al., 2015). It is presumed that wolves, like dogs, down-

regulate enzyme systems associated with protein catabolism to conserve body amino acid stores during periods of low dietary protein intake (Meyer and Stadtfield, 1980; Kreeger, 2003), a trait not shared by obligate carnivores (Morris, 2002). This feast vs. famine lifestyle, not shared with smaller species of wild felids (Bosch et al., 2015), has led to several additional metabolic adaptations that influence differences in nutrient requirements for canids. For example, canids can synthesize active forms of niacin, vitamin A, and vitamin D from precursors and do not have additional taurine or arachidonic acid requirements as observed in felids. (NRC, 2006; Bosch et al., 2015).

The basic digestive anatomy and physiology between dogs and exotic canids, particularly wolves, is thought to be highly conservative (Peterson and Ciucci, 2003). In a literature evaluation by Clauss et al. (2010), apparent CP and fat digestion in exotic carnivores; including exotic canids, hyenids, mustelids, pinnipeds, and ursids; showed similar patterns to domestic dogs. However, apparent carbohydrate digestion in the exotic carnivores was more similar to domestic cats rather than domestic dogs (Clauss et al., 2010). The results of this evaluation suggest that carnivores of different species share similar digestive efficiencies (Clauss et al., 2010). From a research standpoint, this suggests that domestic dogs and cats may serve as valuable models for nutrition studies in exotic carnivores, especially considering RMBDs are typically low in carbohydrates and high in fat and protein. Of important note, it is assumed that, on average, wolves and other exotic canids are generally leaner than most domestic dogs (Peterson and Ciucci, 2003), especially with increasing prevalence of obesity in pets (Courcier et al., 2010). Evidence from studies in humans, companion animals, and rodents

suggest gene expression and metabolism are altered in obese animals (Koza et al., 2006; Ruiz et al., 2011; Soronen et al., 2012; de Godoy and Swanson, 2013). For this reason, obese animals may not be the most appropriate models for exotic carnivores regarding nutrition or health studies.

Due to a relatively short period of domestication, environmental selection pressures faced by wolves, including diet, are observed in the dog's genome (Bosch et al., 2015). Recent evidence has shown three genes involved in starch digestion and glucose uptake (AMY2B, MGAM, and SGLT1) have been upregulated in dogs compared to wolves (Axelsson, 2013). Specifically, a copy number increase in the AMY2B gene that codes for amylase was observed in dogs when compared to their wild predecessors (Axelsson, 2013). Further research has revealed that AMY2B copy number is not fixed in all dog breeds and varies significantly between breeds (Arendt et al., 2014; Freedman et al., 2014). Ancient breeds originating from the Fertile Crescent (e.g., Saluki) show a higher copy number of the AMY2B gene than Arctic or Northern breeds (e.g., Siberian Husky) (Freedman et al., 2014). Arendt et al. (2014) found that amylase activity increased linearly with increasing copy number of the AMY2B gene suggesting that dogs with a higher copy number of AMY2B have a greater starch digestion capacity. For these reasons, breed selection may be an important consideration when using dogs as a nutrition model for exotic canids and for diet type selection in dogs as RMBDs may be more effective for some breeds and not others. For proper nutrition management of exotic canids, diet formulation criteria should combine information from published nutrient requirements of the closest-related domestic model species, natural history of the species, and ecological



studies of food intake and feeding behaviors since published data regarding nutrient requirement of species are few or non-existent (AZA Canid TAG, 2012).

### **Pet Food Trends and Effects of Processing on Nutrients:**

Trends in the pet food industry often follow trends in the human food industry. In recent years, foods and products specifically emphasizing health and wellness have become popular for both pets and humans (Beaton, 2013). Dog food selection and feeding practices have similar cultural and societal influences as selection of food for human family members (Michel, 2006). For purposes of this review, alternative diets are defined as dietary choices that differ from standard extruded or canned dog food.

Several alternative dietary choices for domestic dogs have increased in popularity including vegetarian/vegan diets, natural/organic diets, and RMBDs (Michel, 2006). These diets can either be made at home or purchased commercially but serve to satisfy niche markets in the pet food industry. Motivation behind owner selection of these diets varies. For example, vegetarian/vegan diets may be selected by owners because they choose to follow a vegetarian/vegan lifestyle themselves (Michel, 2006). These vegan/vegetarian diets also may have diagnostic application in food elimination trials for animals with animal protein sensitivities (Michel, 2006). Selection of natural/organic diets may be due to perceived quality and safety of ingredients. Furthermore, selection of a RMBD may result from perceived health benefits or desire to feed an ancestral diet or limit the amount of processing the diet is exposed to (Michel, 2006; Freeman et al., 2013). Feeding RMBDs, specifically, has caused controversy

because perceived health benefits are not well documented and by nature these diets have potential for animal and human health concerns resulting from presence of large populations of microbes (Freeman et al., 2013).

In developed nations, processing via extrusion is the primary manufacturing method for commercial pet foods (Lankhorst et al., 2007; Crane et al., 2010). During extrusion processing, the raw diet mixture is subjected to very high temperatures and pressure for a relatively short duration of time; typically 80.0 – 200.0°C for 10 – 270 seconds (Lankhorst et al., 2007). Canning, like extrusion, subjects raw feed ingredients to high temperatures and pressure; however, the product contains greater moisture, typically 74.0 – 78.0% (Williams et al., 2006) compared with 10.0 – 12.0% in extruded foods. Extrusion and other processing methods have benefited the pet food industry with high production throughput of commercial pet foods, increased digestibility of vegetable proteins and starches, destruction of anti-nutrient factors such as trypsin inhibitor, and destruction of microorganisms or spores in feedstuffs (Björk and Asp, 1983; van Rooijen et al., 2013).

Additionally, diet composition of formulated mixtures should be compatible with processing methodology. Extrusion requires starch and limits fat concentrations; therefore, careful consideration is required during formulation (Hendriks et al., 2015). Starch plays an important functional role in extruded foods. Specifically, starch gelatinization followed by rapid cooling of the diet mixture provides structure and texture of the extrudate (Camire et al., 1990; Moscicki et al., 2013). This functional characteristic makes starch a required ingredient for

extruded pet foods. Typical extruded pet foods contain approximately 40.0% starch but may reach greater than 50.0% inclusion (Spears and Fahey, 2004; Crane et al., 2010). Additionally, extrusion converts starch into a form that is more available for hydrolysis within the digestive tract (Galliard and Bowler, 1987); therefore, complete or partial starch gelatinization typically results in increased digestibility and utilization by the animal (Lin et al., 1998; NRC, 2006; Bazolli et al., 2015).

Lipids function in lubrication during extrusion processing but may interfere with extrudate expansion if included at levels higher than 6.0% of the diet (Ilo et al., 2000; Crane et al., 2010). Lipid content of the extrudate is typically reduced compared to the original mixture as a result of lipid oxidation, hydrogenation, and isomerization during processing (Björck and Asp, 1983; Ilo et al., 2000). Additionally, an increase in temperature during extrusion processing results in decreased lipid stability (Rao and Art, 1989). Loss of heat-labile vitamins also may occur during extrusion processing (Singh et al., 2007). Specifically, vitamins A, E, C, B<sub>12</sub>, and folic acid are the most susceptible to extrusion processing (Riaz et al., 2009). Studies have documented losses up to 29.0% for pyridoxine and up to 100.0% for thiamin, as well (Lombardi-Boccia et al., 2005; Singh et al., 2007). In order to correct for these nutrients lost during extrusion, lipids and deficient vitamins are typically sprayed onto the exterior of the extrudate following extrusion (Crane et al., 2010).

Protein quality also is a concern during processing of dog food. Protein quality of a feedstuff is dependent on amino acid composition, bioavailability, and protein digestibility

(Williams et al., 2006). Data from a study by Cramer et al. (2007) comparing raw versus rendered animal meals showed that rendered meals generally had lower protein quality as indicated by differences in lysine bioavailability (86.0 – 107.0%, 70.0 – 99.0%), total sulfur-containing amino acid bioavailability (64.0 – 99.0%, 61.0 – 78.0%), and total amino acid digestibility (90.3 – 95.5%, 73.2 – 84.8%), respectively. Dietary and physiological factors influence digestibility of protein and other nutrients. Dietary factors that influence protein digestibility include concentrations of macro/micronutrients within the diet, source and quality of ingredients, presence or absence of dietary fiber and anti-nutrient factors, particle size, and processing technique (Johnson et al., 1998; Hamper et al., 2015). Physiological factors that influence protein digestibility include species, breed, age, physiological state, illness, and alterations in gut microflora (Sá et al., 2014; Hamper et al., 2015). Specifically, heating can have several negative effects that decrease protein quality in the finished product and present many unique challenges from an animal feeding perspective. For example, extrusion processing can lead to destruction of amino acids, formation of protein aggregates, protein crosslinking, protein oxidation, and formation of Maillard products (Björck and Asp, 1983; Promeprat et al., 2010).

#### **Advantages of RMBDs:**

A major potential benefit of feeding RMBDs to pets is eliminating the subsequent effects of intensive processing with extrusion or canning. Several studies have demonstrated RMBDs have greater macronutrient digestibility compared to extruded or canned diets (Kendall et al., 1982; Hendricks et al., 1999; Vester et al., 2008; Vester et al., 2010a, 2010b; Kerr et al., 2012;

Kerr et al., 2013; Hamper et al., 2015). Crude protein digestibility was 9.0% greater ( $P<0.05$ ) in African wildcats ( $n=6$ ) fed a commercial RMBD compared with cats fed a high-protein extruded diet although no other differences in digestibility were observed (Vester et al., 2010b). It was also observed that cats on the extruded diet consumed, on average, 58% more ( $P<0.05$ ) nitrogen per day and excreted 0.7g more ( $P<0.05$ ) nitrogen in their feces than cats consuming the RMBD indicating improvements in nitrogen balance when fed RMBDs (Vester et al., 2010b).

Björck et al. (1983) reported that although extrusion decreased digestibility of a biscuit sample by up to 11.0%, the decrease was less pronounced with increasing moisture content of mixture going into the extruder. When comparing macronutrient digestibility in kittens, Hamper et al. (2015) reported greater ( $P<0.001$ ) DM (90.6%), OM (93.5%), CP (94.7%), and GE (94.8%) digestibility values when fed a commercial RMBD compared with a commercial canned diet (DM: 83.8%, OM: 88.4%, CP: 88.9%, GE: 90.2%) with comparable nutrient composition and moisture level. Similar results were documented in a study contrasting digestibility of various diet types using dogs and cats. In this study, a fresh minced (FM) meat diet had greater ( $P<0.05$ ) DM (95.7%), CP (96.9%), and GE (96.2%) digestibility than two canned dog diets (DM: 74.8%, CP: 77.7%, GE: 77.1%), two canned cat diets (DM: 80.5%, CP: 84.9%, GE: 84.0%), and an extruded cat diet (DM: 73.25%, CP: 79.7%, GE: 79.6%); acid-hydrolyzed ether extract was not significantly different between the FM and the processed diets and OM digestibility was not determined (Kendall et al., 1982). Although processing of the canned cat food did not result in decreased amino acid concentrations, results from a study by Hendriks et al. (1999) demonstrated that true ileal amino acid digestibility coefficients were higher in unprocessed canned cat food and decreased with increasing heat treatment ( $P<0.05$ ), with the majority of

effects significant at  $P < 0.001$ . Digestibility differences observed between diets cannot be attributed to processing alone because these experimental diets differed in ingredient composition and processing methods (Kerr et al., 2012). These studies combined, document and support use of RMBDs for improvement of nutrient digestibility compared with typical processed extruded or canned diets.

Recent studies also have documented that carnivores consuming RMBDs typically have decreased feed intakes and lower fecal outputs compared to those consuming extruded diets (Crissey et al., 1997; Vester et al., 2010b; Kerr et al., 2012). This likely results from the digestibility improvements of RMBDs as outlined above. Sand cats (*Felis margarita*) fed a RMBD consumed approximately 22.0% less feed on a dry matter basis than cats fed an extruded diet. In spite of this overall reduction in dry matter intake, Sand cats eating the RMBD consumed, on average, 31.7% more digestible protein and 3.9% more digestible energy than cats eating an extruded diet (Crissey et al., 1997). In a study published by Kerr et al. (2012), domestic cats fed a high-protein extruded diet (57.0% CP) had 14.3 and 34.4% greater ( $P < 0.05$ ) feed intake (g of DM/d) than domestic cats fed a raw or cooked beef-based diet, respectively. Domestic cats fed the extruded diet also had approximately 50.0 and 20.0% greater fecal output ( $P < 0.05$ ) and softer stools ( $P < 0.05$ ) compared to cats fed a raw or cooked beef-based diet, although stools for all diets were close to ideal, indicated with fecal score of 3 out of 5 (Kerr et al., 2012). Similar trends were observed in a study using African wildcats (*Felis sylvestris*) by Vester et al., (2010b). In this study, cats fed a high-protein (55.0% CP) extruded diet consumed 41% more dry matter intake ( $P < 0.05$ ) than cats consuming the raw beef-based diet; however, caloric intake between

the two dietary treatments was not significantly different ( $P>0.05$ ) due to differences in water content between diets. Fecal output on a dry matter basis was 92.5% greater ( $P<0.05$ ) for cats fed the high-protein extruded diet but no significant differences ( $P>0.05$ ) were observed when the increase in feed intake was accounted for in fecal output (Vester et al., 2010b). These differences in feed intake and fecal output between diets are important management considerations for both domestic and managed exotic carnivores alike and are considered major benefits to feeding RMBDs.

In contrast to extruded or canned diets, research has shown conflicting results for digestibility differences between RMBDs and cooked diets (minimal processing). As previously stated, in the study by Kerr et al. (2012), macronutrient digestibility did not differ significantly when domestic cats were fed either a raw beef-based diet or the same diet lightly cooked to an internal temperature of at least 71.0°C. All cooked digestibility coefficients were numerically lower than raw counterparts but differences were minor, did not reach statistical difference, and were less than 10% different for all measures. Cooking caused a significant decrease ( $P<0.05$ ) in *in vitro* digestibility of pork meat treated with both pepsin and trypsin when cooking temperatures reached 100.0°C although temperatures of 60.0°C, 65.0°C, and 70.0°C did not have a significant effect ( $P>0.05$ ) (Wen et al., 2015). Data from these studies suggest that cooking RMBDs at more moderate temperatures (approximately 70.0°C) likely does not result in significant decreases in macronutrient digestibility and may be a viable option when pathogen exposure from raw meat is a concern.

**Disadvantages of RMBDs:**

Several government and veterinary agencies have released statements regarding the feeding of RMBDs to pets. The statement from the Food and Drug Administration (FDA) indicates feeding pets raw foods is not in agreement with their goal of protecting the public from significant health risks (FDA Consumer Health Information, 2014). Additionally, the FDA has a zero-tolerance policy for presence of *Salmonella spp.* in pet foods (FDA, 2015) and adulterated pet foods are subject to regulatory action from the FDA due to human health risk perceived by the likelihood of direct human contact with pet food (FDA, 2013). Similarly, the Centers for Disease Control (CDC), American Veterinary Medical Association (AVMA), and American Animal Hospital Association (AAHA) discourage feeding raw foods to dogs and cats because of salmonellosis risk and other infections to both pets and owners (CDC, 2007). The American College of Veterinary Nutrition (ACVN) also released a statement cautioning about the health risks associated with RMBDs and recommended clients work with their veterinarian to determine if feeding a RMBD is the most appropriate choice (ACVN, 2016). These statements primarily focus on human and animal health risks associated with feeding RMBDs. These risks include the potential for infection from pathogenic microbes or parasites, nutritional inadequacies, and other contaminants that can cause harmful physiological changes (Freeman et al., 2013).

Microbial contamination, by either bacteria or viruses, is the most frequently documented disadvantage of feeding RMBDs. Specifically, *Salmonella spp.* have received the most attention; however, other harmful bacterial contaminants may include *Escherichia coli*,



*Listeria*, *Clostridium*, and *Campylobacter spp.* These bacteria are sometimes commonly associated with various species of domestic livestock (Jenkins et al., 2016). Control measures, such as carcass trimming and washing, are often implemented by packing plants to reduce bacterial contamination on meat products but these practices are unable to completely eliminate all potential contaminants in raw meats (LeJeune and Hancock, 2001). Also, in the absence of gross changes in pathology, contamination of meat by bacteria, viruses, or even parasites can go unnoticed during inspection (LeJeune and Hancock, 2001).

Dogs, as carnivores, possess many physiological adaptations (e.g., short gastrointestinal tract, low stomach pH, commensal bacteria, etc.) that allow them to tolerate relatively high levels of microorganisms in their diet. As a result, dogs do not frequently exhibit clinical illness when colonized by potentially pathogenic bacteria (NRC, 2006; Lenz et al., 2009). As these microorganisms are shed in dog feces, they present potential human health concerns as many of these microorganisms also cause varying degrees of illness in humans (Lenz et al., 2009). Prevalence of *Salmonella spp.* in feces of clinically normal dogs, independent of diet, is estimated between 1.0 and 18.0% but actual prevalence of infection is suspected to be much higher because dogs can be sporadic shedders of *Salmonella spp.* (Sanchez et al., 2002).

Bacterial species found in RMBDs have the capacity to cause illness in dogs. Like in humans, dogs that are immuno-naïve, immunocompromised, or geriatric are at greatest risk for infection from microorganisms present in RMBDs (Freeman et al., 2013). Alterations of gut microenvironment, initiated by a physiological stressor, antibiotics, or other cause, may result

in an increased risk of illness from food-borne pathogenic contaminants (LeJeune and Hancock, 2001). Although not common, clinical salmonellosis can occur in dogs and most often manifests as acute diarrhea with septicemia resulting only in rare instances (Gruenberg, 2015). Clinical cases of salmonellosis in companion animals, caused either directly or indirectly by feeding of a RMBD, have been documented in scientific literature (Striver et al., 2003; Morley et al., 2006). Additionally, asymptomatic carriers of pathogens can develop clinical disease if their immune systems become compromised or overwhelmed (Gruenberg, 2015). Moreover, fecal shedding of pathogens, including but not limited to *Salmonella spp.*, is increased in dogs and other animals during times of illness and or hospitalization (Cummings et al., 2010; AVMA, 2012).

