

## Evaluation of raw pork as a commercially manufactured diet option for zoo-managed African wildcats (*Felis silvestris lybica*)

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**ABSTRACT:** Second to beef, pork is a major protein source produced in the US. Properly sourced and handled pork could be utilized as a protein option for zoo-managed carnivores. Concerns of high levels of microbial populations in raw meat diets are common. The objectives of this study were to determine apparent total tract macronutrient and energy digestibility and fecal scores from cats fed a commercially manufactured raw pork-based diet compared with commercially available raw carnivore diets formulated with either horse or beef and evaluate typical microbial population variation among the diets. Dietary treatments consisted of 4 raw meat-based diets: Horse, Beef, Pork, and beef/horse Blend. All diets were highly digestible, especially fat digestibility (98.6 to 99.7%) in which there were no statistical differences among diets. Digestibility

of organic matter (OM) was greater ( $P = 0.05$ ) when cats consumed the Blend diet (97.2%) compared to the Pork diet (93.1%). Fecal scores ranged from 1.6 to 2.6 (on a 5-point scale), with Beef (2.6) being greater than ( $P = 0.01$ ) Horse (1.6) and ( $P = 0.02$ ) Pork (1.9). *E. coli* counts ranged from 110 to 10,000 cfu/g; total coliforms: 150 to 28,000 cfu/g; yeast: 20 to 4,000 cfu/g; mold count: not detectable to 10 cfu/g; and aerobic plate count: 23,000 to 26,000,000 cfu/g. *Staphylococcus aureus* was not detected in any of the diets. *Salmonella* was presumptive positive in the Pork and Blend diet, and was negative in the other 2 diets. In conclusion, commercially manufactured diets have varying microbial counts. All diets, including the raw pork-based diet were well utilized by exotic small cats and can be included among dietary options for managed felids.

**Key words:** African wildcat (*Felis silvestris lybica*), digestibility, microbes, pork, raw meat diet

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### INTRODUCTION

Managed small exotic cat species in the United States are typically fed combinations of extruded or canned commercial cat foods, commercial raw meat formulations, and whole prey. Raw diets are more digestible than canned or extruded cat foods mainly due to reduced nutrient quality caused by processing (Kendall et al., 1982; Crissey et al., 1997; Vester et al., 2010b; Kerr et al., 2012). Raw meat diets provide a more natural, bioavailable diet compared to processed cat foods, and avoid concerns facing exclusive use of whole prey

feeding such as public perception and meeting nutrient requirements (Kerr, 2012; Robinson, 1998). Beef and horse-meat comprise the majority of raw diet formulations commercially manufactured for exotic carnivores in the US. Previously, our research team reported a raw pork diet was well utilized by large exotic felids when palatability and digestibility were measured and compared with other common diets (Iske et al., 2016). Previous studies have seen differences in digestibility between large and small cat species, therefore, further evaluation in other species is warranted to provide data that may aid diet purchasing decisions at zoological institutions (Kerr et al., 2013b). Additionally, there are no published regulations or recommendations for allowable microbial loads in raw carnivore diets, but the ability of cats to remain asymptomatic after consuming raw

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meat contaminated with *Salmonella* suggests they tolerate high numbers of microorganisms in their diets (Carter and Quinn, 2000; Finley et al., 2006). Evaluation and presentation of typical microbe ranges observed in raw carnivore diets would also benefit animal managers to understand expected ranges. The objectives of this study were to determine if a commercially available raw pork-based diet produced similar apparent total tract macronutrient digestibility and fecal scores as standard, commercially available zoological carnivore diets formulated with either horse or beef. Results may indicate the use of properly handled raw pork as a dietary option for small managed exotic cats. Additionally, general microbial population screens in dietary treatments were evaluated to determine typical variation among commonly used products. The aims of this study were not to formulate or compare diets varying strictly in protein source, but rather to evaluate and compare a raw pork diet with beef and horse-based diets commonly utilized to assist animal managers in making informed decisions regarding commercial products in the US.

## MATERIALS AND METHODS

Animal procedures were approved by the Lee G. Simmons Conservation Park and Wildlife Safari Animal Care and Use Committee (IACUC) before animal experimentation.

