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Apparent nutrient digestibility of two raw diets in domestic kittens

Beth A Hamper, Claudia A Kirk and Joseph W Bartges

Abstract

Objectives The aim of the study was to evaluate overall dry matter, organic matter, crude protein, crude fat and gross energy digestibility of a feline commercial raw diet and a homemade raw diet compared with a canned, heat-processed diet.

Methods Six domestic shorthair kittens (20–28 weeks old) were fed three different diets in a Latin square crossover design. Diet A was a commercially available, canned, heat-processed diet. Diet B was a complete commercial, prefrozen, raw diet (commercial raw), and diet C was a raw diet supplement mixed with ground raw meat obtained locally (homemade raw). Both diets A and B were formulated to meet nutritional profile levels for cats at all life stages. Kittens were given specific diet amounts to maintain a 2–4% weight increase per week. Food was measured before and after feedings to determine the amount eaten, and all feces were collected, weighed and frozen prior to submission. Composite food samples and all feces were submitted to a national laboratory for proximate analysis of crude protein, crude fiber, ash, crude fat, moisture and caloric density.

Results Significantly higher digestibility of dry matter ($P < 0.001$), organic matter ($P < 0.001$), crude protein ($P < 0.001$) and gross energy ($P < 0.001$) was seen in the raw diets compared with the heat-processed diets. This difference resulted in significantly less fecal matter ($P < 0.001$) despite similar levels of intake and kcal ingested, and evidence of no difference in fecal scores.

Conclusions and relevance Higher dry matter, organic matter and protein digestibility was seen in two commercial raw diets compared with a heat-processed diet. Digestibility differences could have been due to variance in dietary protein, fat and carbohydrate concentrations between the diets, variance in dietary ingredients or quality, alterations in protein structure secondary to heat processing, as well as alterations in gastrointestinal flora. Future research examining digestibility in diets with the same macronutrient proportions and ingredients, and mechanisms for any differences, is warranted.

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Introduction

Nutrient digestibility of raw diets has not been well characterized in domestic cats. Owing to cats' strict carnivorous nature, protein and fat are key components in the feline diet. Factors affecting the digestibility of protein in the diet include protein quantity, quality and amino acid composition; the presence of any antinutritional factors such as trypsin inhibitors, phytates, tannins or fiber; and the storage and processing of the diet itself.[1] Factors affecting fat digestibility include animal age, fat and calcium concentrations in the diet, and type of fat (ie, long vs short chain fatty acids, saturated vs monosaturated vs polyunsaturated).[2,3] The lower the concentration of fat in the diet, the lower the apparent digestibility owing to contribution from endogenous fecal fat.[4] Saturated fats have the lowest apparent digestibility, followed by monosaturated and polyunsaturated fatty acids.[2] High dietary calcium has been shown to decrease the digestibility of fat owing to production of Ca–fatty acid soaps.[3] Kittens reach adult levels of apparent fat digestibility at 24 weeks of age.[5]

Processing modifications of proteins can lead to formation of bioactive compounds or loss of nutritional value.[6] Processing conditions include application of heat, fermentation or chemical treatments such as use of oxidizing agents. Heat treatment in general decreases protein bioavailability through a variety of biochemical

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Corresponding author:
Beth Hamper DVM, PhD, DACVN, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA
Email: bhamper@utk.edu

reactions, including proteolysis, protein crosslinking, amino acid racemization, protein–polyphenol reactions, oxidative reactions and browning or Maillard reactions.[6] Changes in digestibility secondary to processing depend on the type, duration and severity of the processing conditions applied to the food. Processed commercial canned pet foods undergo treatment with pressurized steam or water at temperatures between 116°C and 129°C and are maintained at these temperatures long enough (60–90 mins) to kill pathogenic bacteria.[7]