Feeding RMBDs to dogs may increase fecal shedding of *Salmonella* and other bacterial pathogens. The results of one small-scale study (n=10 dogs per group) showed that 30.0% of dogs fed RMBDs had positive fecal cultures for *Salmonella spp.* (P=0.105) and RMBDs were more likely (P<0.001) to contain *Salmonella spp.* than an extruded diet (8 of 10 RMBDs positive for *Salmonella spp.*) (Joffe and Schlesinger, 2002). Results were similar in a larger study (n=80 dogs per group) indicating dogs consuming raw meat diets tested positive for *Salmonella spp.* [odds ratio (OR) 22.7; 95% confidence interval (CI) 3.1-58.8; P<0.001] and extended-spectrum cephalosporinase *Escherichia coli* (OR 17.2; CI 9.4-32.3; P<0.001) at least once during the past year compared with dogs not consuming raw meat; however, wide confidence interval ranges suggest high degree of variability in the results (Lefebvre et al., 2008). Diets evaluated in the study varied and differences in protein type (e.g., muscle meat, organ meat, etc.), overall diet formulation, species of meat used, handling and preparation of diets, and other factors could

potentially explain the large observed variability in presented data. Interestingly, this study did not find any differences between dietary groups for *Clostridium difficile*, Methicillin-resistant *Staphylococcus aureus*, or Vancomycin-resistant *Enterococci*. Furthermore, a 2015 study documented that dogs fed either a control (extruded diet) or raw meat diet shed *Campylobacter spp.* in their feces, with no significant differences in *Campylobacter* shedding detected between groups (Olkola et al., 2015). These previous data demonstrate high variability in pathogen shedding in dogs fed RMBDs and that incidence of shedding is likely lower than frequency of *Salmonella spp.* exposure through diet. Further, these studies evaluate associations between diet types and pathogen shedding in client-owned dogs; however, a multitude of factors separate from diet may contribute to pathogen shedding in feces (e.g., livestock exposure). Future studies need to evaluate causal relationship between dietary pathogen exposure and subsequent shedding in the feces of dogs by controlling for potential confounding environmental factors.

To date, no documented cases of human illness associated with feeding of RMBDs to companion animals have been documented (Finley et al., 2006; AVMA, 2012). However, individual cases of dog to human transmission of *Salmonella spp.* have been documented in scientific literature indicating a potential for transmission (Morse et al., 1976; Sato et al., 2000). In both of the aforementioned cases, *Salmonella spp.* were transmitted between dogs and children. Further, a study published by MacDonald et al. (2015) indicated that risk of *Campylobacter* infection was significantly increased in children that had contact with dogs and/or dog feces (interaction OR 2.4; 95% CI 1.1-5.3). This increased risk of infection in children

is likely due to several factors. Children's immune systems are relatively naïve compared with adults and they also have less stability in gut microflora (Jaspan et al., 2006; Vangay et al., 2015). Additionally, children generally have poorer personal hygiene habits and understanding of potential harm from coming into contact with dog or animal feces (Siegal, 1988). It is important to note that all dogs, regardless of diet, pose a risk for transmission of zoonotic diseases. As previously mentioned, dogs consuming extruded diets can still shed bacteria in their feces that may cause illness in humans. Many parks, schools, and playgrounds have adopted rules that prohibit dogs from walking on the grounds due to risk of zoonoses transmission of tapeworms, roundworms, giardia, and other parasites that dogs can harbor (Traversa et al., 2014).

Risk of parasitic infection is another documented disadvantage of RMBDs. Feeding RMBDs to dogs may result in parasitic infections of protozoans, nematodes, cestodes, or trematodes. Examples of parasites associated with RMBDs include, but are not limited to: *Toxoplasma gondii*, *Trichinella*, *Sarcocystis*, *Neospora*, *Echinococcus*, *Cryptosporidium*, and many others (AVMA, 2012; CDC, 2012). Although these parasites have been documented to cause clinical illness in dogs (Rice et al., 1990; Moré, 2013; Jenkins et al., 2014; Hamel et al., 2016), to date no confirmed cases have been linked with the practice of feeding RMBDs. Specifically, freezing any raw meat portion of the diet at -4.0°C for approximately a week or -20.0°C for a minimum of 1 day is often an effective measure to reduce or eliminate potential parasite concerns (Sotelo et al., 1986; Tenter, 2009; FDA, 2011; Moré, 2013; Wilson et al., 2015).

Concerns about nutritional inadequacies of homemade or commercially-available RMBDs have been a major critique of RMBDs in scientific literature. A study from 2010 evaluated owner responses to a dietary questionnaire regarding feeding practices of RMBDs. Based on self-reported data from owners, authors estimated nutrient content for each ration. Based on these estimates, greater than 60.0% of RMBDs had marked nutrient imbalances such as an imbalanced Ca:P ratio (0.6:1). Approximately 10.0% of diets provided less than 25.0% of recommended allowance of calcium and about 50.0% of diets were inadequate in vitamin D. Additional nutrient imbalances also were found for vitamin A and iodine (Dillitzer et al., 2011). Although owners did not report any adverse health effects associated with the nutritional imbalances, it is possible that symptoms may have gone unnoticed by owners or that nutrient deficiencies were subclinical at the time of investigation. A similar study estimated nutrient content of home-prepared diets for dogs and cats with chronic kidney disease (CKD) based on recipes published in books or available via internet. Even though the majority of recipes published were created by veterinarians, most were determined inappropriate for managing an animal with CKD based on established nutrient recommendations for dietary intervention. However, additional inquiry to determine efficacy of these diets was not performed. The authors emphasized that ambiguity in published recipes likely resulted in prepared diets highly variable in nutrient content (Larsen et al., 2012).

Individual published case studies also have shown varying degrees of clinical illness associated with improperly formulated RMBDs. For example, two domestic cats developed

subclinical pansteatitis due to consuming a diet consisting primarily of pig brain (approximately 39.5 – 42.5% fat, DMB) (Niza et al., 2003). Another case reported that a domestic cat presented to a veterinary hospital for lameness and sudden-onset paralysis. The cat was consuming a homemade diet consisting primarily of raw pork liver and diagnosed with hypervitaminosis A, as serum levels were three times the upper limit. After correcting the inadequacy by switching to a commercial canned diet, the cat made an almost complete, functional recovery (Polizopoulou et al., 2005). Nutritional imbalances resulting in clinical disease also have been observed in dogs. One case reported diffuse osteopenia and myelopathy in a Shetland sheepdog puppy resulting from hypocalcemia and hypophosphatemia caused by an improperly formulated RMBD (Taylor et al., 2009). The calcium to phosphorus ratio of the diet fed was 1.0:5.5 [minimum allowance for growth (MAFG) 1.0 – 2.0:1.0] and absolute concentrations of both calcium (0.08%) and phosphorus (0.44%) were below MAFG, 1.0 – 2.5% and 0.8 – 1.6%, respectively (Taylor et al., 2009). Similarly, a RMBD containing excessive levels of phosphorus fed to German shepherd puppies induced a nutritional secondary hyperparathyroidism that resulted in moderate to severe fibrous osteodystrophy (Kawaguchi et al., 1993).

Separate from nutritional imbalances, RMBDs can cause other potential adverse health effects related to source of meat and bioactivity of ingredients in the diet. For example, two dogs were diagnosed with dietary hyperthyroidism caused by feeding ground beef head meat that contained thyroid gland tissue after presenting with polyuria, polydipsia, excessive panting, restlessness, and bloodwork indicating elevated serum thyroxine (Zeugswetter et al., 2013). Diet samples were analyzed and revealed elevated iodine concentrations (mean +/- standard

deviation) of 9.4 +/- 2.4 mg/kg (average range for iodine in muscle 0.02 – 0.15 mg/kg). Removal of visible thyroid tissue during trimming reduced iodine levels in ground samples to below 0.08mg/kg. An additional five dogs consuming head meat from the same supplier also showed elevated serum thyroxine (193.0 nmol/L). Following dietary elimination or reduction of head meat from this supplier, serum thyroxine levels in all dogs normalized (Zeugswetter et al., 2013). In two separate but related cases, similar clinical symptoms were observed in dogs consuming RMBDs. Although the authors did not analyze the diet for elevated iodine levels, clinical symptoms subsided following a dietary change to a commercial diet and serum thyroxine levels returned to normal levels (17.0 – 58.0 nmol/L) (Köhler et al., 2012; Cornelissen et al., 2014; IDEXX, 2015).

The consequences of nutritional imbalances and inappropriate ingredient selection can cause potentially life-threatening clinical issues in companion animals. Nonetheless, these examples illustrate the impact and importance that nutrition can have on health and wellbeing of an animal. However, it is important to note that these examples are not necessarily disadvantages reflective of feeding RMBDs but, instead, indicate an improper diet formulation or inappropriate ingredient selection or handling. Consulting with a companion animal nutritionist and licensed veterinarian, to formulate an appropriate diet that addresses both nutritional needs and health status of the patient, can circumvent many aforementioned issues.

**Assessing Nutritional Implications on Canine Health:**

Health effects of diet are poorly described in scientific literature for companion and exotic carnivores. For purposes of this review, health is defined as the absence of any disease or impairment. Most feeding and digestibility studies are short in duration (less than 3 months) and do not provide insight into long-term health effects associated with diet type. Further, general health status is often assessed using serum biochemistry, electrolyte, and complete blood count (CBC) values. These values are limited in ability to assess canine health as these values are often laboratory specific and typically established with a sample population of dogs (eClinpath, 2016). Differences in hematology and serum biochemistry values have been documented between extruded diets with either animal-based or plant-based protein sources (Swanson et al., 2004). Further, elevations in serum metabolites such as blood urea nitrogen (BUN), total cholesterol, and serum alanine aminotransferase (ALT), have been documented in both companion animals and exotic felids consuming RMBDs (Vester et al., 2010b; Beloshapka et al., 2011; Kerr et al., 2012; Kerr et al., 2013). For these aforementioned reasons, variations outside of established reference intervals may not necessarily indicate a health impairment but rather a dietary change.

Additional measures of nutritional influences on health status need evaluated in companion and exotic animals. Ethical considerations limit and often prevent use of invasive techniques of assessment. Gastrointestinal histological samples can be obtained through biopsy in diseased patients already undergoing procedures or may be obtained following euthanasia (Allenspach, 2015; Cassmann et al., 2016). Alternatives, such as ultrasonographic imaging of



intestines, have shown some correlation with histological layering (Le Roux et al., 2016); however, immune cell infiltration of gastrointestinal tissues cannot be determined using the technique.

Measures of intestinal integrity and barrier function, as assessed by intestinal transepithelial electrical resistance (TER) and macromolecule permeability, using Ussing chambers are common in livestock and rodent studies (Ueno et al., 2011; Pearce et al., 2013; Pearce et al., 2014; Müller et al., 2016) but are limited in canines (Neirinckx et al., 2011; Hill and Bliklager, 2012). Intestinal TER measures ion flux across a membrane. Decreased ion flux, resulting in an increased TER, is indicative of increased intestinal integrity and barrier function (He et al., 2013). Decreased macromolecule permeability, as determined by relative fluorescence, is another indicator of increased intestinal integrity and barrier function (He et al., 2013). TER and macromolecule permeability values are typically inversely correlated. Application of Ussing chamber technology may offer a novel approach to evaluating diet type on intestinal integrity and barrier function in canines. Studies using these methodologies should be limited to opportunistic tissue sample collection from animals already scheduled for euthanasia or careful consideration of experimental design and objectives as euthanized dogs or exotic animals for research are typically not positively perceived by public. Alternative methods such as measurement of urinary excretion of lactulose mannitol or similar compound following oral administration can also be used to assess intestinal permeability *in vivo* (Wijten et al., 2011; Sequeira et al., 2014); however, ex vivo studies utilizing Ussing chambers allows for regional specific evaluation of intestinal integrity (Westerhout et al., 2015).

### **Meat Sourcing for Carnivore Management:**

Livestock species including poultry, beef, lamb, and pork have all been utilized in the pet food industry (Taylor, 2014). Dressing percentages, the amount of carcass weight used for human-consumption, for most livestock species average between 45.0-73.0% (Rentfrow, 2010). The remaining carcass portions are typically sent to rendering for production of animal fats and animal-meals for use in animal feeds or for other purposes (e.g., hides, tanning, etc.) (Meeker, 2009). The rendering process is an important part of the pet food and animal feed industry; however, rendering can result in destruction of important nutrients (Singh et al., 2007).

Although not the only factor considered, diet cost for zoo-managed carnivores must be considered being that feed costs impact successful operations. With rising costs of meat products, zoos must consider meat source for carnivore diets. Due to cost and historical accessibility, beef and horse-based RMBDs have dominated the zoo industry. With the loss of inspected horse meat processing facilities in the United States in 2007 and rising beef prices, feed costs have become an even larger concern. Some commercially manufactured zoological formulations have utilized more economical meats in order to meet a demand for lower cost products. This raises concerns due to use of 3D/4D meats or plant proteins to formulate some products. Although these meats are more economical, they raise concerns because meats labeled as 3D (diseased, dying, or downed) or 4D (dead on arrival) are unfit for human consumption and are denatured with charcoal to minimize potential risks of feeding (USDA, 2006). These products may be higher in bacterial contamination although research is needed to determine differences between human grade and 3D/4D meats. Zoos may feed a mixture of

these lower quality meat sources with higher quality products to offset costs while attempting to maintain diet quality.

Animal by-products have been an important ingredient source for the pet food industry over the years. However, recent humanization in the pet food industry has led to negative marketing of animal by-products, equating by-products to low-quality, unhealthy ingredients (Phillips-Donaldson, 2014). Animal by-products can have high nutritional value to both domestic and exotic carnivores. Moreover, wolves preferentially select viscera (e.g., lungs, heart, liver, brain, etc.) that are classified as “animal by-products” when consuming a fresh kill (Peterson and Ciucci, 2003; Stahler et al., 2006). This nutritional value is further retained if these products are used in RMBDs instead of going to rendering for production of by-product meals (Singh et al., 2007). Additionally, utilization of animal by-products in RMBD formulation increases the sustainability of associated livestock industries as a greater proportion of the carcass is used and less is sent to rendering facilities.

Safety of horsemeat-based RMBDs has been questioned due to regulation concerns. Specifically, since horses are predominately raised for racing, working, and companionship, record keeping for drug administration varies and drug withdrawal periods have not been established for horse slaughter as with other livestock species (JAVMA news, 2015). Increasing concerns over the safety of horsemeat arose following the deaths of two carnivores at a wildlife sanctuary in Colorado after consumption of horsemeat contaminated with barbiturates, likely from a commonly used euthanasia solution (Corona, 2015). Horsemeat listed as “human grade”

still poses safety concerns due to poor regulation in slaughter facilities located outside the United States. Because horse slaughter is illegal in the United States, horsemeat for dietary management of exotic carnivores must be imported from either Canada or Mexico. Currently; due to safety, traceability, and public relations concerns; horsemeat is no longer used in pet food in the United States.

Diets formulated with novel protein sources are only recently emerging into the zoological market with a pork-based RMBD formulated for managed exotic carnivores (Carnivore Essentials, 2014). Increasing the variety of protein sources available to managed exotic carnivores is an important form of nutritional enrichment and variety may prevent neophobic behaviors in managed animals (AZA Canid TAG, 2012). Additional options may also help manage feed costs.

Unfortunately, when novel products are manufactured, lack of research and objective evaluation often limit use of new products until information is available. Further, protein source selection should take into consideration environmental sustainability as marked differences exist between livestock species regarding feed conversion ratios (FCR), the amount of feed required for one pound of gain (Wilkinson, 2011). Grain-fed beef have a FCR of approximately 8.8 while pork and poultry have estimated FCRs of 4.0 and 2.4, respectively (Wilkinson, 2011).

Meat source is an important factor for both pet and zoo industries. Human-grade meats may provide increased safety but result in higher feeding costs. Cost of diet is less of a factor in

the pet food industry, as premium and super-premium foods dominate the market (Sprinkle, 2013). Ultimately, a balance needs to be achieved between cost-effectiveness and nutritional quality to ensure health and safety of managed carnivores.

**Conclusions:**

Diet selection for both domestic and managed exotic carnivores is a decision based on multiple factors including processing effects on diet, animal and human health implications of diet, natural history and known nutrient requirements of the animal, product availability, dietary variety, and cost effectiveness. Collectively, veterinarians and nutritionists alike need to objectively evaluate risks and benefits of feeding RMBDs to domestic dogs and exotic canids. This includes proper risk assessment, cost analysis, food safety, feces management practices, and education. Future research goals should aim to expand on these factors especially in reference to animal and human health implications, animal performance, and product comparisons.

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### CHAPTER 3

#### EVALUATION OF DIET COMPOSITION, APPARENT TOTAL TRACT MACRONUTRIENT AND ENERGY DIGESTIBILITY, FEED INTAKE, FECAL OUTPUT, AND MICROBIAL PRESENCE IN DOMESTIC DOGS FED COMMERCIAL RAW MEAT-BASED DIETS.

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#### Abstract

Raw meat-based diets (RMBDs) varying in protein source are commonly fed to zoo carnivores and these diet types are gaining in popularity for pet domestic dogs. Most research has focused on exotic felids with limited information for managed exotic canids. Additionally, research of RMBDs for domestic dogs has focused almost exclusively on microbial concerns and not associated health effects. The objectives of this study were to evaluate diet macronutrient composition, apparent total tract macronutrient and energy digestibility, feed intake, fecal output, and microbial presence of four commercial RMBDs for zoo carnivores using domestic dogs. Four intact male dogs (*Canis lupus familiaris*) were fed dietary treatments including horse (Horse), pork (Pork), and two different beef diets (Beef 1; Beef 2) in a repeated 4x4 Latin square design consisting of 14-day periods. Dog saliva, feces, and diets were swabbed for microbial testing. Treatment nutrient concentrations ranged for dry matter (DM) (32.2 – 36.2%), organic matter (OM) (91.1 – 94.9%), crude protein (CP) (50.3 – 61.7%), fat (25.1 – 38.3%), and gross energy (GE) (5805.0 – 6419.8 kcal/kg). Fecal scores across treatments ranged from 1.2 (Horse) to 3.1 (Beef 1). Digestibility of nutrients and energy ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.2%, and 91.3 – 95.5% for DM, OM, CP, fat, and energy, respectively. Dogs consuming Beef 2 had greater digestibility of ( $P<0.05$ ) DM (92.4%), OM (95.3%), CP

(97.7%), and GE (95.5%) but lower ( $P<0.05$ ) digestibility of fat (94.9%) than all other diets evaluated. Dogs consuming Pork diet had greatest GE (6419.8 kcal/kg DM), DE (6027.1 kcal/kg DM), and calculated ME (5547.2; 5093.1; 5504.5 kcal/kg DM) of all diets. All diet (n=16) and saliva samples (n=36) tested negative whereas two fecal samples (n=36) tested positive for *Salmonella spp.* Diet samples from Beef 2 (n=4) for both replicates and two saliva samples (Pork) tested positive for generic *Escherichia coli* (*E. coli*). Feeding RMBDs to domestic diets did not result in major reductions or differences in nutrient digestibility for three of four diets and fecal scores were maintained at 3.1 or less. Therefore, horse, pork, and beef-based diets can be utilized as effective options for managing dogs and likely exotic canids based on nutrient digestibility. Lastly, microbial presence in saliva and fecal samples was lower than expected and these data suggest a minimal risk of human health implications when feeding these RMBDs to exotic or domesticated carnivores.