## Animals

Four nonbreeding African wildcats (*Felis silvestris lybica*; 2 males, 2 females, average age 9.5 yr, average weight 4.8 kg) were used. Cats received an extruded diet prior to study and were individually housed indoors in 9.2 m<sup>3</sup> enclosures at the Lee G. Simmons Conservation Park and Wildlife Safari. Park staff provided care and monitoring of health. Water was provided ad libitum. Animals were weighed at the start and conclusion of the study to ensure body weight was maintained.

## Diet Composition and Microbes

Four commercially produced raw meat diets formulated to meet or exceed nutrient requirements for domestic cats were used in the study (National Research Council, 2006). Diets were stored frozen at -18°C until 24 h prior to feeding, at which time they were moved to a cooler (2°C). Dietary treatments were sourced from single manufactured lots. Ingredient compositions of diets are listed in Table 1 and chemical compositions of diets are included in Table 2. Treatments included a horse-based [Nebraska Brand, North Platte, NE; Premium Feline Diet (Horse)], beef-based [Nebraska Brand; Special Beef Feline Diet (Beef)], beef and horse-based blend [Triple A Brand Meat Company, Burlington, CO; Complete Feline Diet (Blend)] and pork-based [Carnivore Essentials, Sheboygan Falls, WI; Carnivore Essentials (Pork)]. Pork

**Table 1.** Ingredient composition of horse-, beef-, pork-, and horse/beef blend-based raw meat diets fed to managed African wildcats<sup>1</sup>

Treatment	Ingredient composition
Horse (Nebraska Brand; Premium Feline, Nebraska Packing Inc., North Platte, Nebraska)	Horsemeat, powdered cellulose, dicalcium phosphate, calcium carbonate, Vitamin Premix [Roughage Products, Vitamin E Supplement, Mineral Oil, Niacin Supplement, Biotin, Menadione Sodium Bisulfite Complex (source of Vitamin K Activity), Vitamin A Supplement, Riboflavin, Pyridoxine Hydrochloride, Folic Acid, Calcium Pantothenate, Thiamine Mononitrate, Vitamin D3 Supplement] Trace Mineral Premix (Copper Sulfate, Manganese Sulfate, Ethylenediamine dihydriodide, Sodium Selenite), Choline chloride, taurine, salt
Beef (Nebraska-Brand; Special Beef Feline, Nebraska Packing Inc., North Platte, Nebraska)	Beef, meat by-products, fish meal, soy bean meal, dried beet pulp, calcium carbonate, dicalcium phosphate, dried egg, brewers dried yeast, salt, Vitamin Premix (Choline chloride, vitamin E supplement, niacin, vitamin B-12 riboflavin, folic acid, vitamin A acetate, thiamine mononitrate, d-calcium pantothenate, mineral oil, biotin, pyridoxine hydrochloride, vitamin D-3 supplement), taurine, Trace Mineral Premix, (zinc oxide, manganous oxide, copper oxide, mineral oil, sodium selenite, calcium iodate)
Pork (Sustainable Swine Resources, LLC; Carnivore Essentials, Sheboygan Falls, Wisconsin)	Pork, Pork By-products, Vitamins [Beet Pulp, Cellulose, Calcium Carbonate, Rice Hulls, Sodium Chloride, Mineral Oil, Vitamin E Supplement, d-alpha-Tocopheryl Acetate (Source of Natural Vitamin E), Biotin, Niacin Supplement, Thiamine Mononitrate, Vitamin B12 Supplement, Vitamin A Acetate, Vitamin D3 Supplement, Pyridoxine Hydrochloride, Riboflavin Supplement, d-Calcium Pantothenate, Folic Acid], Minerals (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)
Blend (Triple A Brand Meat Company; Feline Complete Diet, Burlington, Colorado)	Beef muscle meat, Horse muscle meat, KanTech Feline Complete vitamin/mineral premix

<sup>1</sup>Ingredient compositions are listed as given by manufacturers of each diet.

**Table 2.** Chemical composition of horse-, beef-, pork-, and horse/beef blend-based raw meat diets fed to managed African wildcats (DMB)

Item <sup>1</sup>	Horse	Beef	Pork	Blend
DM, % as-fed	36.9	34.6	33.0	28.7
OM, %	90.4	91.3	94.0	91.1
CP, %	50.3	52.4	51.0	65.7
Fat, %	31.6	33.1	39.9	25.4
NFE, %	1.1	0.3	0	0
CF, %	3.1	2.3	2.4	1.2
TDF, %	7.3	5.5	5.6	1.2
GE, kcal/g	6.1	6.3	6.7	6.1