Digestibility refers to the percentage of foodstuff taken into the digestive tract that is absorbed and used by the body. The effect of heat-processed vs raw diets on digestibility has been previously examined in exotic felids. Crissey et al compared a raw meat diet with a dry kibble diet in sand cats and found the raw meat diet to have 10% higher digestibility in dry matter (DM) and energy and 15% higher digestibility in crude protein compared with the kibble extruded diet.[8] A more recent study looked at a commercial raw meat vs an extruded, high-protein kibble diet in the African wildcat (*Felis lybica*), the domestic cat's wild ancestor.[9] Crude protein digestibility in the raw diet was 8% higher than the extruded diet.[9] In a study with domestic cats, Kerr et al compared energy and macronutrient digestibility in a raw, beef-based diet and the same diet microwaved to an internal temperature of at least 71°C.[10] No differences were found between the raw and microwaved diets with respect to macronutrient and energy digestibility. Another study evaluating protein quality of various raw and rendered animal products found total essential amino acid and total amino acid digestibility ranging from 93.7–96.7% and 90.3–95.5%, respectively, in the raw diets, and 84.0–87.7% and 79.2–84.8%, respectively, in the rendered animal meats.[11] Ingredient composition and processing conditions were not reported in these studies, and nutrient composition sometimes differed markedly among test diets.

The objective of this study was to compare apparent digestibility in kittens of two commonly available raw diets with a premium heat-processed, high-protein, canned diet. The hypothesis was that apparent digestibility of DM, organic matter (OM) and crude protein would be greater in the raw diets than in the heat-processed diet, but there would be no difference in crude fat apparent digestibility between the three treatment groups. A further objective was to compare fecal quantity and quality between the three diets, using fecal scores and fecal weights. The hypothesis was that raw feeders would have less fecal volume but no difference in fecal quality/score.

Materials and methods

All kittens were born and raised at the University of Tennessee Veterinary Medical Center and research facility; their care was in compliance with the Guide for the Use and Care of Laboratory Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

Animals

Six 20-week-old kittens (five males, one female) were used for this study. All kittens were given a physical examination before and after the digestibility trial and were deemed healthy. Prior to entry into the digestibility trial, all kittens had been fed one of the raw diets for a 10 week growth trial, following recommendations of the Association of American Feed Control Officials (AAFCO). The kittens were housed in individual metabolism cages during the digestibility trial. Body weights were recorded biweekly on all kittens to ensure adequate but not excessive weight gain. At the end of the study, the kittens were transferred to a permanent feline colony that is used for dietary and other non-invasive research or adopted to private homes.

Diets

Diet A was a commercial heat-processed canned diet (Evo Turkey and Chicken Formula Canned Cat and Kitten Cat Food; Natura Pet Foods). This canned food diet was chosen to match closely a raw diet's moisture and nutrient content. Diet B was a commercial frozen raw diet (Wild Kitty Raw All Natural Cat Food Chicken and Clam frozen raw diet; Wild Kitty Cat Food). Both diets A and B were formulated to meet nutritional levels established by AAFCO for all cat life stages. Diet C was a home-prepared raw diet made from raw, boneless, skinless chicken breast (Tyson Foods) obtained from a local grocery store and mixed, according to manufacturer's instructions, with a popular commercial food supplement (TCFeline Plus Cat Food Premix with beef liver; TCFeline) designed to balance a raw meat diet. This diet has no AAFCO statement of nutritional adequacy. The homemade diet was prepared every 2 weeks and immediately frozen. All raw foods were kept frozen until 1 day before feeding, when they were transferred to a refrigerator in preparation for feeding the next day. Each kitten was fed twice daily a quantity of food to allow for a 2–4% weight increase per week associated with growth. Water was available at all times.

Food intake

To determine food intake, all food was weighed before and after each offering, and the difference was determined. The majority of food was eaten within 15–20 mins of presentation. Any food not ingested within 4 h of presentation was removed to prevent microbial contamination or proliferation. Previous studies using the same rooms showed evaporation rates of $<5\%$ over 24 h, so any water loss was considered minor (C Kirk, 2015, personal communication). Feeding bowls and feeding area were sanitized between feedings.

Digestibility trial

A Latin square design was used, with each kitten rotating through the three dietary regimens in a random order with a 7 day acclimation period followed by a 7 day total feces collection period. Feces were scored and recorded daily by the same individual (BAH) during the 7 day collection period. Fecal scoring was performed using a five-point scale as follows: 1 = watery, liquid that can be poured; 2 = soft, unformed stool; 3 = soft, moist, formed stool; 4 = dry, well-formed stool; 5 = hard, dry pellets. All feces were collected and weighed daily in individual containers and immediately stored at –20°C. A composite sample of each diet and all feces were submitted to Eurofins laboratory (Des Moines, IA, USA) for proximate analysis of moisture, crude protein, crude fat, ash, crude fiber and calories.