## Introduction

Research regarding raw meat-based diets (RMBDs) is limited for both managed exotic carnivores and companion animals. Focus of companion animal research relating to RMBDs has been pathogen shedding, case studies, and human health implications (Joffe and Schlesinger, 2002; Lefebvre et al., 2008; Olkkola et al., 2015). Few studies have made comparisons of digestibility or health parameters in companion animals fed RMBDs compared to extruded or canned diets, particularly in dogs (Kendall et al., 1982; Kerr et al., 2011; Kerr et al., 2012; Hamper et al., 2015). Research efforts using managed exotic carnivores to evaluate RMBDs have been conducted almost exclusively with felid species not canids (Crissey et al., 1997;

Vester et al., 2010; Kerr et al., 2013a; 2013b; Iske et al., 2016). Further, beef and horse proteins have comprised the majority of RMBDs used in zoological institutions. Our laboratory previously demonstrated effective use of raw pork for managed exotic felids (Iske et al., 2016); however, similar studies have not been conducted in exotic canids.

Exotic canids managed in zoological institutions are often housed in groups or packs, making individual feed intake and fecal output data for digestibility studies difficult to collect. Basic anatomy and physiology of digestion between dogs and wolves is thought to be highly conserved (Peterson and Ciucci, 2003). Apparent protein and fat digestion in exotic carnivores (e.g., canids, hyenids, ursids, etc.) follows a similar pattern as domestic dogs (Clauss et al., 2010). Starch digestion has been the primary nutritional target in domestication of the dog (Axelsson et al., 2013; Arendt et al., 2014) which is further evidenced by an increased capacity for apparent total tract digestibility of starch (Clauss et al., 2010). Because of their conserved anatomy and physiology, along with starch being low or absent in RMBDs, domestic dogs may serve as valuable models in nutrition studies for exotic canids and other exotic carnivores. Additionally, diets for zoological carnivores are typically formulated to meet NRC nutrient requirements for dogs and cats as actual nutrient requirements for exotic species are unknown. Therefore, the objectives of this study were to evaluate diet macronutrient composition, apparent total tract macronutrient and energy digestibility, feed intake, fecal output, and microbial presence of four RMBDs commercially manufactured for zoological carnivores using the domestic dog as a model. We hypothesize all RMBDs 1.) will be comparable in macronutrient and energy composition, 2.) will have high digestibility for macronutrients and

energy in domestic dogs, 3.) will vary in fecal scores and fecal output between diets, and 4.) will pose low microbial risk to human health.

## **Materials and Methods**

### *Animals:*

All animal procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC) prior to animal experimentation. Four intact male domestic dogs (*Canis lupus familiaris*) with predominately hound bloodlines were used in this study ranging in age from 1.0 to 3.0 years, body condition score (BCS) from 4.0 to 7.0 (9-point scale; Nestlé Purina), and body weight from 16.5 to 23.0 kg. Dogs used in this study served as breeding males for a separate, biomedical research colony and were maintained on extruded kibble foods prior to initiation of our study. All dogs were housed individually in (2.5m L x 1.2m W x 1.8 m H) runs at the College of Veterinary Medicine at Iowa State University (Ames, IA, USA) in order to prevent inter-male aggression. Dogs were maintained in accordance with United States Department of Agriculture (USDA) and National Institutes of Health (NIH) guidelines for care of dogs used in biomedical research. To ensure all dogs were free of potential underlying health ailments that may confound data and to obtain baseline values, complete blood count (CBC) and serum chemistry panels with electrolytes were performed prior to initiation of the study. Fecal floatation examinations were performed prior to initiation of the study to ensure all dogs in the facility were free of protozoal and nematode parasites. Dogs were kept on a 14:10 hour light:dark schedule in a temperature controlled room (20°C).

Water was provided ad libitum throughout the study. Dogs were fed twice daily (0400 and 1400) to maintain body weight and BCS based on previous energy intake estimates.

*Experimental Design:*

Dogs were randomly assigned to one of four dietary treatments in a repeated 4x4 Latin square design consisting of 14 d periods. Each period included a 10 d diet adaptation phase (d 1 – 10) followed by a 4 d total fecal collection phase (d 11 – 14). This design allowed each dog to receive each diet for one period during each replicate. Previous energy intake was estimated for each dog prior to beginning the study. Dogs were fed to maintain body weight and body condition throughout the course of the study.

Total feed intake, fecal output, and fecal scores were recorded for each dog during each collection period. Total fecal output for each dog was collected and scored daily and stored at -20.0°C until laboratory analyses. Fecal scores were determined using the following scale: 1 = very hard, dry feces to 7 = watery diarrhea (Nestlé Purina). On d 14 of each period, fecal and saliva samples were obtained and stored at -80.0°C in sterile Whirl-Pak® bags (Nasco; Atkinson, WI, USA) until microbial analysis was performed. On d 14 of each period, blood samples were collected and submitted for serum chemistry with electrolytes and CBC. Dogs were weighed and body condition scored on d 14 of each period and also at initiation and conclusion of the study.

### *Experimental Diets:*

Four commercially manufactured RMBDs commonly fed to managed exotic carnivores were evaluated in this study. Dietary treatments included one horse-based (Nebraska Brand Premium Feline; Central Nebraska Packing Inc.; North Platte, NE, USA (Horse)), one pork-based (Carnivore Essentials; Sustainable Swine Resources; Sheboygan Falls, WI, USA (Pork)), and two beef-based [Nebraska Brand: Special Beef; Central Nebraska Packing Inc.; North Platte, NE, USA (Beef 1); Frozen Beef Zoo Diet; Kennel Supply LLC; Council Bluffs, IA, USA (Beef 2)] diets. All diets were reported as formulated to meet or exceed nutrient requirements of domestic cats and dogs (NRC, 2006) except Beef 2 labeled as a custom specialty pet formulation marketed in the Iowa greyhound racing industry but utilized as a diet option in regional zoos. Ingredients for each dietary treatment are listed in *Table 3.1*. Each dietary treatment was subsampled, swabbed for microbial testing, and stored at -20.0°C until laboratory analyses.

### *Diet and Fecal Chemical Analyses:*

Total fecal collections and dietary treatments were analyzed for macronutrient chemical composition and energy. A sub-sample (50 – 100g) of each diet was collected during each period and pooled for analyses. All chemical analyses except were conducted in the Comparative Nutrition Laboratory at Iowa State University (Ames, IA, USA). Fecal samples and dietary sub-samples were dried at 55.0°C in a forced air drying oven and ground with a coffee grinder to accommodate small sample size (model BCG110B; KitchenAid). Diet and fecal samples were analyzed for dry matter (DM) (Method 934.01, AOAC) and organic matter (OM) (Method 942.05, AOAC). Crude protein (CP) was determined using a LECO Nitrogen Analyzer



(Method 992.15, AOAC) (model TruMacN; LECO Corporation; St. Joseph, MI, USA). Crude fat was determined via acid hydrolysis and hexane extraction (Method 960.39, AOAC). Gross energy (GE) was determined via bomb calorimetry (model 6200; Parr Instrument Co.; Moline, IL, USA). Total dietary fiber (TDF) was determined in diets as a more accurate assessment of fiber than crude fiber, using the Prosky method (Prosky et al., 1984; AOAC, 2003) at the Comparative Nutrition Laboratory at the University of Illinois at Urbana-Champaign. Nitrogen-free extract (NFE) concentrations were calculated using the following equation:  $\text{NFE \%} = 100 - \Sigma (\text{moisture \%}, \text{CP \%}, \text{fat \%}, \text{ash \%}, \text{TDF \%})$ .

#### *Feed Intake and Fecal Output:*

Feed intake was recorded for each dog throughout the experiment. Total fecal output was collected daily during the collection phase of each period and averaged in order to determine daily fecal output (g as-is/d). These values were then used to determine energy and macronutrient digestibilities. Feces collected during the 4 d collection period was pooled for each dog and mechanically ground in order to homogenize samples.

#### *Apparent Total Tract Digestibilities and Energy Calculations:*

Apparent total tract macronutrient and energy digestibilities were determined using the chemical composition data from diet and fecal samples and feed intake/fecal output records.

Apparent total tract macronutrient and gross energy (GE) digestibilities were calculated using

the following equation:  $\text{Apparent digestibility (\%)} = \left( \frac{\text{intake} - \text{fecal output}}{\text{intake}} \right) * 100$

Digestible energy (DE) values were calculated using the following equation:

$$\text{DE} = \text{kcal GE/g of diet} * \% \text{ GE apparent total tract digestibility of diet}$$

Metabolizable (ME) energy values were estimated using Atwater values, modified Atwater values (AAFCO), and the NRC (2006) equation to provide a comparison of the three methods.

Equations for all three ME estimations are listed below:

$$\text{ME} = 9.0 \text{ kcal ME/g of fat} + 4.0 \text{ kcal ME/g of CP} + 4.0 \text{ kcal ME/g of NFE} \quad (\text{Atwater})$$

$$\text{ME} = 8.5 \text{ kcal ME/g of fat} + 3.5 \text{ kcal ME/g of CP} + 3.5 \text{ kcal ME/g of NFE} \quad (\text{AAFCO})$$

$$\text{ME} = \text{DE} - (1.04 * \text{g of CP}) \quad (\text{NRC})$$

The NRC equation is assumed to be the most accurate because it factors in calculated DE from experimental results for a specific diet. Modified Atwater values use digestibility coefficients of 81%, 85%, and 79% for CP, fat, and NFE, respectively. Modified Atwater values are commonly used in the pet food industry as an estimate of ME (Case et al., 2011). However, use of Atwater factors is recommended for estimated ME of homemade diets due to typically high digestibilities (NRC, 2006). Estimated digestibility coefficients of 91%, 96%, and 96% for CP, fat, and NFE, respectively, are used for calculation of Atwater values (Atwater and Bryant, 1900).

#### *Microbial Presence:*

Diet, saliva, and fecal samples were submitted to the Food Microbiology Laboratory in the Department of Animal Sciences at Iowa State University (Ames, IA, USA) for microbial testing. Two samples per dietary treatment were taken for each replicate for *Salmonella spp.* and generic *Escherichia coli* (*E. coli*) testing (n=16). One fecal and one saliva swab were obtained per dog and per period for each replicate and prior to initiation of the study (n=36). Diet and saliva samples were cultured for *Salmonella spp.* and generic *E. coli* and fecal samples

were cultured for *Salmonella* spp. Samples and extraneous fecal material from each sample bag were transferred to sterilized test tubes. For diet and saliva samples, 5mL of Buffered Peptone Water (BPW) was added to each test tube. For fecal samples, 3mL of BPW was added to each test tube.

For *Salmonella* analysis, 1mL was transferred into Neogen® ANSR® *Salmonella* Broth for overnight enrichment at 37°C and streaked onto XLD overly with Triple Sugar Iron Agar (TSA) slant (Kang and Fung, 2000). Samples that produced typical black colonies and tested positive on Neogen® ANSR® were further tested on Neogen® Reveal® 2.0 strips for *Salmonella*. Samples that produced a positive result on Neogen® Reveal® 2.0 strips for *Salmonella* were considered confirmed positive for *Salmonella*. For *E. coli* analysis, samples were vortexed and a 1mL aliquot was transferred to a 9mL peptone tube (1:10 dilution) and vortexed. Further serial dilutions were completed. For each dilution, 1mL of sample was plated onto 3M™ Petrifilm™ *E. coli*/Coliform test plates in duplicate. Plates were incubated at 37°C for 24 h and examined for *E. coli* and enumerated.

#### *Blood Panels:*

A 4mL sample of blood was collected from each dog via cephalic venipuncture at baseline and on d 14 of each period. Samples were submitted to the Clinical Pathology Laboratory at Iowa State University College of Veterinary Medicine (Ames, IA, USA) for a serum chemistry panel with electrolytes (VITROS 5.1 FS Chemistry Analyzer; Ortho Clinical Diagnostics; Raritan, NJ, USA) and CBC (ADVIA 2120i Hematology System; Siemens Healthcare; Erlangen,

Germany). Data from all dogs for both replicates were averaged within diet and reported with corresponding reference intervals for each measure.

### *Statistical Analysis:*

Apparent total tract digestibilities, feed intake, fecal output, fecal chemical composition, serum chemistry, and CBC data were analyzed using the mixed models procedure of SAS<sup>®</sup> (PROC MIXED, SAS Institute; Cary, NC, USA). The fixed effects of period, replicate, and diet were tested and dog was considered a random effect. Differences between diets were determined using least squared means (LSMEANS). A probability of  $P < 0.05$  was considered statistically significant and standard error of the means (SEM) were determined. Microbial data were analyzed as a binomial response (positive or negative) using the generalized mixed models procedure of SAS<sup>®</sup> (PROC GLIMMIX, SAS Institute; Cary, NC, USA). The fixed effects of period, replicate, and diet were tested and dog was considered a random effect.

## **Results**

### *Diet and Fecal Chemical Analyses:*

Chemical compositions for dietary treatments are presented in *Table 3.2*. Dietary treatment nutrient concentrations ranged for DM (32.2 – 36.2%), OM (91.1 – 94.9%), CP (50.3 – 61.7%), fat (25.1 – 38.3%), and GE (5805.0 – 6419.8 kcal/kg). TDF concentrations ranged from 0.0 to 5.9% resulting in calculated NFE concentrations that were very low, ranging from 0.8 to 5.4% for Beef 2 and Beef 1 diets, respectively. Chemical compositions of fecal samples collected from dogs fed each diet are listed in *Table 3.3*. Fecal nutrient concentrations ranged for DM

(27.4 – 46.9%), OM (58.9 – 72.8%), CP (12.7 – 22.9%), fat (2.9 – 21.5%), and GE (3.0 – 3.7 kcal/g). Fecal samples from dogs fed Horse (46.9%) had greater than 71% DM compared to Beef 1 diet (27.4%) ( $P < 0.05$ ). However, fecal samples from dogs fed Beef 1 (22.9%) contained approximately 80.3% more ( $P < 0.05$ ) CP than fecal samples from dogs fed Horse (12.7%). The most marked difference observed between fecal samples based on treatment was in fat concentration. Fecal fat concentrations for dogs fed Beef 2 (21.5% fat on DMB) were over 240.0% greater than Beef 1 and Pork diets (6.3 and 6.1% fat on DMB, respectively) and over 650.0% greater than the Horse diet (2.9% fat on DMB) ( $P < 0.05$ ).

*Feed Intake, Fecal Outputs, and Fecal Scores:*

Feed intake and fecal output data for dietary treatments are presented in *Table 3.4*. Feed intake (g AF/d) was the same across all dietary treatments as per experimental design. When considering the moisture contents, dry matter intake (DMI) varied slightly from a low of 178.0 g/d for Beef 2 to a high of 200.2 g/d for Horse but were not significant. This variation reflected GE intakes (kcal/d) that ranged from 1132.2 to 1251.0, also not significant.

Fecal output (g as-is/d) was 2.5 fold greater ( $P < 0.05$ ) in dogs fed Beef 1 diet (102.4 g/d) than Beef 2 (29.4 g/d) and approximately 0.6 fold higher ( $P < 0.05$ ) than Horse and Pork fed dogs (69.1 and 57.4 g/d, respectively). Fecal output (g DM/d) differed ( $P < 0.05$ ) between all dietary treatments including Beef 2 (13.5 g/d), Pork (21.3 g/d), Beef 1 (27.4 g/d), and Horse (32.3 g/d). Fecal scores of dogs consuming Horse (1.2) were more than one-fold lower ( $P < 0.05$ ) than fecal

scores from dogs consuming either Pork (2.7) or Beef 1 (3.1). Dogs consuming the Beef 2 diet had fecal scores of 1.9, differing ( $P<0.05$ ) from the other treatments.

*Apparent Total Tract Digestibilities and Energy Calculations:*

Apparent total tract digestibility values and energy calculations are listed in *Table 3.4*. Digestibility of nutrients and energy ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.2%, and 91.3 – 95.5% for DM, OM, CP, fat, and energy, respectively. Dogs consuming Beef 2 diet had the greatest ( $P<0.05$ ) digestibility of DM (92.4%), OM (95.3%), CP (97.7%), and GE (95.5%) compared with all other treatments. In contrast, dogs consuming this diet also had the lowest ( $P<0.05$ ) digestibility of fat (94.9%) compared with all other diets evaluated that averaged 97.8% and were not different. Dogs consuming Horse diet had the lowest ( $P<0.05$ ) DM (83.3%), OM (88.4%), and energy (91.3%) digestibility values compared to dogs consuming other treatments. Interestingly, dogs fed Horse had the lowest digestibility values for three of five measures (DM, OM, GE) while dogs fed Beef 2 had the highest digestibility values for four of five measures (DM, OM, CP, GE). Dogs feed Pork and Beef 1 diets had similar digestibility values for OM and fat that averaged 91.1 and 97.7%, respectively. Dogs fed the Beef 1 diet did have the lowest ( $P<0.05$ ) CP (93.8%) digestibility values compared with all other treatments. Dogs fed Pork had the highest ( $P<0.05$ ) fat digestibility (98.2%) compared with all other diets that averaged 96.7%. All diets were considered highly digestible ( $>90.0\%$  digestibility) for CP, fat, and energy.