<sup>1</sup>DM = dry matter; OM = organic matter; CP = crude protein; NFE = nitrogen free extract; CF = crude fiber; TDF = total dietary fiber; GE = gross energy.

was sourced from a bio-secure, integrated swine operation. Each dietary treatment was subsampled once, dried at 55°C, ground through a 2-mm screen (Wiley mill, model 3383-L10, Thomas Scientific, Swedesboro, NJ) and analyzed for chemical composition. Dietary treatments were analyzed for dry matter (DM; Method 934.01) and organic matter (OM; Method 942.05; AOAC, 2006). Crude protein (CP) was determined using a Leco Nitrogen/Protein Determinator (Method 992.15; model FP-528, Leco Corporation, St. Joseph, MI). Fat concentrations were determined by hexane extraction (Method 991.36; AOAC, 1995). Gross energy (GE) was determined by bomb calorimetry (model AC 500, Leco Corporation, St. Joseph, MI). Crude fiber (CF) was analyzed by Midwest Laboratories (Omaha, NE; AOCs Ba6a-05; Thiex, 2008). Total dietary fiber (TDF) was adjusted for high protein samples (Prosky et al., 1994). One representative subsample per diet was collected, frozen at -18°C, and transported without thawing to Midwest Laboratories (for microbial evaluations; Method 991.14; Method 2003.07; AOAC, 1994; Maturin and Peeler, 2001; Tournas et al., 2001; AOAC, 2003). *Salmonella* and *Escherichia coli* (*E. Coli*) were analyzed for microbial presence of serotypes including non-pathogenic strains (presumptive positive or negative). All chemical analyses were conducted in the nutrition lab at Omaha's Henry Doorly Zoo and Aquarium (Omaha, NE) unless otherwise noted.

### Experimental Design

The experimental design was a 4 × 4 Latin square with animals randomly assigned to 1 of 4 dietary treatments. Animals were fed treatment diets isocalorically for GE to maintain body weight, determined prior to start of study. Each treatment period consisted of a 10 d diet adaptation phase followed by a 4 d collection phase. Total food intake, fecal output and fecal scores were obtained daily during each collection phase. Feces were evaluated using a scale of 1 to 5 with: 1 = hard, dry pellets; 2 = dry,

well-formed; 3 = soft, moist, formed; 4 = soft, unformed; 5 = watery liquid (Felid Taxon Advisory Group, 2014).

### Energy and Macronutrient Digestibility

Fecal samples per animal were pooled at conclusion of each period, dried at 55°C, and ground through a 2-mm screen (Wiley mill, model 3383-L10, Thomas Scientific). Fecal samples were analyzed for DM, OM, CP, GE, and fat concentrations using methods previously described for diet analyses. Apparent total tract digestibility values were calculated using the following equation: [(nutrient intake – fecal output) / (nutrient intake)] × 100. Digestible energy (DE) values were calculated using the following equation: (kcal/g gross energy in diet × energy digestibility of respective diet). For method comparison, metabolizable energy (ME) of diets were calculated using the published National Research Council (NRC) equation [ME = DE – (0.77 × g protein in diet)] as well as using modified Atwater values (8.5 kcal/g fat, 3.5 kcal/g protein, 3.5 kcal/g carbohydrate) and unmodified Atwater values (9.0 kcal/g fat, 4.0 kcal/g protein, 4.0 kcal/g carbohydrate) multiplied by fat, protein, and carbohydrate concentration of each diet (National Research Council, 2006). Carbohydrate concentrations of diets were calculated by difference as nitrogen free extract (NFE) using the following equation (DMB): (100 – (% ash + % CP + % fat + % TDF)). Although crude fiber is typically used in calculating NFE, more accurate measures of dietary fiber result in more accurate estimations of NFE; therefore, TDF was used for calculating NFE (Cho et al., 2001; deOliveira et al., 2012). Due to very low fiber and NFE concentrations in diets and high relative error, NFE of some diets produced a slightly negative value; therefore, a value of 0 was used for NFE.

### Statistical Analysis

All data were analyzed using the Mixed Models procedure of SAS (SAS Inst. Inc., Cary, NC). The fixed effect of diet was tested and cat was considered a random effect. All data were reported as least squared means and a probability of  $P < 0.05$  was considered statistically significant. Reported standard error of the means (SEM) were determined according to the Mixed Models procedure of SAS. Data of 1 animal was not included for periods 2 (Blend) and 4 (Horse) because of lack of intake (less than 30% of daily offered diet for 3 consecutive days at any point during the study). This animal did consume adequate amounts (more than 30% of daily offered diet) for periods 1 (Beef) and 3 (Pork); therefore, data were included for those 2 periods with the exception of fat digestibility because of lack of adequate sample volume for fecal fat determination.

## RESULTS

### Diet Composition

Diets contained similar concentrations of OM (90.4 to 94.0%) and GE (6.1 to 6.7 kcal/g DM), but were variable in other nutrients (Table 2). The Blend diet had the lowest DM (28.7%) and fat (25.4%) concentrations and highest CP (65.7%) concentrations. The other diets (Horse, Beef, and Pork) ranged in DM from 33.0 to 36.9% and CP from 50.3 to 52.4%. The Pork diet was highest in fat concentration (39.9%) compared to other diets. Fiber concentrations (CF and TDF) were highly variable among diets ranging from a low in the Blend diet (1.2 and 1.2%, respectively) to a high in the Horse diet (3.1 and 7.3%, respectively; Table 2).

### Diet Microbes

Microbial counts were variable among diets and can be found in Table 3. *E. coli* and total coliform counts were numerically highest in the Beef diet (10,000 and 28,000 cfu/g, respectively) and numerically lowest in the Blend diet (110 and 150 cfu/g, respectively). The Beef diet tested positive for mold (10 cfu/g) while all other diets did not contain detectable levels of mold. Yeast and aerobic plate counts were greatest in the Blend diet (4,000 and 26,000,000 cfu/g, respectively), while yeast was lowest in the Pork diet (20 cfu/g). *Staphylococcus aureus* was not detected in any of the diets (10 cfu/g reporting limit). *Salmonella* was presumptive positive in Pork and Blend diets, and was negative in the other two diets.

### Energy and Macronutrient Digestibilities

Average diet intakes and fecal output and characteristics are found in Table 4. Dry matter intakes (g/d) and GE intake (kcal/d DMB) of all diets were not different ( $P > 0.05$ ). With the exception of the single animal removed

**Table 4.** Intake, fecal output, fecal characteristics, and apparent total tract macronutrient digestibility in managed African wildcats fed horse-, beef-, pork-, and horse/beef blend-based raw meat diets

Item <sup>1</sup>	Diet				SEM
	Horse	Beef	Pork	Blend	
<b>Intake</b>					
Food intake, g DM/d	35.3	46.4	40.9	50.7	11.2
GE intake, kcal/d DMB	209.7	292.8	275.0	305.8	70.7
<b>Fecal output</b>					
Fecal output, g as-is/d	8.8 <sup>a,b</sup>	15.3 <sup>a</sup>	8.8 <sup>a,b</sup>	4.1 <sup>b</sup>	4.0
Fecal output, g DM/d	5.4	5.4	4.6	2.8	1.7
Fecal scores <sup>2</sup>	1.6 <sup>b</sup>	2.6 <sup>a</sup>	1.9 <sup>b</sup>	2.2 <sup>a,b</sup>	0.3
<b>Apparent nutrient digestibility</b>					
DM, %	87.6 <sup>a</sup>	88.9 <sup>a,b</sup>	90.1 <sup>a,b</sup>	94.1 <sup>b</sup>	2.5
OM, %	91.1 <sup>a</sup>	92.2 <sup>a</sup>	93.1 <sup>a</sup>	97.2 <sup>b</sup>	1.8
CP, %	96.8 <sup>b</sup>	93.4 <sup>a</sup>	96.0 <sup>a,b</sup>	98.1 <sup>b</sup>	1.3
Fat, %	98.6	99.4	99.4	99.7	0.5
GE, %	92.1 <sup>a</sup>	93.3 <sup>a</sup>	94.5 <sup>a,b</sup>	97.3 <sup>b</sup>	1.7
DE <sup>3</sup> , kcal/g	5.6	5.9	6.4	6.0	
Calculated ME, kcal/g <sup>4</sup>	4.5	4.7	5.2	4.5	
Calculated ME, kcal/g <sup>5</sup>	4.9	5.1	5.6	4.9	
Calculated ME, kcal/g <sup>6</sup>	5.2	5.5	6.0	5.5	

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

<sup>1</sup>GE = gross energy; DE = digestible energy; ME = metabolizable energy; DMB = dry matter basis.