Digestible energy (DE) values ranged from 5297.7 to 6076.3 kcal/kg for Horse and Beef 2 diets, respectively. The application of the NRC equation yielded metabolizable energy (ME) values of 4728.7, 5504.5, 5066.2, and 5435.1 kcal/kg for Horse, Pork, Beef 1, and Beef 2, respectively, that were similar to calculated values using Atwater factors that yielded 4659.4, 5547.2, 5148.5, and 5414.4 kcal/kg for Horse, Pork, Beef 1, and Beef 2, respectively. The application of AAFCO recommended modified Atwater factors yielded lower values of 4233.9, 5093.1, 4703.5, and 4940.1 kcal/kg for Horse, Pork, Beef 1, Beef 2, respectively.

#### *Microbial Presence:*

Microbial presence in diet, saliva, and fecal samples are presented in *Table 3.5*. All diet samples (n=16) tested negative for *Salmonella spp.* Generic *E. coli* was detected in 2 of 4 diet samples from the Beef 2 treatment and was negative in all other diet samples. *Salmonella spp.* were not detected in any of the saliva samples (n=36); however, two saliva samples obtained from dogs fed Pork, tested positive for generic *E. coli*. *Salmonella spp.* was detected in only two fecal samples (n=36) and both samples were obtained from dogs fed Pork.

#### *Blood Panels:*

Means are reported by diet for both serum chemistry panel with electrolytes (*Table 3.6*) and CBC (*Table 3.7*). Hematology revealed treatment differences ( $P<0.05$ ) for white blood cell (WBC) counts and mean platelet volume (MPV) but all values were within normal reference ranges. Overall, no other treatment differences were detected for CBC values. Slight elevations above reference intervals were observed for blood urea nitrogen (BUN) for dogs fed Beef 2 diet

(31.1mg/dL; reference: 10.0 – 30.0mg/dL) and triglycerides for dogs fed Beef 1 diet (115.5mg/dL; reference: 24.0 – 115.0mg/dL). Dogs fed RMBDs had, on average, an increase of 76.0, 39.1, and 18.7% for BUN, triglycerides, and cholesterol, respectively, compared to baseline values. All dogs exhibited higher than normal concentrations of hemoglobin (range: 18.7 – 19.2g/dL; reference: 12.0 – 18.0g/dL) and high-normal hematocrit during each treatment period and at baseline (range: 56.1 – 56.6%; reference: 37.0 – 57.0%). Treatment differences ( $P < 0.05$ ) were detected for BUN, creatinine, calcium, and magnesium.

## Discussion

### *Diet Chemical Composition and Nutrient Digestibility*

The study objectives included evaluation of various products manufactured for zoos using the domestic dog as a model; therefore, dietary treatments varied in ingredients and chemical composition. As expected, all dietary treatments were high in CP (>50.0% DMB) and fat (>25.0% DMB) and were comparable in nutrient and energy concentrations (*Table 3.2*). Digestibility of nutrients and energy ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.2%, and 91.3 – 95.5% for DM, OM, CP, fat, and energy, respectively (*Table 3.4*). A previous study from our laboratory using large exotic felids reported similar ranges for DM (83.6 – 88.0%), OM (88.5 – 90.8%), CP (92.7 – 95.7%), fat (96.5 – 99.0%), and energy (90.9 – 92.4%) digestibility of RMBDs including the Horse, Pork, and Beef 1 diet used in the present study (Iske et al., 2016). In an alternative study by Kerr et al. (2012), the same Beef 1 diet was evaluated in domestic cats. Apparent total tract digestibility for DM (86.1, 85.6, and 86.7%), OM (90.3, 89.2, and 90.5%), CP (93.8, 93.1, and 93.3%), fat (97.1, 96.5, and 95.5%), and GE (92.0, 90.9, and



91.5%) were similar between the current study, Iske et al. (2016), and Kerr et al. (2012) studies, respectively. These similarities suggest similar digestive efficiencies between domestic dogs and cats and certain species of exotic felids consuming RMBDs (Clauss et al., 2010). Therefore, dogs and cats may have potential application in evaluating raw meat-based dietary options for certain species of exotic carnivores.

Although dietary treatments differed in apparent total tract digestibilities, all diets were highly digestible. Specifically, digestibility of CP, fat, and energy all exceeded 90.0% indicating a high availability to the animal. In comparison, dogs fed processed diets have lower digestibility of nutrients with average apparent total tract digestibility for CP and fat ranging from 78.0 – 81.0% and 77.0 – 85.0%, respectively (Davenport and Remillard, 2010). Kerr et al., (2012) reported apparent total tract digestibilities in a super-premium, extruded diet of 78.2, 83.9, 81.6, 91.3, and 84.7% for DM, OM, CP, fat, and energy, respectively. Hamper et al., (2015) reported slightly higher values in kittens fed a processed canned diet (83.8, 88.4, 88.9, 94.2, and 90.2% for DM, OM, CP, fat, and energy, respectively). Although high for all treatments, digestibility of fat was lower in dogs fed the Beef 2 diet (94.9%) compared with other treatments that averaged 97.8%. Dry fecal output was only 13.5 g/d for Beef 2 compared with an average of 27.0 g/d for the other treatments. Additionally, 21.5% of the fecal DM was fat when dogs consumed the Beef 2 diet compared with an average of 5.1% for the other three treatments. Beef 2 was the only diet that did not include a dietary fiber source. Inclusion of dietary fiber typically decreases macronutrient digestibility of a diet, consistent with data obtained from this study (Kendall et al., 1982; Kerr et al., 2013b). Although lower in fat

digestibility, dogs consuming Beef 2 had greater ( $P < 0.05$ ) DM, OM, CP, and energy digestibility. Studies in dogs and poultry have found that inclusion of moderate amounts of dietary fiber may actually improve fat digestibility (Muir et al., 1996; Kienzle et al., 2001; Jiménez-Moreno et al., 2009). This may provide an explanation for the reduced digestion of fat observed for dogs fed Beef 2. Although Beef 2 diet had superior digestibility, this diet was not formulated to meet or exceed nutrient requirements of domestic cats or dogs. As a result, potential nutrient deficiencies, especially in micronutrients, are probable with extended feeding of this diet. No symptoms of nutrient deficiencies were observed in dogs during this study; however, our experimental design dictated that each dog was rotated through each of the four dietary treatments. Because dogs were not maintained on a single diet for a period greater than one month, this period of time may not have been long enough to elicit symptoms of micronutrient deficiency in the dogs.

*Meat Sourcing and Ingredient Selection for Carnivore Management:*

Diets in our study varied in their main protein ingredient. Purchasing decisions based on selection of primary protein ingredient are influenced by many factors including availability, source variability, safety concerns, cost, and possibly perceptions related to particular meat sources. Perception concerns include misinformation about product labeling, sourcing of meat, and safety of ingredients. Historically, concerns regarding pseudorabies and *Trichinella*-infected pork decreased its use in the human, pet food, and zoological industries (CDC, 2012). However, due to improved farming practices and freezing of raw pork meat, risk from commercially raised pork in the United States is considered negligible (CDC, 2012). Although diets in this study were

named for their main protein source (horse, pork, or beef), Beef 1 also contained meat by-products, fish meal, and soybean meal as additional protein ingredients. Meat by-products are defined by the Association of American Feed Control Officials (AAFCO) as: “The non-rendered, clean, parts, other than meat, derived from slaughtered mammals. This ingredient can include, but is not limited to, lungs, spleen, kidneys, brain, liver, blood, bone, partially defatted low-temperature fatty tissue, stomach, and intestines freed of their contents. It does not include hair, horns, teeth, and hoofs (AAFCO, 2016).” Labeling of this diet with meat by-products allows the manufacturer to use any species of meat that is available potentially increasing variability between different lots of the diet but also likely reducing production and consumer costs (Hendriks et al., 2002). Although providing variety to managed exotic carnivores can be beneficial from a health and enrichment standpoint (AZA Canid TAG, 2012), unknown variability in protein source within a diet may pose potential issues for carnivores with food allergies or aversions. This variation of source may also contribute to nutrient variability across lots when diets are mixed.

All diets used for this study utilized protein ingredients readily available from the agricultural industry including horsemeat, pork, pork by-products, beef, beef by-products, fish meal, soybean meal, and dried egg. Animal agriculture is an important provider of animal-based protein ingredients for both the pet food and zoological industries. Inclusion of by-products in Pork and both beef diet treatments serves to utilize portions of the carcass not in direct competition with the human-food industry. Recent trends in the pet food industry have moved toward exclusion of by-products due to consumer misconceptions about nutritional quality of

these products. Animal by-products (i.e., organ meats) are considered a very high-quality protein source from both an amino acid profile and bioavailability standpoint. Additionally, wild canids, most notably wolves, preferentially consume organ meats from a kill prior to skeletal muscle and other portions of the carcass (Peterson and Ciucci, 2003; Stahler et al., 2006). Inclusion of by-products in dietary treatments still resulted in high apparent total tract digestibilities (DM >86.0%, OM >90.0%, CP >93.0%, fat > 94.0%, and GE > 92.0%) suggesting that inclusion of animal by-products does not negatively impact apparent total tract digestibility of macronutrients and energy. For these reasons, inclusion of animal by-products in carnivore diets for zoological institutions increases the economical sustainability of the livestock industry by utilizing a greater percentage of the carcass while lessening the economic load that is associated with purchasing animal-based protein sources.

Another obvious ingredient difference between the dietary treatments was fiber type. Horse diet contained cellulose, a non-fermentable fiber. Beef 1 diet contained beet pulp, a moderately fermentable fiber while the pork diet contained a mixture of both beet pulp and cellulose. Beef 2 did not contain any added fiber ingredient. The resulting TDF concentrations were 5.9, 4.0, and 3.8% for Horse, Beef 1, and Pork, respectively, while Beef 2 did not contain measurable concentrations of TDF. These differences in dietary fiber type and concentrations may have contributed to observed differences in stool quality, appearance, fecal scores, and consistency (*Figure 3.1*) which is in agreement with existing literature (Kendall et al., 1982). Specifically, feeding Horse (highest TDF and non-fermentable cellulose only) resulted in stools with the lowest fecal score (1.2) compared to other diets (2.6). Stools were very hard and

several samples were observed with frank blood visible indicating the dogs had difficulty passing stools (*Figure 1*). The ideal fecal score on a 7-point scale (Nestlé Purina) would be a score of 2 to 3 because these scores reflect fecals that are firm but not hard and leave little to no residue on the ground when picked up. As expected, fecal score had positive correlation with fecal moisture % for Horse, Pork, and Beef 1 diets. Differences in dietary fiber sources likely contributed to differences in fecal score and moisture content in these diets as Pork and Beef 1 diets included beet pulp, a soluble dietary fiber, which can increase fecal moisture. Further, Pork diet also included cellulose, which likely contributed to greater fecal DM % and lower fecal scores. Beef 2 did not have the same correlation, likely due to the relatively high fat content and absence of additional fiber.

On an as-is basis, dogs fed Beef 1 produced greater ( $P<0.05$ ) fecal output (102.4 g as-is/d) than all other dietary treatments. Stools from dogs fed Beef 1 were wetter in appearance (*Figure 1*) and had lesser ( $P<0.05$ ) DM (27.4%) than all other diets (*Table 3.3*). These data are important considerations for manure management and enclosure cleaning. Similar results were obtained in a study by Kerr et al. (2013b) where cats fed raw beef-based diets containing beet pulp as a fiber source had greater ( $P<0.05$ ) fecal output than cats fed a raw beef-based diet substituted with cellulose as the fiber source. Further, the authors of this study indicated that smaller species of exotic felids (e.g., cheetahs, jaguars) tolerated beet pulp well whereas large species of exotic felids (e.g., Malayan and Siberian tigers) were more sensitive to the inclusion of beet pulp and had more ideal fecal scores with cellulose (Kerr et al., 2013b). Similar

sensitivities relating to colonic fermentation and fiber source have been noted in large breeds of domestic dogs (Zentek et al., 2002; Nery et al., 2012).

Dietary fiber plays an important role in gastrointestinal health by acting as substrate for large intestinal fermentation to promote production of short-chain fatty acids, maintaining stool quality and consistency, modulating metabolism, and aiding in removal of putrefactive byproducts (Kerr et al., 2013b). For exotic carnivores, animal-derived indigestible dietary components (e.g., skin, bone, hair) termed “animal fiber” are suspected to have fiber-like functions in the gastrointestinal tract (Depauw et al., 2013). Research regarding animal fiber is just beginning and initial studies have focused solely on managed exotic felids (Depauw et al., 2013). Dogs fed Beef 2 produced stools that were greasy in appearance and contained 21.5% fat on a DMB compared to Horse, Pork, and Beef 1 (*Figure 1*). Due to exclusion of fiber in Beef 2 and high fat-content feces, changes in gut microbiota and fecal fermentative products may have occurred but were not analyzed as part of this study. Future diet formulations for managed exotic carnivores should consider selection of fiber type based on individual species tolerance or feeding a mixture of fermentable and non-fermentable fibers (Kerr et al., 2013b). As previously discussed, long term effects of feeding the Beef 2 diet to managed exotic canids should be considered as absence of fiber in diets of dogs and cats is not considered to be nutritionally sustainable (Swanson et al., 2013). Additionally, previous and current data may support formulation of species specific or size specific diets for carnivores rather than general carnivore formulations.

### *Economics of Energy:*

The Pork diet provided the highest GE (64198.8 kcal/kg DM) and calculated ME values of 5547.2, 5504.5, and 5093.1 kcal/kg DM using Atwater, NRC, and modified Atwater values, respectively, compared to all other diets even though it was not the highest in apparent total tract digestibility for energy (*Table 3.4*). Given these data, a cost comparison can be made between diets based on dollars per 1000 kcal energy provided to the animal. Cost estimates on an as fed basis are included in *Table 3.8* based on recent delivery data for each of the diets. Cost (\$) per kg of diet ranged greatly from approximately \$2.20 for Beef 1 to approximately \$4.40 for Pork. Due to differences in nutrient composition and diet digestibility, the same as fed weight of each diet provides notably different amounts of energy. When cost (\$) is calculated per 1000 kcal DE (Horse: 1.72, Pork: 2.07, Beef 1: 1.10, Beef 2: 1.69) or per 1000 kcal ME (Horse: 1.96, 2.15, 1.93; Pork: 2.25, 2.45, 2.27; Beef 1: 1.19, 1.31, 1.21; Beef 2: 1.89, 2.07, 1.89), the diets are closer in cost. Given diet costs are an important management consideration for zoological institutions, estimating feeding costs based on energy content may provide additional insight into purchasing and budgeting decisions. Further, it is important to note the differences observed in calculated ME values for each diet based on Atwater, modified Atwater, and NRC equations (Horse: 4659.4, 4233.9, 4728.7 kcal/kg DM; Pork: 5547.2, 5093.1, 5504.5 kcal/kg DM; Beef 1: 5148.5, 4703.5, 5066.2 kcal/kg DM; Beef 2: 5414.4, 4940.1, 5435.1 kcal/kg DM for Atwater, modified Atwater, and NRC equations, respectively). Interestingly, the values between Atwater and NRC values were very similar whereas modified Atwater values markedly differed. Similarly, Clauss et al. (2010) demonstrated that dietary ME estimates using Atwater factors more similarly compared to ME values calculated using experimental results for exotic

carnivores. Current AAFCO labeling recommendations for the pet food industry in the United States recommend the use of modified Atwater values to estimate energy content of a diet. Commercially manufactured exotic diets are also under the recommendations of AAFCO in the U.S. However, these data and results obtained by Clauss et al. (2010) indicate that modified Atwater values may be an underestimate of ME, especially in highly digestible diets. This underestimation of ME content may further exacerbate obesity issues that are common among companion animals and animals managed in zoological institutions.

#### *Microbial Presence:*

Diet, saliva, and fecal samples were obtained from all dogs prior to initiation of the study when dogs were consuming a commercial, extruded diet and all samples tested negative. During the study, only 2 fecal samples tested positive for *Salmonella spp.* (Table 3.5) and 3 saliva samples and 2 diet samples tested positive for generic *E. coli*. Literature surrounding RMBDs in dogs has primarily focused on fecal shedding of *Salmonella spp.* and other microorganisms of concern from a public health standpoint. Several studies have documented increased shedding of *Salmonella spp.* and other microorganisms in feces of dogs fed RMBDs compared to dogs fed extruded diets (Joffe and Schlesinger, 2002; Lefebvre et al., 2008; Lenz et al., 2009). Over 2500 serotypes of *Salmonella* have been described and less than 100 of those are considered pathogenic in humans (CDC, 2015). Further, overall shedding of *Salmonella spp.* in dogs, regardless of diet, is suspected to be low but estimates up to 18.0% in clinically normal dogs have been documented (LeJeune et al., 2001; Lowden et al., 2015). This notion is consistent with data obtained from our study with less than 5.6% of total fecal samples (n=36)



testing positive for *Salmonella spp.* and none of the saliva samples (n=36) testing positive for *Salmonella spp.* Samples for this study were taken at single time points at the end of each period rather than serial samples taken over the course of several days that were cost prohibited in this study. Therefore, our data may present an underestimate of true fecal shedding of *Salmonella spp.* but do appear low from data obtained. Future studies should aim to further quantify the transmission risk associated with *Salmonella spp.* through animal feces or saliva using serial sampling. Although the overall incidence of shedding is low, common sense practices should be considered when handling RMBDs and feces of any species consuming any diet. Safe handling of RMBDs for zoos has been well outlined and published by Crissey et al., (2001).

#### *Blood Panels:*

Mild elevations above reference interval for BUN (31.1; reference interval: 10.0 – 30.0 mg/dL) for dogs fed Beef 2 and triglycerides (115.5; reference interval: 24.0 – 115.0 mg/dL) for dogs fed Beef 1 were reported but all other serum metabolite and electrolyte concentrations were within normal limits suggesting no change in health status of dogs during this study based on blood parameters. BUN (Horse: 28.0, Pork: 23.1, Beef 1: 23.4, Beef 2: 31.1 mg/dL) for all treatments was greater ( $P < 0.05$ ) than baseline (15.0 mg/dL) (reference interval: 10.0 – 30.0 mg/dL). Serum increase in BUN may be indicative of late stage renal dysfunction especially if elevations in serum creatinine and inorganic phosphorus are also observed (Brown, 2013). However, elevations in BUN in the absence of elevated creatinine can be caused by consumption of a high-protein diet or be associated with upper gastrointestinal (GI) bleeding

(eClinpath, 2013). Melena was not observed in the dogs during this study indicating that upper GI bleeding was unlikely. As a result, the elevation observed in BUN was likely dietary related (CP >50% for all dietary treatments) and not caused by an underlying pathologic change. Although numerical increases in triglycerides and cholesterol were observed in dogs fed the RMBDs, the differences did not reach statistical significance; therefore, expected within reference ranges.

Cats used in a related study by Kerr et al. (2012) had elevations in serum alanine aminotransferase (ALT) across dietary treatments; extruded (57.0 U/L), raw beef (67.8 U/L), and cooked beef (70.1 U/L) diets (reference interval: 8.3-52.5 U/L) (Kerr et al., 2012). Baseline bloodwork was not reported for this study so it is unknown whether diet affected serum ALT concentrations. Elevations in serum ALT in dogs were not observed in this study. Hematology revealed mild elevations in hemoglobin and hematocrit above reference intervals that were observed across dietary treatments. However, these elevations were consistent with baseline values reported prior to study initiation; 18.7g/dL and 56.4% for hemoglobin and hematocrit, respectively (reference intervals: 12.0 – 18.0g/dL; 37.0 – 55.0%). These mild elevations in hemoglobin and hematocrit may indicate mild dehydration in the dogs used in this study.

Reference intervals for serum chemistry screening and CBC are laboratory specific and are typically established with a sample population of dogs (eClinpath, 2016). These values provide an indication of internal health status (e.g., organ function, immune status, etc.). Since feeding extruded diets to dogs predominates in developed nations, these reference intervals

were likely established in extruded-fed dogs. Swanson et al. (2004) found differences in serum chemistry values in dogs associated with diets that were animal-product based (APB) or plant-product based (PPB). The authors reported a tendency ( $P < 0.01$ ) towards increased blood cholesterol concentrations in dogs fed an APB diet, speculating that this was related to the increased fat content of this diet. The results of this study and others indicate that differences in serum chemistry values may occur between dogs fed diets differing markedly in macronutrient concentrations, such as with extruded- and RMBDs.

### *Conclusions:*

Results from this study indicate these raw meat dietary options varying in protein source and ingredients were all comparable in nutrient composition and highly digestible in the domestic dog. In addition, fecal scores were at 3.1 or less and most blood chemistry values were within normal reference intervals. Elevations seen in BUN and triglycerides for Beef 2 and Beef 1, respectively, were likely dietary related and not related to an underlying pathologic change, indicating dog health was not negatively impacted by feeding these RMBDs. Variations between diets in energy content, fecal output, stool characteristics, and fiber type exist and are important considerations for nutrition management of exotic canids. Further research is needed to determine a greater understanding of dietary options for management of exotic carnivores in order to increase dietary options for nutrition management taking into account species natural history, body size, and ingredient selection. In addition, our comparison of ME calculations provided additional support for the use of Atwater or NRC equations over the AAFCO recommended modified Atwater equations when evaluating energy content of RMBDs.

Generating apparent total tract digestibility data for a variety of diets across various species provides valuable information for zoological institutions to make informed decisions regarding available commercial diets. As expected, this study indicated a low risk of pathogen exposure to humans from RMBDs and the dogs consuming them as evidenced by positive fecal swabs for *Salmonella spp.* and positive diet swabs for generic *E. coli*. However, overall incidence was low (5.6% for *Salmonella spp.*). Additionally, utilizing domestic dogs as a nutrition model for exotic canids allows researchers to gain valuable insight into exotic canid nutrition while advancing our understanding of similar diets and tolerances in order to benefit the pet food industry, as well.

Dietary selection for managed exotic carnivores and companion animals should consider nutrition, economics, and environmental sustainability (Swanson et al., 2013) and decisions will vary across institutions in light of these criteria. Ingredient selection also should be evaluated to ensure diets appropriately address long-term nutrition needs of animals, economic feasibility, minimization of negative impacts on the environment, and goals of the feeding program. Further, ingredient supply ideally should be consistently available and free of potential safety concerns. Although all dietary treatments were similar in macronutrient and energy concentrations and considered highly digestible, ingredient differences had marked differences on fecal characteristics and digestibility of macronutrients and energy. Based on our results, we conclude that all diets evaluated may be suitable for the nutritional management of exotic canids; however, long-term feeding of Beef 2 is not advised due to likely micronutrient deficiencies and absence of fiber in the diet.

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**Table 3.1:** Ingredient composition of horse-, pork-, and beef-based raw meat diets fed to domestic dogs (*Canis lupus familiaris*).

Diet	Ingredients
<p>Horse</p> <p>Nebraska Brand ®, Nebraska Packing Inc.; North Platte, NE, USA;</p> <p>Premium Feline</p>	<p>Horsemeat, powdered cellulose, dicalcium phosphate, calcium carbonate, vitamin premix (roughage products, vitamin E supplement, mineral oil, niacin supplement, biotin, menadione sodium bisulfite complex, vitamin A supplement, riboflavin, pyridoxine hydrochloride, folic acid, calcium pantothenate, thiamine mononitrate, vitamin D<sub>3</sub> supplement), trace mineral premix (copper sulfate, manganese sulfate, ethylenediamine, dihydriodide, sodium selenite), choline chloride, taurine, salt.</p>
<p>Pork</p> <p>Sustainable Swine Resources , LLC; Sheboygan Falls, WI, USA;</p> <p>Carnivore Essentials</p>	<p>Pork, pork-byproducts, vitamin premix (beet pulp, cellulose, calcium carbonate, rice hulls, sodium chloride, mineral oil, vitamin E supplement, d-α-tocopheryl acetate, biotin, niacin supplement, thiamine mononitrate, vitamin B<sub>12</sub> supplement, vitamin A acetate, vitamin D<sub>3</sub> supplement, pyridoxine hydrochloride, riboflavin supplement, d-calcium pantothenate, folic acid), mineral premix (beet pulp, cellulose, calcium carbonate, rice hulls, mineral oil, choline chloride, calcium phosphate, magnesium oxide, potassium chloride, ferrous sulfate, zinc sulfate, copper sulfate, manganese sulfate, zinc oxide, sodium selenite, cobalt carbonate, calcium iodate).</p>
<p>Beef 1</p> <p>Nebraska Brand ®, Nebraska Packing Inc.; North Platte, NE, USA;</p> <p>Special Beef Feline</p>	<p>Beef, meat by-products, fish meal, soybean meal, dried beet pulp, calcium carbonate, dicalcium phosphate, dried egg, brewers dried yeast, salt, vitamin premix (choline chloride, vitamin E supplement, niacin, vitamin B<sub>12</sub>, riboflavin, folic acid, vitamin A acetate, thiamine mononitrate, d-calcium pantothenate, mineral oil, biotin, pyridoxine hydrochloride, vitamin D<sub>3</sub> supplement,), taurine, trace mineral premix (zinc oxide, manganous oxide, copper oxide, mineral oil, sodium selenite, calcium iodate).</p>
<p>Beef 2</p> <p>Kennel Supply, LLC; Council Bluffs, IA, USA</p> <p>Frozen Beef Diet</p>	<p>Beef, kidney, heart, liver, calcium carbonate.</p>

**Table 3.2:** Chemical composition (DM basis) of horse-, pork-, and beef-based raw meat diets fed to domestic dogs (*Canis lupus familiaris*).<sup>1</sup>

Item	Diet				SEM
	Horse	Pork	Beef 1	Beef 2	
DM %	36.2	35.2	35.8	32.2	1.9
Moisture %	63.8	64.8	64.2	67.9	1.9
OM %	91.1	94.7	93.0	94.9	0.3
ASH %	8.9	5.3	7.0	5.1	0.3
CP %	54.7	50.3	51.8	61.7	3.7
Fat %	25.1	38.3	31.8	32.4	3.7
TDF %	5.9	3.8	4.0	0.0	0.4
NFE %	5.3	2.3	5.4	0.8	1.4
GE (kcal/kg DM)	5805.0	6419.8	6090.4	6363.7	141.6

<sup>1</sup> Abbreviations: SEM, standard error of the mean; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; GE, Gross Energy; kcal, kilocalories; g, grams.

**Table 3.3:** Chemical composition (DM basis) of fecal samples collected from domestic dogs (*Canis lupus familiaris*) fed horse-, pork-, and beef-based raw meat diets.<sup>1</sup>

Item	Diet				SEM
	Horse	Pork	Beef 1	Beef 2	
DM %	46.9 <sup>c</sup>	37.4 <sup>b</sup>	27.4 <sup>a</sup>	46.7 <sup>c</sup>	1.4
OM %	63.7 <sup>b</sup>	72.8 <sup>c</sup>	64.7 <sup>b</sup>	58.9 <sup>a</sup>	0.5
ASH %	36.3 <sup>b</sup>	27.2 <sup>a</sup>	35.3 <sup>b</sup>	41.1 <sup>c</sup>	0.5
CP %	12.7 <sup>a</sup>	19.3 <sup>b</sup>	22.9 <sup>c</sup>	18.9 <sup>b</sup>	0.7
Fat %	2.9 <sup>a</sup>	6.1 <sup>b</sup>	6.3 <sup>b</sup>	21.5 <sup>c</sup>	0.7
GE (kcal/g DM)	3.0 <sup>a</sup>	3.7 <sup>c</sup>	3.5 <sup>b</sup>	3.7 <sup>c</sup>	0.1

<sup>a-c</sup> Means within a row lacking a common superscript letter are different ( $P < 0.05$ ).

<sup>1</sup> Abbreviations: SEM, standard error of the mean; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; GE, Gross Energy; kcal, kilocalories; g, grams.

**Table 3.4:** Feed intake, fecal output, fecal scores, apparent total tract macronutrient and energy digestibilities, and energy calculations in domestic dogs (*Canis lupus familiaris*) (n=4) fed horse-, pork-, and beef-based raw meat diets.<sup>1</sup>

	Diet				
Item	Horse	Pork	Beef 1	Beef 2	SEM
Intake					
Feed intake (g AF/d)	553.5	553.5	553.5	553.5	--
Feed intake (g DM/d)	200.3	194.8	198.1	178.0	--
GE intake (kcal/d)	1162.8	1250.8	1206.5	1132.2	--
Output					
Fecal output (g as-is/d)	69.1 <sup>c</sup>	57.4 <sup>c</sup>	102.4 <sup>d</sup>	29.4 <sup>a</sup>	7.2
Fecal output (g DM/d)	32.3 <sup>d</sup>	21.3 <sup>b</sup>	27.4 <sup>c</sup>	13.5 <sup>a</sup>	1.2
Fecal scores	1.2 <sup>a</sup>	2.7 <sup>c</sup>	3.1 <sup>c</sup>	1.9 <sup>b</sup>	0.2
Apparent Digestibilities					
DM %	83.3 <sup>a</sup>	89.4 <sup>c</sup>	86.1 <sup>b</sup>	92.4 <sup>d</sup>	0.6
OM %	88.4 <sup>a</sup>	91.8 <sup>b</sup>	90.3 <sup>b</sup>	95.3 <sup>c</sup>	0.4
CP %	96.2 <sup>b</sup>	95.9 <sup>b</sup>	93.8 <sup>a</sup>	97.7 <sup>c</sup>	0.3
Fat %	98.1 <sup>b</sup>	98.2 <sup>b</sup>	97.1 <sup>b</sup>	94.9 <sup>a</sup>	0.3
GE %	91.3 <sup>a</sup>	93.9 <sup>b</sup>	92.0 <sup>a</sup>	95.5 <sup>c</sup>	0.4
Energy Calculations					
DE (kcal/kg)	5297.7	6027.1	5605.2	6076.3	--
ME <sup>1</sup> (kcal/kg)	4659.4	5547.2	5148.5	5414.4	--
ME <sup>2</sup> (kcal/kg)	4233.9	5093.1	4703.5	4940.1	--
ME <sup>3</sup> (kcal/kg)	4728.7	5504.5	5066.2	5435.1	--

<sup>a-c</sup> Means within a row lacking a common superscript letter are different (P<0.05).

<sup>1</sup> Abbreviations: SEM, standard error of the mean; AF, As Fed; GE, Gross Energy; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; DE, digestible energy; ME, metabolizable energy; g, grams; d, day; kcal, kilocalories.

ME<sup>1</sup> = 9.0 kcal of ME/g of fat + 4.0 kcal of ME/g of CP + 4.0 kcal of ME/g of nitrogen free-extract

ME<sup>2</sup> = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of nitrogen free-extract

ME<sup>3</sup> = DE - (1.04 \* g CP of diet)

**Table 3.5:** *Salmonella* spp. and generic *E. coli* presence in diet (n=16), saliva (n=36), and fecal (n=36) samples obtained from domestic dogs (*Canis lupus familiaris*) fed commercially available horse-, pork-, and beef-based raw meat diets.<sup>1</sup>

Item			Baseline	Treatment			
				Horse	Pork	Beef 1	Beef 2
Replicate 1:	Salmonella	Diet:	--	Negative	Negative	Negative	Negative
		Saliva:	Negative	Negative	Negative	Negative	Negative
		Fecal:	Negative	Negative	Positive	Negative	Negative
	E. Coli	Diet:	Negative	Negative	Negative	Negative	Positive
		Saliva:	Negative	Negative	Positive	Negative	Negative
Replicate 2:	Salmonella	Diet:	--	Negative	Negative	Negative	Negative
		Saliva:	--	Negative	Negative	Negative	Negative
		Fecal:	--	Negative	Positive	Negative	Negative
	E. Coli	Diet:	--	Negative	Negative	Negative	Positive
		Saliva:	--	Positive	Positive	Negative	Negative

<sup>1</sup> Abbreviations: spp, species; *E. coli*, *Escherichia coli*.

**Table 3.6:** Serum metabolite and electrolyte concentrations of domestic dogs (*Canis lupus familiaris*) fed commercially available horse-, pork-, and beef-based raw meat diets.<sup>1</sup>

Item	Diet					SEM	Reference Interval <sup>2</sup>
	Baseline	Horse	Pork	Beef 1	Beef 2		
BUN (mg/dL)	15.0 <sup>a</sup>	28.0 <sup>c</sup>	23.1 <sup>b</sup>	23.4 <sup>b</sup>	31.1 <sup>c</sup>	1.6	10.00 – 30.00
Creatinine (mg/dL)	0.9 <sup>b</sup>	0.9 <sup>b</sup>	0.8 <sup>b</sup>	0.7 <sup>a</sup>	0.9 <sup>b</sup>	0.0	0.50 - 1.50
Glucose (mg/dL)	89.0	87.1	89.3	89.0	86.0	2.6	68.00 - 115.00
Total Protein (g/dL)	6.3	6.3	6.5	6.5	6.4	0.1	5.20 - 7.10
Albumin (g/dL)	3.6	3.5	3.5	3.5	3.5	0.1	2.70 - 4.00
Alk Phos (U/L)	34.3	24.6	26.1	25.1	26.1	2.5	20.00 - 150.00
ALT (U/L)	52.8	40.3	35.8	42.1	46.0	4.0	24.00 - 90.00
Total Bilirubin (mg/dL)	0.2	0.4	0.3	0.3	0.3	0.1	0.10 - 0.60
Cholesterol (mg/dL)	181.3	208.8	227.0	193.6	231.5	20.7	132.00 - 300.00
Triglycerides (mg/dL)	76.3	107.5	98.6	115.5	103.0	13.9	24.00 - 115.00
Sodium (mEq/L)	144.0	143.9	144.6	140.6	144.4	1.9	141.00 - 151.00
Potassium (mEq/L)	4.9	4.8	4.8	4.8	4.9	0.1	3.90 - 5.3
Chloride (mEq/L)	110.3 <sup>a</sup>	114.4 <sup>b</sup>	114.9 <sup>b</sup>	114.5 <sup>b</sup>	114.5 <sup>b</sup>	0.6	112.00 - 121.00
Bicarbonate (mEq/L)	25.0	22.0	22.1	22.3	22.6	0.7	19.00 - 25.00
Calcium (mEq/dL)	10.6	10.4 <sup>a</sup>	10.5 <sup>a,b</sup>	10.7 <sup>b</sup>	10.5 <sup>a,b</sup>	0.1	9.70 - 11.30
Phosphorus (mEq/dL)	4.3	4.1	4.2	4.3	4.0	0.2	3.20 - 6.00
Magnesium (mEq/dL)	2.0 <sup>b</sup>	1.7 <sup>a</sup>	1.9 <sup>b</sup>	1.9 <sup>b</sup>	2.0 <sup>b</sup>	0.0	1.70 - 2.50

<sup>a-c</sup> Means within a row lacking a common superscript letter are different (P<0.05).

<sup>1</sup> Abbreviations: SEM, standard error of the mean; BUN, blood urea nitrogen; Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; mg, milligrams; g, grams; U, standardized units; mEq, milliequivalent; L, liters; dL, deciliters.

<sup>2</sup> Reference intervals for canines are laboratory specific.