<sup>2</sup>Fecal scores were evaluated using a scale of 1 to 5 with: 1 = hard, dry pellets; 2 = dry, well-formed; 3 = soft, moist, formed; 4 = soft, unformed; 5 = watery liquid.

<sup>3</sup>DE = calculated using following equation: kcal/g gross energy  $\times$  energy digestibility.

<sup>4</sup>ME = Calculated using modified Atwater: 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of N-free extract.

<sup>5</sup>ME = Calculated using unmodified Atwater: 9 kcal of ME/g of fat + 4 kcal of ME/g of CP + 4 kcal of ME/g of N-free extract.

<sup>6</sup>ME = Calculated using NRC equation:  $DE - (0.77 \times \text{g protein of diet})$ .

from the study for 2 periods, all animals maintained body weight within 5% throughout the study. Fecal dry matter outputs (g/d) were not different among animals but as-is

**Table 3.** Microbial population counts of commercially available horse-, beef-, pork-, and horse/beef blend-based raw meat diets fed to managed African wildcats<sup>1</sup>

Diet	Cfu <sup>2</sup> /g						Org <sup>3</sup> /25g	
	E. Coli (generic)	Total coliforms	Yeast	Mold count	Staphylococcus aureus	APC <sup>4</sup>	Salmonella	
Horse	400	1,000	480	n.d.	n.d.	$2.3 \times 10^4$	Negative	
Beef	10,000	28,000	840	10	n.d.	$4.4 \times 10^6$	Negative	
Pork	3,900	9,600	20	n.d.	n.d.	$3.9 \times 10^4$	Presumptive Positive	
Blend	110	150	4,000	n.d.	n.d.	$2.6 \times 10^7$	Presumptive Positive	

<sup>1</sup>Reporting limits: E. Coli = 10 cfu/g, Total Coliforms = 10 cfu/g, Yeast = 10 cfu/g, Mold Count = 10 cfu/g, Staphylococcus aureus = 10 cfu/g, Salmonella = 1 org/25g.

<sup>2</sup>Cfu = colony-forming units.

<sup>3</sup>Org = organisms.

<sup>4</sup>APC = Aerobic plate count.

grams of fecal output (g/d) were greater ( $P = 0.01$ ) when animals were fed Beef (15.3 g) compared to when fed the Blend diet (4.1 g). Fecal scores ranged from 1.6 to 2.6, with the Beef treatment producing higher fecal scores than Horse ( $P = 0.01$ ) and Pork ( $P = 0.02$ ).

All diets were highly digestible, especially regarding fat (98.6 to 99.7%), and were not different. Most statistical differences were seen between Horse, Beef, and Blend diets. The Blend diet was 7.4% more digestible ( $P = 0.04$ ) in DM than Horse, 5.0% more digestible ( $P = 0.01$ ) in CP than Beef, 6.7% and 5.4% more digestible in OM compared to Horse ( $P = 0.01$ ) and Beef ( $P = 0.02$ ), respectively, and 5.6% and 4.3% more digestible in GE compared to Horse ( $P = 0.02$ ) and Beef ( $P = 0.04$ ), respectively. Digestibility of OM was the only difference detected between the Pork diet (93.1%) and the Blend diet (97.2%), with the Blend diet being 4.4% more digestible ( $P = 0.05$ ). No other differences in nutrient digestibility were detected for Pork compared with the other diets. Using energy digestibility values from the current study, DE values for Horse, Beef, Pork, and Blend diets were 5.6, 5.9, 6.4, and 6.0 kcal/g DM, respectively. The NRC equation predicted ME of 5.2, 5.5, 6.0, and 5.5 kcal/g DM for the Horse, Beef, Pork, and Blend diets, respectively. Modified Atwater factors yielded ME predictions of 4.5, 4.7, 5.2, and 4.5 kcal/g DM, respectively, while unmodified Atwater factors yielded 4.9, 5.1, 5.6, and 4.9 kcal/g DM of ME for each respective diet (Table 4).