**Table 3.7:** Plasma complete blood count of domestic dogs (*Canis lupus familiaris*) fed commercially available horse-, pork-, and beef-based raw meat diets.<sup>1</sup>

Item	Diet					SEM	Reference Interval <sup>2</sup>
	Baseline	Horse	Pork	Beef 1	Beef 2		
WBCs (x10 <sup>3</sup> /μL)	12.5 <sup>b</sup>	10.8 <sup>a</sup>	10.7 <sup>a</sup>	11.2 <sup>b</sup>	10.8 <sup>a</sup>	0.3	6.0 - 17.0
Neutrophils (x10 <sup>3</sup> /μL)	7.6	6.8	6.4	6.9	6.5	0.3	3.0 - 11.4
Lymphocytes (x10 <sup>3</sup> /μL)	3.7	3.5	3.5	3.3	3.4	0.1	1.0 - 4.8
Monocytes (x10 <sup>3</sup> /μL)	0.5	0.5	0.5	0.5	0.5	0.0	0.2 - 1.4
Eosinophils (x10 <sup>3</sup> /μL)	0.6	0.4	0.4	0.4	0.4	0.0	0.0 - 0.8
Basophils (x10 <sup>3</sup> /μL)	0.03	0.03	0.03	0.03	0.03	0.0	0.00 - 0.10
RBCs (x10 <sup>3</sup> /μL)	8.0	7.9	7.8	7.8	7.9	0.1	5.5 - 8.5
Hemoglobin (g/dL)	18.7	19.2	19.0	19.1	19.1	0.2	12.0 - 18.0
Hematocrit (%)	56.4	56.5	56.1	56.3	56.6	0.9	37.0 - 55.0
MCV (fL)	70.8	71.6	71.6	71.9	71.8	0.4	60.0 - 77.0
MCH (pg)	23.5 <sup>a</sup>	24.3 <sup>b</sup>	24.2 <sup>b</sup>	24.3 <sup>b</sup>	24.2 <sup>b</sup>	0.1	19.5 - 30.0
MCHC (g/dL)	33.2	34.0	33.9	33.8	33.7	0.2	32.0 - 36.0
RDW (%)	12.8	12.4	12.4	12.4	12.4	0.2	11.6 - 14.8
Platelets (x10 <sup>3</sup> /μL)	237.0	238.9	256.9	251.8	245.9	11.5	200.0 - 500.0
MPV (fL)	9.9 <sup>b</sup>	9.2 <sup>a,b</sup>	9.0 <sup>a,b</sup>	9.1 <sup>a,b</sup>	8.7 <sup>a</sup>	0.2	7.00 - 11.00

<sup>a-b</sup> Means within a row lacking a common superscript letter are different (P<0.05).

<sup>1</sup> Abbreviations: SEM, standard error of the mean; WBCs, white blood cells; RBCs, red blood cells; MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume; g, grams; pg, picograms; μL, microliter; dL, deciliter; fL, femtoliter.

<sup>2</sup> Reference intervals for canines are laboratory specific.

**Table 3.8:** Cost analysis and total GE, DE, and estimated ME provided by commercially available horse-, pork-, and beef-based raw meat diets when fed to domestic dogs (*Canis lupus familiaris*).<sup>1</sup>

Item	Diet			
	Horse	Pork	Beef 1	Beef 2
Intake				
Feed intake (g AF/d)	553.5	553.5	553.5	553.5
Diet % DM	36.2	35.2	35.8	32.2
Feed intake (g DM/d)	200.3	194.8	198.1	178.0
DE Estimates				
DE (kcal/d)	1061.2	1174.3	1110.4	1081.3
ME Estimates				
ME <sup>1</sup> (kcal/d)	933.3	1080.8	1019.9	963.5
ME <sup>2</sup> (kcal/d)	848.1	992.3	931.8	879.1
ME <sup>3</sup> (kcal/d)	947.2	1072.5	1003.6	967.2
Approximate Cost of Diets				
Cost (\$/kg AF)	3.30	4.40	2.20	3.30
Cost (\$/1000 kcal DE)	1.72	2.07	1.10	1.69
Cost (\$/1000 kcal ME <sup>1</sup> )	1.96	2.25	1.19	1.89
Cost (\$/1000 kcal ME <sup>2</sup> )	2.15	2.45	1.31	2.07
Cost (\$/1000 kcal ME <sup>3</sup> )	1.93	2.27	1.21	1.89

<sup>1</sup> Abbreviations: AF, As Fed; DM, Dry Matter; DE, digestible energy; ME, metabolizable energy; g, grams; d, day; kcal, kilocalories.

ME<sup>1</sup> = 9.0 kcal of ME/g of fat + 4.0 kcal of ME/g of CP + 4.0 kcal of ME/g of nitrogen free-extract

ME<sup>2</sup> = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of nitrogen free-extract

ME<sup>3</sup> = DE – (1.04 \* g CP of diet)



**Figure 3.1:** Stool samples from domestic dogs (*Canis lupus familiaris*) fed commercially available horse-, pork-, and beef-based raw meat diets and associated fecal scores.



## CHAPTER 4

### EX VIVO EVALUATION OF INTESTINAL INTEGRITY AND GASTROINTESTINAL HISTOLOGY TO DETERMINE POTENTIAL GASTROINTESTINAL HEALTH IMPLICATIONS BETWEEN DOMESTIC DOGS FED COMMERCIAL EXTRUDED OR RAW MEAT-BASED DIETS.

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#### **Abstract:**

Feeding raw meat-based diets (RMBDs) to companion animals has continued to increase over the years due to perceived nutritional benefits on health. However, few data are published regarding the health effects of feeding RMBDs compared to extruded diets beyond nutrient digestibility. The objective of this pilot study was to evaluate the use of gastrointestinal histology and Ussing chamber technology for evaluations of transepithelial electrical resistance (TER) and macromolecule permeability in determining intestinal health effects associated with feeding extruded- versus RMBDs to domestic dogs (*Canis lupus familiaris*). Two intact male dogs were fed a rotation of four different RMBDs and two intact female dogs were fed a rotation of two different extruded diets for a minimum of seven months prior to euthanasia. Following euthanasia, tissue samples of the gastrointestinal tract and associated tissues were collected from each dog for histological examination. Additional intestinal samples were collected and used for Ussing chamber evaluation of TER and macromolecule permeability. As expected, diet macronutrient and energy concentration varied greatly between extruded- and RMBDs for dry matter (DM) (89.0, 34.8%), crude protein (32.2, 54.6%), fat (16.1, 31.9%), and gross energy (GE) (3.9, 6.2 kcal GE/g DM), respectively. Histology revealed that all dogs on study had mild

inflammation throughout the gastrointestinal tract and associated tissues but no clear differences in degree of inflammation were observed between dietary groups and mild inflammation was expected in adult dogs within the range obtained. Intestinal TER and macromolecule permeability data was highly variable across all dogs but numerical increases in apparent permeability coefficient ( $P_{app}$ ) of FITC-Dextran transport were observed in extruded-fed dogs suggesting lesser intestinal barrier function. The results of this pilot study suggest that gastrointestinal histology and Ussing chamber evaluation of intestinal membrane integrity may be used to characterize internal health effects of differing diet types and additional health benefits of feeding RMBDs may extend beyond nutrient digestibility.

**Introduction:**

Feeding raw meat-based diets (RMBD) has increased in popularity among pet owners in developed nations (Michel, 2006). Owner motivations for feeding RMBDs include perceived nutritional superiority and health benefits compared to processed diets such as extruded kibbles (Freeman et al., 2013). However, current published research efforts related to RMBDs fed to companion animals have focused primarily on macronutrient digestibility and microbial contamination. Health effects of feedings RMBDs, including diet-related histological changes, have not been evaluated and normal reference ranges for blood work have been established using primarily extruded-fed dogs. Currently, it is unknown if these ranges are appropriate for RMBD-fed dogs.

Ethical considerations for using companion animals in research have made it difficult to obtain tissue samples to better understand the influence of diet on internal health of the animal. Previous research has utilized opportunistic tissue collections from animals scheduled for euthanasia at animal shelters or research facilities. Ussing chamber ex vivo evaluation of intestinal integrity, as assessed by intestinal transepithelial electrical resistance (TER) and macromolecule permeability, has been used commonly in both porcine and murine models (Schulzke et al., 2005; Clarke, 2009; Boudry et al., 2011; He et al., 2013; Pearce et al., 2013; Pearce et al., 2014). Intestinal TER measures ion flux across a membrane. Increased TER due to decreased ion flux across a membrane is an indicator of increased intestinal integrity and barrier function (He et al., 2013). Ussing chambers also can be utilized to determine macromolecule permeability. Decreased macromolecule permeability, as determined by relative fluorescence, is another indicator of increased intestinal integrity and barrier function (He et al., 2013). TER and macromolecule permeability are typically inversely related. Ussing chamber evaluation may provide insight into intestinal barrier integrity and function. The objectives of this experiment were to compare histology of gastrointestinal tract and associated tissues, intestinal integrity, and intestinal barrier function of domestic dogs tended for euthanasia that have been fed extruded diets or RMBDs. We hypothesize no difference in histology of gastrointestinal tract and associated tissues, intestinal integrity, and intestinal barrier function between dogs fed extruded- versus RMBDs.

## Materials and Methods:

### *Animals:*

A total (n=4) of two intact males and two intact female domestic dogs (*Canis lupus familiaris*) with predominately hound bloodlines were used in this study ranging in age from 2 to 3 years, body condition score (BCS) from 4 – 7 (9-point scale), and body weight from 12.5 – 23.0 kg. Male dogs (n=2) were housed individually and female dogs (n=2) were co-housed in grated (2.5m L x 1.2m W x 1.8 m H) runs at the College of Veterinary Medicine at Iowa State University (Ames, IA, USA). Dogs used in this study served as breeding animals for a separate research colony and were scheduled for management euthanasia prior to conducting this experiment and had no diagnosed disease or known clinical symptoms. All dogs were used in accordance with the Iowa State University Institutional Animal Care and Use Committee (IACUC). Dogs were kept on a 14:10 hour light-dark schedule in a temperature controlled room (20°C). Water was provided ad libitum and dogs were fed to maintain body weight and BCS.

### *Experimental Design and Diets:*

All dogs were provided and maintained on either a standard laboratory extruded diet after reaching one year of age (Envigo: Teklad 8653 Laboratory Dog Diet; Madison, WI, USA) or an extruded puppy food (Royal Canin: Medium Puppy Dry Dog Food; Sioux City, SD, USA) (*Table 4.1*). Female dogs served as the control group and were maintained on a combination of the extruded diets. Male dogs were enrolled in a separate nutrition study evaluating digestibility of RMBDs in domestic dogs and served as the treatment group. Male dogs were provided a combination of one of four rotating RMBDs for a total of seven months prior to euthanasia and

were previously fed the same extruded diets as the females. RMBDs included one horse-based (Nebraska Brand Premium Feline; Central Nebraska Packing Inc.; North Platte, NE, USA (Horse)), one pork-based (Carnivore Essentials; Sustainable Swine Resources; Sheboygan Falls, WI, USA (Pork)), and two beef-based [Nebraska Brand: Special Beef; Central Nebraska Packing Inc.; North Platte, NE, USA (Beef 1); Frozen Beef Zoo Diet; Kennel Supply LLC; Council Bluffs, IA, USA (Beef 2)] diets (*Table 4.1*). Chemical analyses of RMBDs were conducted according to AOAC methodology as part of an alternative experiment (Chapter 3). Diet ingredients are presented in *Table 4.1* and chemical compositions are presented in *Table 4.2*.

#### *Gastrointestinal Histology:*

All dogs were euthanized via administration of barbiturate overdose. Immediately following euthanasia, tissue samples were taken of liver, stomach, duodenum, jejunum, ileum, proximal colon, and mesenteric lymph nodes and placed into 10% neutral-buffered formalin for subsequent histological examination. Fixed tissue samples were trimmed, embedded in paraffin, sliced into 5µm sections, mounted onto slides in duplicate, and stained with hematoxylin and eosin (H&E). Following mounting and staining, tissue samples were evaluated by a single, board-certified veterinary histopathologist. The evaluator was blinded to dietary treatment and instructed to evaluate histological differences between dogs.

#### *Intestinal Transepithelial Electrical Resistance & Macromolecule Permeability:*

Immediately following euthanasia, intestinal samples from all dogs were taken of duodenum, jejunum, ileum, and proximal colon and transferred to chilled, oxygenated Krebs-

Henseleit buffer (KHB) solution (25 mM NaHCO<sub>3</sub>, 120mM NaCl, 6.3 mM KCl, 1 mM MgSO<sub>4</sub>, 0.32 mM NaH<sub>2</sub>PO<sub>4</sub>, and 2 mM CaCl<sub>2</sub>; pH = 7.4). Each intestinal section was trimmed into approximate 2.5cm<sup>2</sup> sections in duplicate. *Tunica muscularis* and *tunica serosa* layers were removed by blunt and sharp dissection. Intestinal sections were rinsed with KHB prior to mounting into modified Ussing chambers (Physiological Instruments; San Diego, CA, USA) in order to remove residual intestinal contents. Time from euthanasia to mounting did not exceed 30 minutes per dog.

All intestinal sections from each individual dog were processed together so only one dog was euthanized per day. Intestinal sections were pinned flat, clamped at a voltage of 0 mV, and mounted between the halves (serosal side and mucosal side) of each chamber. All chamber slides had a surface area of 1.0 cm<sup>2</sup>. Each chamber was connected to dual channel current and voltage electrodes submerged in 3.0% noble agar and filled with 3 M KCl for electrical conductance as described by Pearce et al. (2013). Both serosal and mucosal sides of each chamber were filled with 4mL warm KHB solution. KHB solution was continuously oxygenated and circulated in water-jacketed reservoirs throughout the experiment to maintain a solution temperature of 37°C. Transepithelial electrical resistance (TER,  $\Omega\cdot\text{cm}^2$ ) was recorded for 30 minutes.

Following collection of TER data, KHB was removed from the luminal side of each chamber and replaced with 2.2mg/mL of 4.4 kDa fluorescein isothiocyanate labeled dextran (FITC-Dextran). Samples from both sides of each chamber were obtained in duplicate every 20 minutes for a total of 80 minutes. The relative fluorescence was determined using a fluorescent

plate reader (Bio-Tek, USA). Excitation and emission wavelengths were 485 and 520 nm, respectively. An apparent permeability coefficient ( $P_{app}$ ) was calculated for each tissue sample using the following equation:

$$P_{app} = dQ/(dt \times A \times C_0); \text{ where: } dQ/dt = \text{transport rate } (\mu\text{g}/\text{min}); C_0 = \text{initial concentration in donor chamber } (\mu\text{g}/\text{mL}); A = \text{surface area of membrane } (\text{cm}^2).$$

#### *Statistical Analyses:*

Statistical analyses were not performed on these data due to small sample size and high expected variability of the data. TER and  $P_{app}$  values are reported individually for each dog and sample means were averaged across treatment group. Ranges across all dietary treatments are also reported.

## **Results**

#### *Diet Compositions:*

Diet macronutrient and energy profiles for extruded diets are presented in Table 4.2. On average, RMBDs were 83.1, 41.0, 49.4, and 36.9% greater in moisture, crude protein (CP), fat, and gross energy (GE) than extruded diets, respectively. On average, extruded diets contained a calculated nitrogen-free extract (NFE) content of 36.2% whereas the RMBDs had a calculated NFE content of 3.5%.



### *Gastrointestinal Histology:*

The histopathologist concluded that all dogs (n=4) in this study had mild inflammation noted throughout the gastrointestinal tract and accessory organs (*Tables 4.3*). Clear differences in degree of inflammation between extruded-fed or RMBD-fed dogs were not observed by the veterinary histopathologist. Dog 4 (RMBD-fed) had a focal liver change as characterized by irregular vacuolization by clear to eosinophilic flocculent material. No other histological abnormalities were noted.

### *Intestinal Transepithelial Electrical Resistance & Macromolecule Permeability:*

Average intestinal TER ranged from 156.4 to 268.5  $\Omega\cdot\text{cm}^2$  between the two dogs fed the extruded diet (*Table 4.4*). TER values ranged for duodenum (151.0 – 302.2  $\Omega\cdot\text{cm}^2$ ), jejunum (152.8 – 339.0  $\Omega\cdot\text{cm}^2$ ), ileum (242.8 – 235.7  $\Omega\cdot\text{cm}^2$ ), and proximal colon (78.9 – 197.0  $\Omega\cdot\text{cm}^2$ ). Average TER for extruded-fed dogs was 212.4  $\Omega\cdot\text{cm}^2$ . Unfortunately, TER data were not obtained from the RMBD-fed dogs because tissue samples were not clamped at a voltage of 0 mV. Values for  $P_{\text{app}}$  (arbitrary units) ranged for duodenum (3.1 – 43.8), jejunum (3.8 – 91.1), ileum (4.5 – 76.6), and proximal colon (0.8 – 118.8) (*Table 4.4*). Marked differences were observed in  $P_{\text{app}}$  values between extruded- and RMBD-fed dogs. Average  $P_{\text{app}}$  for extruded-fed dogs was 57.9 (20.3 – 118.8) and 8.6 (0.8 – 23.9) for RMBD-fed dogs. Further, Ussing chamber technology was utilized in this study to evaluate region-specific differences in intestinal integrity and barrier function. Average  $P_{\text{app}}$  values for extruded-fed dogs were 36.3 (28.8 – 43.8), 71.2 (51.2 – 91.1), 48.5 (20.3 – 76.6), and 75.7 (32.5 – 118.8) while  $P_{\text{app}}$  values for RMBD-fed dogs were 6.9 (3.1 – 10.7), 8.4 (3.8 – 13.0), 14.2 (4.5 – 23.9), and 5.1 (0.8 – 9.4) for

duodenum, jejunum, ileum, and proximal colon, respectively. Although  $P_{app}$  values for RMBD-dogs were lower than extruded-fed dogs for all sections measured, the most marked difference was observed in the colon with a 13.8-fold lower average  $P_{app}$  for RMBD-fed dogs (*Table 4.4, Figure 4.1*).

## Discussion

### *Diet Composition and Implications:*

The extruded and RMBDs used in this study differed notably in nutrient profiles and ingredients as expected. Raw meat diets contained high moisture concentrations (63.8 – 67.9%), CP (50.3 – 61.7%, dry matter basis (DMB)), and fat (25.1 – 38.3%, DMB) with little inclusion of dietary fiber and low NFE. In contrast, extruded diets were very low in moisture (10.0 – 12.0%), moderate in CP (31.1 – 33.3%, DMB) and fat (12.3 – 20.0%, DMB), and high in NFE (34.4 – 38.0%, DMB). Dogs do not have a metabolic requirement for dietary glucose and can meet their requirement through gluconeogenesis for both growth and maintenance (Romsos et al., 1976). However, dogs can efficiently digest dietary NFE when processed in extruded diets with apparent total tract digestibility of NFE as high as greater than 95% (Bazolli et al., 2015) and NFE can be used as an economical source of energy in the diet especially during lactation or illness (Romsos et al., 1976).

Studies have documented high digestibility of RMBDs fed to exotic and domestic carnivores (Kendall et al., 1982; Hendricks et al., 1999; Vester et al., 2008; Vester et al., 2010a, 2010b; Kerr et al., 2012; Kerr et al., 2013; Hamper et al., 2015; Iske et al., 2016). This increase in

digestibility can result in decreased feed intake and fecal output aiding in overall management and husbandry; however, increased digestibility may provide additional health benefits to the animal. Apparent total tract macronutrient and energy digestibilities were calculated as part of an alternative experiment (Chapter 3) for all RMBDs fed. Digestibility of nutrients and energy for RMBDs ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.2%, and 91.3 – 95.5% for DM, organic matter (OM), CP, fat, and energy respectively. Apparent total tract digestibility averages for commercial processed dog and cat diets range from 78.0 – 81.0% and 77.0 – 85.0% for CP and fat, respectively. Commercial, processed diets considered to be low-residue, high-digestible diets have apparent total tract digestibilities exceeding 87.0 and 90.0% for CP and fat, respectively (Davenport and Remillard, 2010). Apparent total tract digestibilities were not determined for extruded-fed dogs in this study; however, an alternative study reported apparent total tract digestibilities in dogs fed a super-premium, extruded diet of 78.2, 83.9, 81.6, 91.3, and 84.7% for DM, OM, CP, fat, and energy, respectively (Kerr et al., 2012). Suspected differences in apparent total tract macronutrient digestibility, especially CP digestibility, between extruded- and RMBD-fed may have implications on gastrointestinal health.

Specifically, undigested dietary proteins and proteins from endogenous losses (e.g., digestive enzymes, sloughed intestinal cells, mucin, mesenteric blood, etc.) enter the large intestine and are subjected to microbial fermentation (Fuller and Reeds, 1998). Bacterial protein amino acid metabolism occurs through proteolysis, peptide degradation, deamination, and decarboxylation (Vince and Burrige, 1980; Jha and Berrocoso, 2016). Microbial

fermentation of protein in the large intestine produces putrefactive compounds that are associated with decreased intestinal integrity, gastrointestinal distress, and gastrointestinal disease across species (MacFarlane and Cummings, 1991; Ramakrishna et al., 1991; Hughes et al., 2000; Jha and Berrococo, 2016).

Putrefaction of proteins entering the large intestine leads to an accumulation of fermentative products including but not limited to: ammonia, biogenic amines, phenols, indoles, branched-chain fatty acids (BCFA), and short-chain fatty acids (SCFA) (MacFarlane and Cummings, 1991; Hughes et al., 2000; Kerr et al., 2012). Although SCFAs, such as butyric acid, play an important role in colonocyte energy metabolism; ammonia, phenols, indoles, and BCFAs can contribute to inflammation both in the large intestine and systemically (Bone et al., 1976; Nollet and Verstraete, 1996; Bikker et al., 2006). This indicates a balance between putrefactive and saccharolytic catabolism in the large intestine and has implications for intestinal and potentially whole body health (MacFarlane and MacFarlane, 1997).

Some studies have indicated CP digestibility, rather than CP content, has the biggest influence on concentration of fecal putrefactive compounds because decreases in CP digestibility results in increased protein entering the large intestine acting as a substrate for microbial fermentation (Kerr et al., 2013). Kerr et al. (2013) documented that fecal ammonia and BCFA concentrations were greater ( $P < 0.05$ ) in domestic cats fed an extruded beef diet ( $190.4 \mu\text{mol/g DM}$ ,  $43.7 \mu\text{mol/g DM}$ ) compared to cats fed a raw beef ( $69.4 \mu\text{mol/g DM}$ ,  $17.6 \mu\text{mol/g DM}$ ) or cooked beef ( $72.0 \mu\text{mol/g DM}$ ,  $16.8 \mu\text{mol/g DM}$ ) diet, respectively. Although

these diets did not differ in CP concentration, apparent digestibility of protein from the extruded beef diet was (81.6%) compared with ( $P<0.05$ ) the raw beef (93.3%) and cooked beef (92.9%) diets (Kerr et al., 2013). Considering dogs, Nery et al. (2012) demonstrated that CP concentration and CP digestibility both influenced concentration of fecal putrefactive compounds as dogs fed a medium-digestibility poultry meal (PM) diet had greater ( $P<0.05$ ) fecal concentrations of ammonia ( $1.0\mu\text{mol/g DM}$ ) and BCFAs ( $14.4\mu\text{mol/g DM}$ ) than dogs fed a high-digestibility wheat gluten (WG) diet ( $0.8\mu\text{mol/g DM}$ ;  $9.7\mu\text{mol/g DM}$ , respectively). Dogs in this study fed a high protein (38.2-39.2% CP) diet had greater ( $p<0.001$ ) concentrations of fecal ammonia ( $1.0\mu\text{mol/g DM}$ ) and BCFAs ( $13.5\mu\text{mol/g DM}$ ) compared with dogs fed a low-protein (21.4-21.6% CP) diet ( $0.7\mu\text{mol/g DM}$ ;  $10.6\mu\text{mol/g DM}$ , respectively), regardless of protein source (Nery et al., 2012).

Interestingly, other studies have found that diets differing in CP digestibility did not result in higher concentrations of fecal putrefactive compounds. Vester et al. (2010b) documented that fecal ammonia and total BCFA concentrations did not differ significantly ( $p>0.05$ ) between African Wildcats (*Felis lybica*) fed extruded ( $190.3\mu\text{mol/g DM}$ ,  $31.1\mu\text{mol/g DM}$ ) or raw meat ( $137.2\mu\text{mol/g DM}$ ,  $21.2\mu\text{mol/g DM}$ ) diets. Moreover, fecal ammonia concentrations of dogs fed a raw beef diet ( $125.9\mu\text{mol/g DM}$ ) or raw chicken diet ( $105.1\mu\text{mol/g DM}$ ) were not different and numerically similar to values obtained in the wildcats by Vester et al., (2010b) (Beloshapka et al., 2012). In contrast, fecal total BCFA concentrations in dogs ( $16.4$ - $17.6\mu\text{mol/g DM}$ ) were numerically lower than values obtained in wildcats by Vester et al., (2010b) ( $21.2$ - $31.1\mu\text{mol/g DM}$ ) (139). Likewise, a study by Swanson et al. (2002) found that

fecal ammonia concentrations and total BCFA concentrations for adult female dogs fed extruded diets were 134.4 $\mu$ mol/g DM and 18.9 $\mu$ mol/g DM, respectively. Although there are no established reference ranges for concentrations of protein fermentation products, these published values suggest some similarities across dogs and cats and across various diet types. However, these values can be highly variable as they measure by-products of fermentation and not fermentation directly. These fermentation by-products (e.g., phenols, indoles, SCFAs, etc.) are constantly being absorbed across the intestinal epithelium and values present in the feces may not be reflective of the degree of macronutrient fermentation.

#### *Gastrointestinal Histology:*

Histopathology indicated all dogs exhibited mild inflammation throughout the digestive tract (*Table 4.3*). Inflammation in the gastrointestinal tract is triggered by the binding of pathogen-associated molecular patterns (PAMPs) that are molecular patterns shared by a variety of microorganisms, to pattern recognition receptors (PRRs) located on the cell membrane or within cellular vesicles in the gastrointestinal parenchyma (Gourbeyre et al., 2015). Low numbers of inflammatory cells (e.g., lymphocytes, plasma cells, eosinophils) are expected within the lamina propria of the gastrointestinal tract due to continuous exposure to microorganisms and ingesta (Gourbeyre et al., 2015). As a result, a relatively broad range of inflammation in the gastrointestinal tract is considered normal (Jergens et al., 2014). As previously indicated, there were no observable differences in gastrointestinal inflammation between dietary groups even though the bacterial load in the RMBDs was suspected to be much greater than in the extruded diets because they did not undergo an antimicrobial, kill

step as is common in commercial, extruded diets. Dogs, as carnivores, have several anatomical and physiological adaptations that allow them to tolerate the consumption of bacteria that would otherwise cause illness in humans (Bosch et al., 2015). As a result, dogs do not frequently exhibit clinical illness when colonized by potentially pathogenic bacteria (NRC, 2006; Lenz et al., 2009). Continuous exposure of PRRs to their associated PAMPs can cause a desensitization of the PRRs and subsequent attenuation of down-stream inflammatory signaling (Lotz et al., 2006). For this reason, long-term consumption of a diet high in microorganisms may not elicit an increased inflammatory response due to the desensitization of PRRs. With no differences detected in histology after 7 mo of feeding raw, it does not appear that RBMDs increased risk of inflammation in the gastrointestinal tract.

Liver histology from Dog 4 indicated a mild focal vacuolar change. Interpretation from the histopathologist indicated that the focal nature of the lesion suggests that it was unlikely to be diet related as a systemic response typically manifests in a generalized manner. It is suspected that this lesion resulted from a prior liver insult or may represent an early hyperplastic change.

#### *Intestinal Transepithelial Electrical Resistance & Macromolecule Permeability:*

Marked differences were observed in intestinal TER values (extruded-fed only) and  $P_{app}$  values between dogs and tissue types. Previous studies in humans and non-human primates have indicated a correlation between aging and decreased intestinal integrity as indicated by decreased TER and increased macromolecule permeability (Tran and Greenwood-Van

Meerveld, 2013; Man et al., 2015). Dogs used in this study were young adults and approximately the same age at time of euthanasia (2.0-3.0 years old); therefore, age of dog likely did not influence results. A limitation of this study is that extruded-fed dogs were both female whereas RMBD-fed dogs were both male. Sex differences in mucosal immune activation have been documented in humans and rodent models (Al-Nakkash et al., 2011; Sankaran-Walters et al., 2013; Shastri et al., 2015). As a result, differences in TER and macromolecule permeability may have been influenced by sex hormones. The TER values obtained from the extruded-fed dogs are similar to existing canine data (range approximately 80 – 260  $\Omega\text{cm}^2$ ) in the literature (Neirinckx et al., 2011; Hill and Bliklager, 2012). However, use of the Ussing chamber technique in canines is minimal and used biomedically, not for nutritional application. Limited use of this technology is likely due to ethical considerations involving euthanasia and tissue collection.

Numerically, RMBD-fed dogs had an almost 6-fold lower  $P_{\text{app}}$  (8.6; range: 0.8 – 23.9) compared to extruded-fed dogs (57.9; range: 20.3 – 118.8). Greater  $P_{\text{app}}$  values indicate increased intestinal macromolecule permeability and are typically associated with decreased intestinal membrane integrity. The results of this preliminary study suggest that dogs fed extruded diets had decreased intestinal membrane integrity compared to RMBD-fed dogs as evidenced by a 85% reduction of  $P_{\text{app}}$  between extruded- and RMBD-fed dogs. Interestingly, these values compared to data from pigs were notably higher. Pearce et al. (2013) reported  $P_{\text{app}}$  of 3.61 +/- 0.93 and 2.74 +/- 3.55 for pigs housed under thermoneutral conditions and 7.92 +/- 1.08 and 15.67 +/- 3.55 for heat stressed pigs for ileum and colon, respectively. Alternative



studies are consistent indicating lower  $P_{app}$  values in pigs than obtained for the dogs in this study for 4.4 kDa FITC-Dextran (Pearce et al., 2014).

Given these data, the hypothesis that diet type affects gastrointestinal health and integrity in dogs should be evaluated in a larger scale experimental study. Human and non-human primate studies have utilized biopsies in order to obtain tissue samples for Ussing chamber evaluation of intestinal integrity and barrier function (Tran and Greenwood-Van Meerveld, 2013; Man et al., 2015). Using biopsies for tissue collection would allow additional evaluation without using a terminal patient. Other studies in various species have measured urinary excretion of lactulose mannitol (or similar compound) following oral administration in order to assess intestinal barrier function *in vivo* (Wijtten et al., 2011; Sequeira et al., 2014). Although lactulose mannitol tests can provide an indication of gastrointestinal permeability, these results are not typically correlated with macromolecule permeability on Ussing chamber evaluation or bacterial translocation into intestinal epithelium (Wijtten et al., 2011). Further, *ex vivo* studies utilizing Ussing chambers allows for regional specific evaluation of intestinal integrity and therefore provides additional knowledge regarding gastrointestinal health (Westerhout et al., 2015). Differences in observed macromolecule permeability between lactulose mannitol tests and Ussing chamber evaluation may related to increased absorptive area of total tract *in vivo* measurements versus *ex vivo* regional specific differences.

*Conclusions:*

After seven months on rotated raw diets, dogs did not show changes in inflammatory status but did exhibit a potential improvement in intestinal integrity as evidenced by an 85.0% lower  $P_{app}$  value compared to extruded-fed dogs. These data suggest that raw diets may, in fact, provide some health benefit beyond increased digestibility that warrant further investigation. Overall, additional studies need to be performed in order to explore the effects of diet type on intestinal health and integrity in dogs either from longitudinal studies using donated tissues or *ex vivo* experimental studies with differing simulated dietary conditions. Opportunistic tissue collection from the gastrointestinal tract and associated tissues can provide valuable insight into the internal health of dogs on varying diet types. Moreover, establishing baseline TER and  $P_{app}$  values for dogs of varying ages and sex may provide needed baselines for determining expected variability and analyzing experimental results in future studies. Our data provide some initial information and knowledge obtained using a novel application of Ussing chamber technology to evaluate intestinal integrity and barrier function differences in relation to diet type.

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**Table 4.1:** Ingredient composition of extruded- and RMBDs fed to domestic dogs (*Canis lupus familiaris*).

Diet	Ingredients
Extruded 1 Envigo ®, Teklad 25% Lab Dog Diet; 8653	Ground corn, porcine meat and bone meal, dehulled soybean meal, corn gluten feed, wheat middlings, porcine fat, poultry digest, dried beet pulp, ground wheat, dried whey, iodized salt, choline chloride, calcium propionate, kaolin, ferrous sulfate, vitamin E acetate, manganous oxide, zinc oxide, niacin, thiamin mononitrate, copper sulfate, calcium pantothenate, vitamin A acetate, menadione sodium bisulfite complex, riboflavin, pyridoxine hydrochloride, cobalt carbonate, ethylenediamine dihydroiodide, vitamin B <sub>12</sub> supplement, folic acid, vitamin D <sub>3</sub> supplement, biotin.
Extruded 2 Royal Canin ®; Medium Puppy Dry Dog Food	Chicken by-product meal, brewers rice, corn gluten meal, check fat, corn, wheat gluten, dried plain beet pulp, wheat, natural flavors, brewers rice flour, fish oil, calcium carbonate, grain distillers dried yeast, sodium silico aluminate, vegetable oil, potassium phosphate, salt, fructooligosaccharides, L-lysine, hydrolyzed yeast, choline chloride, taurine, DL-methionine, vitamins [DL-alpha tocopherol acetate, L-ascorbyl-2-polyphosphate, biotin, D-calcium pantothenate, vitamin A acetate, niacin supplement, pyridoxine hydrochloride, thiamine mononitrate, vitamin B <sub>12</sub> supplement, riboflavin supplement, folic acid, vitamin D <sub>3</sub> supplement, zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, calcium iodate, copper proteinate, sodium selenite, rosemary extract, mixed tocopherols, citric acid.
Horse Nebraska Brand ®, Nebraska Packing Inc.; North Platte, NE, USA; Premium Feline	Horsemeat, powdered cellulose, dicalcium phosphate, calcium carbonate, vitamin premix (roughage products, vitamin E supplement, mineral oil, niacin supplement, biotin, menadione sodium bisulfite complex, vitamin A supplement, riboflavin, pyridoxine hydrochloride, folic acid, calcium pantothenate, thiamine mononitrate, vitamin D <sub>3</sub> supplement), trace mineral premix (copper sulfate, manganese sulfate, ethylenediamine, dihydriodide, sodium selenite), choline chloride, taurine, salt.

**Table 4.1 continued:** Ingredient composition of extruded- and RMBDs fed to domestic dogs (*Canis lupus familiaris*).

<p>Pork</p> <p>Sustainable Swine Resources , LLC;</p> <p>Carnivore Essentials</p>	<p>Pork, pork-byproducts, vitamin premix (beet pulp, cellulose, calcium carbonate, rice hulls, sodium chloride, mineral oil, vitamin E supplement, d-<math>\alpha</math>-tocopheryl acetate, biotin, niacin supplement, thiamine mononitrate, vitamin B<sub>12</sub> supplement, vitamin A acetate, vitamin D<sub>3</sub> supplement, pyridoxine hydrochloride, riboflavin supplement, d-calcium pantothenate, folic acid), mineral premix (beet pulp, cellulose, calcium carbonate, rice hulls, mineral oil, choline chloride, calcium phosphate, magnesium oxide, potassium chloride, ferrous sulfate, zinc sulfate, copper sulfate, manganese sulfate, zinc oxide, sodium selenite, cobalt carbonate, calcium iodate).</p>
<p>Beef 1</p> <p>Nebraska Brand ®, Nebraska Packing Inc.; North Platte, NE, USA;</p> <p>Special Beef Feline</p>	<p>Beef, meat by-products, fish meal, soybean meal, dried beet pulp, calcium carbonate, dicalcium phosphate, dried egg, brewers dried yeast, salt, vitamin premix (choline chloride, vitamin E supplement, niacin, vitamin B<sub>12</sub>, riboflavin, folic acid, vitamin A acetate, thiamine mononitrate, d-calcium pantothenate, mineral oil, biotin, pyridoxine hydrochloride, vitamin D<sub>3</sub> supplement,), taurine, trace mineral premix (zinc oxide, manganous oxide, copper oxide, mineral oil, sodium selenite, calcium iodate).</p>
<p>Beef 2</p> <p>Kennel Supply, LLC;</p> <p>Frozen Beef Diet</p>	<p>Beef, kidney, heart, liver, calcium carbonate.</p>



**Table 4.2:** Chemical composition of extruded- and RMBDs fed to domestic dogs (*Canis lupus familiaris*).<sup>1, 2</sup>

Item	Extruded Diets			RMBDs				
	Extruded 1	Extruded 2	Average	Horse	Pork	Beef 1	Beef 2	Average
DM %	88.0	90.0	89.0	36.2	35.2	35.8	32.2	34.8
Moisture %	12.0	10.0	11.0	63.8	64.8	64.2	67.9	65.2
OM %	90.0	92.4	91.3	91.1	94.7	93.0	94.9	93.4
ASH %	11.4	8.3	9.8	8.9	5.3	7.0	5.1	6.6
CP %	31.1	33.3	32.2	54.7	50.3	51.8	61.7	54.6
Fat %	12.3	20.0	16.1	25.1	38.3	31.8	32.4	31.9
<sup>3</sup> Fiber %	3.9	4.0	4.0	5.9	3.8	4.0	0.0	0.4
NFE %	38.0	34.4	36.2	5.3	2.3	5.4	0.8	1.4
GE (kcal/g DM)	3.8	4.0	3.9	5.8	6.4	6.1	6.4	6.2

<sup>1</sup> Abbreviations: DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; NFE, Nitrogen-Free Extract; GE, Gross Energy; kcal, kilocalories; g, grams.