## DISCUSSION

Reluctance to feed raw pork originated from concerns associated with *Trichinae* and pseudorabies (Catty, 1969; Nauwynck et al., 2007). In the US, herd biosecurity, improved farm practices, microbial interventions, inspection, vaccination programs, and freezing of raw pork products have drastically reduced these concerns if not eradicated them altogether (Hwang and Beuchat, 1995; Farkas, 1998; Rastogi et al., 2007; Greve, 2012; Lindsay et al., 2012). In the US, pseudorabies was considered eradicated in all 50 states by 2004; however, it still exists in some feral hogs and in other countries (Anderson et al., 2008). Between 2008 and 2012 only 90 cases of human *Trichinae* were confirmed by the Centers for Disease Control (CDC) of which only 22 were reported from pork and the remainder associated with wild game consumption (Wilson et al., 2015). Similar reductions are documented in other developed countries including Denmark and the Netherlands that consider themselves free of *Trichinae* (Gamble, 2001). Freezing pork at  $-23.3^{\circ}\text{C}$  will kill *Trichinae*; therefore, pork sourced from integrated swine operations in the US, that are frozen prior to feeding, do not pose risk of these 2 concerns (Gamble, 2001). However, fresh pork not frozen prior to

feeding, or pork sourced from bio-insecure or feral sources, are of concern and should not be fed without proper testing. Additionally, the USDA has published proper handling and storage procedures for raw meat and prey fed to captive exotic animals to control microorganism growth and contamination (Crissey et al., 2001).

The objectives of this study were to determine if a commercially manufactured raw pork diet had similar apparent total tract macronutrient and energy digestibility and fecal scores as other common zoological formulations in small exotic cats, and to evaluate typical microbial population ranges in the diets. Cat ages ranged in this study, and though differences in digestibility may exist in animals of varying ages, separating age effects was not an intention of this study (Taylor et al., 1995; Teshima et al., 2010). Additionally, significant differences in nutrient digestibility between sexes are not typically observed (Wynne, 1989; Vester et al., 2008).

Prior to the study, cats received an extruded diet. Three of 4 cats transitioned from kibble to raw diets within 1 wk. One cat consumed less than 30% of daily offered diet when offered Blend and Horse treatments. While DM intake did not differ statistically, large standard error (11.2) indicated a wide range (35.3 to 50.7 g/d) across animals. Lack of consumption of Blend and Horse diets shown by one cat may have resulted from novelty of raw meat since an extruded diet was previously fed. This is characteristic of cats fed 1 diet or diet type long term who display an initial aversion to other apparently palatable foods, termed neophobia (Bradshaw, 2006). Another explanation could be preference of protein source because the cat consumed Beef and Pork diets without intake reduction. Microbe levels in the Horse diet were lower than the other diets (Table 3); therefore, bacterial contamination was an unlikely explanation.

## Diet Composition

Dietary treatments were largely composed of raw meat or raw animal by-products, commercially formulated and manufactured to meet domestic cat NRC requirements and Association of American Feed Control Officials (AAFCO) recommendations for commerce in the US (National Research Council, 2006). All diets contained similar DM (28.7 to 36.9%), OM (90.4 to 94.0%), and CP (50.3 to 65.7%) concentrations as reported in previous studies analyzing raw diets (29.0 to 38.2% DM, 91.5 to 94.6% OM, and 44.9 to 64.5% CP; Crissey et al., 1997; Vester et al., 2008; Vester et al., 2010a; Vester et al., 2010b; Kerr et al., 2013a). From these previous studies, fat (22.2 to 36.9%) and GE (5.9 to 6.4 kcal/g DM) concentrations also were similar to the current study with the exception of the Pork diet that was slightly greater (39.9% and 6.7 kcal/g DM GE, respectively). When re-

ported, TDF concentrations were similar in previous studies (range of 4.8 to 8.4%) compared to the current study with the exception of the Blend diet having the lowest TDF (1.2%) concentration which would be expected as it did not contain labeled added fiber ingredients (cellulose, beet pulp; Vester et al., 2008; Kerr et al., 2013a).

### **Diet Microbes**

Pathogenic *Salmonella* and *E. coli* contamination are common concerns associated with raw meat diets. Although USDA published a document outlining proper handling and storage of raw meat to control microorganism growth and contamination, there are no publications outlining allowable microbial loads of raw carnivore diets for zoos (Crissey et al., 2001). Results from the current study indicated “presumptive positive” (Pork and Blend) or “negative” (Beef and Horse) for evaluation of *Salmonella* species. Aerobic colony counts and *E. coli* counts in diets were 5 and 20 times greater, respectively, than allowable levels set by the European Commission issued for human grade raw meat products (European Commission, 2005). A lack of consumption was seen with the Horse and Blend diets in one cat; as discussed previously. No other clinical symptoms (vomiting, diarrhea, lethargy, lameness) or diseases related to foodborne pathogens were observed or reported during the experiment. Fecal shedding of *Salmonella* was documented in up to 95% of exotic cats with no clinical symptoms or signs of illness (Clyde et al., 1997). The ability of cats to remain asymptomatic after consuming raw meat containing *Salmonella* suggests they tolerate high numbers of foodborne microorganisms, compared to humans (Carter and Quinn, 2000; Finley et al., 2006). Therefore, it is likely healthy carnivores are not clinically affected by presence of pathogens such as *Salmonella* and standards set for human grade meat products may not be appropriate.

Microbial levels in carnivore diets may pose potential risks to humans through preparation and handling of raw meat diets. Animal managers should adhere to USDA published standard operating procedures for handling raw meat to reduce risks (Crissey et al., 2001). Additionally, the CDC advises the immediate washing of hands after contacting animal fecal material to reduce the risk and contraction of Salmonellosis (Center for Disease Control and Prevention, 2015).

In the current study, only general microbes typically evaluated in quality control programs in zoos were considered to demonstrate the expected range of properly handled raw meat diets. It is critical to understand that “presumptive positive” results for *Salmonella* indicates presence of any of 2,400 serotypes of *Salmonella* species, only a handful of which are pathogenic (Carlson et al., 2012). Similarly, *E. coli* counts include all species,

totaling more than 50,000 serotypes, of which few are pathogenic (Ørskov and Ørskov, 1992). For this reason, high levels do not inherently infer a diet is dangerous to a carnivore. Serotyping samples would be necessary to evaluate specific strains of concern. Bacteria such as *Listeria monocytogenes* and *Campylobacter jejuni* have not been critically evaluated in zoo raw diets and likely warrant research (Eisel et al., 1997). If clinical signs are observed in an animal consuming a raw meat diet, it may be necessary to evaluate the diet for microbes beyond general screens typically used.

### **Energy and Macronutrient Digestibilities**

Digestibility of nutrients is simply a measure of how efficiently a diet is digested by the animal. In animal production systems, higher digestibility typically results in less feed and reduced costs of production. In pets or exotic animals managed in zoos, higher diet digestibility may not be necessary but could reduce feeding costs. Goals of animal feeding programs vary across institutions; therefore, digestibility measures can be considered in tandem with feeding goals. All diets evaluated were highly digestible across nutrients, regardless of protein source. Values reported in the current study were similar to those previously reported in studies analyzing raw meat diets (horse or beef). Previous studies reported similar ranges of digestibility for OM (87.4 to 96.5%), CP (91.7 to 97.2%), fat (90.5 to 99.0%), and GE (89.6 to 97.3%; Crissey et al., 1997; Vester et al., 2008; Vester et al., 2010a; Vester et al., 2010b; Kerr et al., 2013a; Iske et al., 2016). However, DM digestibility values (83.6 to 90.0%) reported in the previous studies were slightly lower than those observed in the present study (87.6 to 94.1%). These ranges include data from our previous work feeding similar diets in large exotic cats (Iske et al., 2016). Macronutrient digestibility values of Pork were similar in large cats with 88.0, 90.8, 95.7, 98.5, and 92.4% digestibility for DM, OM, CP, fat, and GE, respectively (Iske et al., 2016). These values are within 3% of those observed in the current study.

Crude protein digestibility of Beef (93.4%) was lower than Horse (96.8%) and Blend (98.1%) diets. Protein digestibility could have been affected by protein content and dietary fiber (Clauss et al., 2010). Kienzle et al. (1991) demonstrated an 18.9% reduction in protein digestibility of a raw meat diet fed to domestic cats when 15% horn meal was added as a fiber source. Protein digestibility differences may be more profound when beet pulp is included compared with cellulose. In a study evaluating raw meat diets containing 9.5% TDF from various fiber sources, fed to 30 domestic cats, protein digestibility was approximately 5.8% lower when beet pulp was used compared with cellulose (Sunvold et al., 1995). High

fermentative characteristics of beet pulp (compared to cellulose) in the large intestine consequentially increase bacterial protein, leading to more protein excretion and underestimation of apparent total tract protein digestibility (Kerr et al., 2013b). This is supported in the current study as the Beef diet contained beet pulp and may have contributed to lower observed protein digestibility.