<sup>2</sup> Based on proximate analyses values provided by the manufacturer for extruded diets; laboratory determination of proximate analyses values determined for RMBDs.

<sup>3</sup> Fiber for extruded diets is expressed as a Crude Fiber value; fiber of RMBDs is expressed as a Total Dietary Fiber value

**Table 4.3:** Histopathology results for extruded- and RMBD- fed domestic dogs (*Canis lupus familiaris*).<sup>1</sup>

		Tissue:	Findings:
DOG 1 – EXTRUDED-FED	Results:	Liver:	Within normal limits.
		Stomach:	In widely scattered locations, superficial lamina propria infiltrated by low numbers of lymphocytes and plasma cells.
		Pancreas:	Within normal limits.
		Duodenum:	Multifocally, the superficial villus lamina propria is expanded by low numbers of lymphocytes and plasma cells.
		Jejunum/Ileum:	Within normal limits.
		Proximal Colon:	Superficial colonic lamina propria is multifocally expanded by low to focally moderate numbers of lymphocytes and plasma cells.
		MLN:	Medullary sinuses are moderately expanded by clear spaces.
	Conclusions:	Stomach:	Mild, multifocal, lymphoplasmacytic gastritis.
		Duodenum:	Mild, multifocal, lymphoplasmacytic enteritis.
		Proximal Colon:	Mild, multifocal, lymphoplasmacytic colitis.
		MLN:	Moderate medullary edema.
DOG 2 – EXTRUDED FED	Results:	Liver:	Within normal limits.
		Stomach:	In widely scattered locations, superficial lamina propria infiltrated by low numbers of lymphocytes, plasma cells, and occasional eosinophils.
		Pancreas:	Within normal limits.
		Duodenum:	Multifocally, the superficial villus lamina propria is expanded by low numbers of lymphocytes, plasma cells, and occasional eosinophils. Low numbers of lymphocytes are migrating through the surface epithelium.
		Jejunum/Ileum:	Within normal limits.
		Proximal Colon:	Superficial colonic lamina propria is multifocally expanded by low numbers of plasma cells and lymphocytes.
		MLN:	Medullary sinuses are mildly expanded by clear spaces.
	Conclusions:	Stomach:	Mild, multifocal, lymphoplasmacytic and eosinophilic gastritis.
		Duodenum:	Mild, multifocal, lymphoplasmacytic and eosinophilic enteritis.
		Proximal Colon:	Mild, multifocal, lymphoplasmacytic colitis.
		MLN:	Mild, medullary edema.

**Table 4.3 continued:** Histopathology results for extruded- and RMBD- fed domestic dogs (*Canis lupus familiaris*).<sup>1</sup>

DOG 3 – RMBD-FED	Results:	<p>Liver: Within normal limits.</p> <p>Stomach: --</p> <p>Pancreas: Within normal limits.</p> <p>Duodenum: Multifocally, the superficial villus lamina propria is expanded by low numbers of lymphocytes and plasma cells.</p> <p>Jejunum/Ileum: Within normal limits.</p> <p>Proximal Colon: Within normal limits.</p> <p>MLN: --</p>
	Conclusions:	<p>Duodenum: Mild, multifocal lymphoplasmacytic enteritis.</p>
DOG 4 – RMBD-FED	Results:	<p>Liver: Sections of liver have a small, irregular focus in which hepatocyte cytoplasm is diffusely distended by clear to eosinophilic flocculent material.</p> <p>Stomach: In widely scattered locations, the superficial lamina propria is infiltrated by low numbers of lymphocytes and plasma cells.</p> <p>Pancreas: Within normal limits.</p> <p>Duodenum: --</p> <p>Jejunum/Ileum: Multifocally, the superficial villus lamina propria is expanded by low numbers of lymphocytes and plasma cells.</p> <p>Proximal Colon: Superficial colonic lamina propria is multifocally expanded by low to focally moderate numbers of plasma cells and lymphocytes.</p> <p>MLN: Medullary sinuses, and to a lesser extent the subcapsular sinuses, are moderate to markedly expanded by clear spaces.</p>
	Conclusions:	<p>Liver: Mild, focal, vacuolar change.</p> <p>Stomach: Mild, multifocal, lymphoplasmacytic gastritis.</p> <p>Jejunum/Ileum: Mild, multifocal, lymphoplasmacytic enteritis.</p> <p>Proximal Colon: Mild, multifocal, lymphoplasmacytic colitis.</p> <p>MLN: Moderate edema.</p>

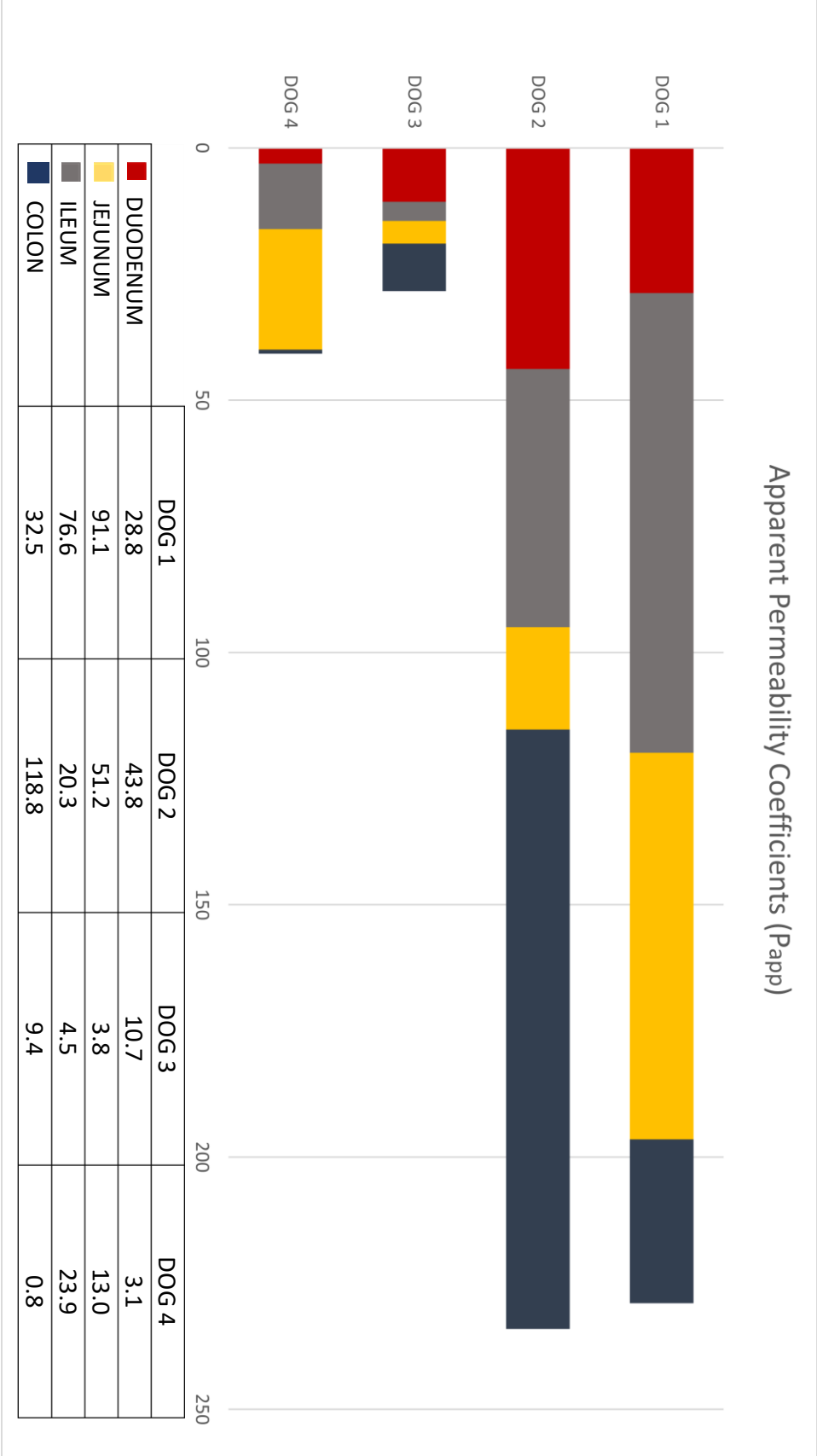
<sup>1</sup> Abbreviations: MLN, Mesenteric Lymph Node.

**Table 4.4:** Intestinal transepithelial electrical resistance (TER,  $\Omega\cdot\text{cm}^2$ ) and apparent permeability coefficients ( $P_{\text{app}}$ ) for domestic dogs (*Canis lupus familiaris*) fed extruded- versus RMBDs.

		Extruded-fed			RMBD-fed				
Item		Dog 1	<i>Tissue Average</i>	Dog 2	Dog 3	<i>Tissue Average</i>	Dog 4	<i>Average</i>	<i>Range</i>
Duodenum									
	TER (Ω.cm <sup>2</sup> )	151.0	226.6	302.2	--	--	--	226.6	151.0 - 302.2
	P <sub>app</sub>	28.8	36.3	43.8	10.7	6.9	3.1	21.6	3.1 - 43.8
Jejunum									
	TER (Ω.cm <sup>2</sup> )	152.8	245.9	339.0	--	--	--	245.9	152.8 - 339.0
	P <sub>app</sub>	91.1	71.2	51.2	3.8	8.4	13.0	39.8	3.8 - 91.1
Ileum									
	TER (Ω.cm <sup>2</sup> )	242.8	239.3	235.7	--	--	--	239.2	235.7 - 242.8
	P <sub>app</sub>	76.6	48.5	20.3	4.5	14.2	23.9	31.3	4.5 - 76.6
Proximal Colon									
	TER (Ω.cm <sup>2</sup> )	78.9	138.0	197.0	--	--	--	137.9	78.9 - 197.0
	P <sub>app</sub>	32.5	75.7	118.8	9.4	5.1	0.8	40.4	0.8 – 118.
TER (Ω.cm <sup>2</sup> )	<i>Dog Average</i>	156.4		268.5	--		--		
	<i>Dog Range</i>	78.9 - 242.8		197.0 - 339.0	--		--		
	<i>Diet Average</i>	212.4			--		--		
	<i>Diet Range</i>	78.9 - 339.0			--		--		
P <sub>app</sub>	<i>Dog Average</i>	57.3		58.5	7.1		10.2		
	<i>Dog Range</i>	28.8 - 91.1		20.3 – 118.8	3.8 – 10.7		0.8 – 23.9		
	<i>Diet Average</i>	57.9			8.6				
	<i>Diet Range</i>	20.3 - 118.8			0.8 - 23.9				

<sup>1</sup> Abbreviations: TER, transepithelial electrical resistance; apparent permeability coefficient,  $P_{\text{app}}$ .

**Figure 4.1:** Regional specific apparent permeability coefficients ( $P_{app}$ ) for domestic dogs (*Canis lupus familiaris*) fed extruded- versus RMBDs.



## **CHAPTER 5**

### **CONCLUSIONS**

Dietary options and protein source, including pork, in RMBDs has been evaluated in exotic felids (Vester et al., 2010; Kerr et al., 2012; Iske et al., 2016). Few data exist regarding digestibility and fecal characteristics associated with feeding raw zoological diets to exotic canids. Conducting nutrition studies with managed exotic canids is often difficult because animals are group-housed, preventing accurate individual feed intake and fecal output collection. Instead, domestic dogs may serve as an experimental model for nutrition studies for some exotic canid species.

From a nutritional standpoint, domestication gave dogs an increased ability to digest and utilize starch in the diet (Axelsson et al., 2013). However, dogs still possess physiological adaptations (e.g., short gastrointestinal tract, low stomach pH, commensal bacteria, etc.) characteristic of carnivores (Bosch et al., 2015). The functional and anatomical similarities between dogs and exotic canids suggest that dogs may be appropriate models for nutrition studies when diets are low in starch.

As RMBDs increase in popularity among pet owners, concerns increase regarding nutritional appropriateness and also human and animal health concerns associated with feeding these diets. Suspected pathogen exposure to humans from saliva of dogs consuming RMBDs is one concern that has recently been evaluated. Fecal shedding of microorganisms in dogs fed RMBDs has been documented and may expose humans to these pathogens. To date,

the author has found no documented cases of human illness from a dog fed a RMBD. However, these risks should be considered and common sense hygiene practices should be implemented when handling these diets.

As the field of companion animal nutrition expands, increasing emphasis is being placed on effects of nutrition on health and longevity. Nutrition research in companion animals not only offers benefits to pets and owners alike, it may also provide needed insight into exotic canids and felids. Future studies should be designed to maximize application of research to encompass both companion animals and exotic carnivores. The overall objectives of this research were to evaluate four existing raw meat-based dietary options available for exotic canids and to evaluate human and animal health implications of feeding these diets to domestic dogs. We hypothesized all RMBDs evaluated: 1.) would be highly digestible in domestic dogs, 2.) microbial risk to humans would be low, and 3.) there would be no adverse implications on canine health as a result of feeding RMBDs.

**Specific Aim 1 (chapter 3): Evaluate diet composition, apparent total tract macronutrient and energy digestibility, feed intake, fecal output, and microbial presence in domestic dogs fed RMBDs commercially manufactured for zoological carnivores.**

Nutrient and energy concentrations for all diets ranged for dry matter (DM) (32.2 – 36.2%), organic matter (OM) (91.1 – 94.9%), crude protein (CP) (50.3 – 61.7%), fat (25.1 – 38.3%), and gross energy (GE) (5.8 – 6.4 kcal/g). Digestibility of nutrients and energy ranged from 83.3 – 92.4%, 88.4 – 95.3%, 93.8 – 97.7%, 94.9 – 98.23%, and 91.3 – 95.5% for DM, OM,

CP, fat, and energy respectively, with Beef 2 diet having greater ( $P<0.05$ ) DM (92.4%), OM (95.3%), CP (97.7%), and GE (95.5%) digestibilities but lesser ( $P<0.05$ ) fat digestibility (94.9%) than all other diets evaluated. These data suggest although dietary treatments varied they were comparable in diet composition and apparent total tract macronutrient and energy digestibilities, indicating that all RMBDs evaluated may be effective dietary options for exotic canids from a digestibility standpoint.

Average fecal scores across treatments were 2.2 out of 7 (Nestlé Purina). Fecal output was greatest ( $P<0.05$ ) for dogs consuming Beef 1 diet (102.4 g/d) and lowest for dogs consuming Beef 2 diet (29.4 g/d) on an as-is basis. Fecal dry matter ranged from 27.4% (Beef 1) to 46.9% (Horse). These data indicate that RMBDs evaluated did not cause poorly formed stools or diarrhea. Because marked differences in fecal output on both a dry matter and as-is basis were observed, dietary selection may have implications on feces management and frequency of defecation for animals.

All diet samples ( $n=16$ ) tested negative for *Salmonella spp.* while both diet samples from the Beef 2 diet tested positive for *E. coli*. All saliva samples ( $n=36$ ) tested negative for *Salmonella spp.*; two saliva samples tested positive for *E. coli*. Two fecal samples ( $n=36$ ) tested positive for *Salmonella spp.* These data indicate that dogs consuming RMBDs, as well as RMBDs themselves, may serve as vectors for bacterial illness in a small percentage of cases. As a result, common sense hygiene practices should be followed when handling RMBDs or when coming into contact with saliva or feces of animals, regardless of diet.



**Specific Aim 2 (chapter 4): Application of novel technology to compare gastrointestinal histology, intestinal transepithelial electrical resistance, and intestinal macromolecule permeability between domestic dogs fed commercial extruded versus RMBDs.**

Histological examination showed that all dogs had mild inflammation throughout the gastrointestinal tract and associated tissues; however, no clear differences in the degree of inflammation were observed between extruded- versus RMBDs. These data suggest that consumption of RMBDs, assumed to be comparatively high in bacteria, did not contribute to increased inflammation in the gastrointestinal tract or in associated tissues. High variability existed in the intestinal transepithelial electrical resistance (TER) and macromolecule permeability data. Substantial numerical increases in apparent permeability coefficient ( $P_{app}$ ), indicating increased macromolecule permeability and decreased intestinal integrity, were documented in extruded-fed dogs. These data suggest potential differences in intestinal permeability between dogs consuming different diet types and may indicate some benefit to RMBDs beyond digestibility improvements. Larger scale studies are warranted due to observed high variability between animals and tissues within and across dietary treatments. Moreover, histological evaluation of gastrointestinal tract and associated tissues, in addition to Ussing chamber evaluation of intestinal TER and macromolecule permeability, may provide additional insight to the internal health of dogs on varying diet types.

**Future Research:**

Although this research provided new insights in the area of canid nutrition as it relates to feeding RMBDs on human and animal health, many questions still remain that should be

targets of future research efforts. Additional studies are needed to evaluate specific diet ingredients and nutrient concentrations for management of exotic carnivores, specifically in reference to ingredient types (e.g., fiber, protein sources, etc.). For this research, domestic dogs were used as an experimental model for exotic canids because of functional and anatomical similarities between their gastrointestinal tracts. Future research should aim to make a direct comparison between digestion efficiencies of domestic dogs and different species of exotic canids for varying diet types.

To date, research efforts surrounding health effects associated with RMBDs have focused almost exclusively on improperly formulated diets. Short-term feeding trials of RMBDs, for the purpose of digestibility studies, have provided some insight on the ability of domestic dogs and cats to tolerate RMBDs. To our knowledge, this study provides the first look at the potential health effects of long-term feeding of RMBDs to domestic dogs. Preliminary data suggest potential intestinal integrity benefits associated with feeding RMBDs compared to extruded diets. Additional long-term feeding studies need to be performed in order to assess the internal health implications of varying diet types. When possible, obtaining tissue samples can provide added insight to internal health of companion and exotic animals but should only be taken opportunistically due to ethical considerations.

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