Beyond fiber source, differences in protein digestibility could be attributed to protein ingredients and processing. The Beef diet also contained fish meal and soybean meal as protein sources whereas other treatment diets contained only raw muscle meat or raw animal meat by-products. Plant-based protein sources may contain as much as 18.8% NFE which cats are less efficient at digesting compared to protein (McDonald, 2002; Karr-Lilienthal et al., 2004). Heat processing to produce meal ingredients may cause Maillard reactions that could reduce protein digestion (Camire et al., 1990). Maillard reactions and cross-linkages of amino acids, may reduce amino acid retention and availability as well as protein digestibility and quality (Björck et al., 1983; Björck et al., 1984).

In the US, commercially manufactured companion and exotic animal feeds for interstate commerce are regulated at state levels by the AAFCO, which suggests modified Atwater values for product labeling of ME (Association of American Feed Control Officials, 2014). Other methods of predicting ME include unmodified Atwater values and the NRC equation that uses digestible energy and protein content (National Research Council, 2006). Modified Atwater values reflect 85.0, 95.0, and 80.0% digestibility for carbohydrate, fat, and protein, respectively, based on standard kibble diets (Kienzle, 2002). In contrast, unmodified Atwater factors reflect 98.0, 98.0, and 90.0% digestibility for carbohydrate, fat, and protein, respectively which is more representative of digestibility values obtained from raw diets (Kienzle, 2002).

Differences between DE and ME of diets were 25.1, 17.5, and 8.5% using modified and unmodified Atwater factors, and the NRC equation, respectively. Urine energy losses in domestic cats were 6.5% of energy intake when fed a high protein (61.5%) canned diet (mixed with beef heart) and 4.6% when fed a high fat diet (49.1% fat, 39.9% protein; Riond et al., 2003). This, along with negligible gas loss because of limited fermentation in cats, suggests differences between DE and ME should be around 6.5%. The current study is supported by previous studies demonstrating modified Atwater values underestimate ME for raw meat diets. Animal managers should evaluate the ME content on labels of diets produced in the US to manage body weight and condition more accurately (Clauss et al., 2010). Although AAFCO suggests modified Atwater

values to predict ME for commercial labeling, unmodified Atwater values or NRC methodology better predicts ME of raw meat diets and should be considered for labeling of raw meat diets.

Fecal scores of cats consuming Beef (2.6) were greater than scores from cats consuming Horse (1.6) and Pork (1.9). Fiber sources varied among the treatment diets and likely contributed to differences observed in fecal scores. Differences may exist among exotic cat size as smaller species including cheetah appear to tolerate fermentable beet pulp (4% of diet) while larger species, such as tigers appear to have more ideal fecal scores when fed cellulose (4% of the diet; Kerr et al., 2013b). Domestic cats also show more ideal fecal scores with beet pulp inclusion, opposed to cellulose (Sunvold et al., 1995). The Beef diet in the current study contained beet pulp and had a TDF concentration of 5.5%, resulting in fecal scores closest to what is considered ideal (3.0). Cellulose, a non-fermentable fiber, provides the bowel with tactile stimulation inducing colonic motility and weight while fermentable fibers such as beet pulp induce chemical changes and production of short chain fatty acids (SCFA), that can be absorbed by colonocytes for energy and aid in colon health (Bueno et al., 2000a; Bueno et al., 2000b). Too much fermentable fiber, likely above 4% of diet for cats, may result in excess production of SCFA, increasing passive transport absorption and possibly resulting in soft stools (Kerr et al., 2013b). It has been suggested that raw diets for zoo carnivores should include a combination of beet pulp and cellulose (2% each) to achieve optimal intestinal health (Kerr et al., 2013b).

## Conclusions

Similar macronutrient and energy digestion was observed in African wildcats fed a pork-based raw meat diet compared with other common diets varying in protein and fiber source. Additionally, cats tolerated high and variable ranges of microbial loads in raw meat diets without impact on fecal scores. Beyond lack of consumption, clinical signs of infection and disease were absent in cats fed diets and nutrient digestibilities fell within expected ranges despite the microbe variation, indicating guidelines for appropriate and expected levels of microbes in carnivore diets need further evaluation and clarification to aid animal managers and diet manufacturers alike. Additionally, because raw meat diets are digested more efficiently than processed foods, predictions of ME for labeling of commercial products should utilize either the NRC equation or unmodified Atwater values as AAFCO suggested modified Atwater values will underestimate ME and could contribute to animal obesity. In conclusion, if properly sourced and handled, pork can serve as a protein option in raw meat diets for zoo-managed carnivores.

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