Digestible carbohydrate, presumed to be nitrogen-free extract (NFE), was estimated based on the calculated difference between 100% and the percentage amounts of everything else in the food (100%: % moisture – % crude protein – % crude fat – % crude fiber – % ash). Briefly, food samples were put into a Cuisinart to mix for a composite sample. Portions of fecal samples were weighed in a dish and then placed onto a sheet and put into a vacuum oven (Association of Analytical Communities [AOAC] methods 934.06, 925.45, 920.151) for at least 5 h at 65°C. Samples were then removed from the oven and cooled in a desiccator. Once cooled, dried sample weight was determined, and moisture was determined by calculating the difference between the weight of the undried portion and the dried portion.

Crude protein was determined by entering a portion of the sample into the combustion chamber of a protein analyzer (AOAC method 990.03); gas from combustion was analyzed for nitrogen content and calculated to protein.

Crude fat was determined by acid hydrolysis (AOAC method 954.02), in which a portion of the sample was hydrolyzed with HCl. The hydrolyzed sample was extracted in a liquid extraction with a combination of ethyl and petroleum ethers. The ethers containing the fat were collected and dried, and the resulting extracted fat was used to calculate the crude fat in the sample. Ash was determined by weighing 2 g sample into a crucible, then drying the sample in an oven, ashing it in a muffle furnace and determining the weight of the ash (AOAC method 942.05).

Crude fiber was determined by digesting a portion of the sample with acid and base. The weight of the residue minus the ash from the residue determines crude fiber (AOAC method 962.09).

Calorie content was determined with a bomb calorimeter, in which a portion of the sample was exploded in a water jacketed, closed vessel and the increase in heat in the water was measured. NFE was determined by the following formula:

$$\text{NFE} = 100 - (\text{DM crude protein\%} + \text{DM crude fat\%} + \text{DM crude fiber\%} + \text{DM ash\%})$$

Apparent digestibility was calculated using the following equation:

$$\text{apparent digestibility} = (\text{intake} - \text{output}) / \text{intake}$$

Statistical analysis

A completely randomized design was used to compare mean differences in apparent digestibility (DM, OM, crude protein, crude fat, NFE and gross energy), DM food intake, DM fecal output and kcal ingested in kittens, with blocking by treatment (diet). Mixed model ANOVA (SAS, version 9.2) was used to compare least squares means.

The two testable assumptions of ANOVA, normally distributed residuals and equal variances between groups, were tested for all dependent variables. No variables failed to meet these assumptions. Normality was tested using the Shapiro–Wilk test, and homogeneity of variance was tested using Levene's F test. Statistical significance was defined as $P \leqslant 0.05$, while trend was defined as $P \leqslant 0.10$.

Results

Table 1 lists the analyzed macronutrient composition of each diet fed during the digestibility trial on an energy basis (nutrient g/1000 kcal as fed). Crude protein content of both the heat-processed and commercial raw diet was similar. All crude protein concentrations were greater than the National Research Council (NRC) feline growth minimum requirement of 45 g/1000 kcal metabolizable energy (ME).[2] The homemade raw diet had a substantially higher concentration of crude protein and lower concentration of crude fat than the other two diets. All diets had crude fat concentrations greater than the NRC feline growth recommended allowance of 22.5 g/1000 kcal ME.[2] There was no detectable NFE (carbohydrate) in the homemade raw diet, while the heat-processed and commercial raw both had <14 g/1000 kcal concentration of carbohydrate. Kitten mean apparent digestibility values are shown in Table 2. Kitten mean apparent total tract DM ($P <0.0001$), OM ($P <0.001$), crude protein ($P = 0.001$) and gross energy ($P <0.001$) digestibility were significantly greater in the homemade raw diet compared with the commercial raw diet and heat-processed diet ($P <0.001$). The commercial raw diet had significantly greater apparent DM, OM, crude protein and gross energy digestibility compared with the heat-processed diet ($P <0.001$). A trend toward significantly greater crude fat digestibility ($P = 0.056$) was found in the commercial raw diet compared with the homemade and heat-processed diets.

Table 1 Calculated dietary nutrient composition (g/1000 kcal) and metabolizable energy (ME)

	Heat-processed*	Commercial raw†	Homemade raw‡
ME (kcal/g)**	1.46	1.56	1.0
Protein	89.5	93.3	176.4
Fat	72.5	64.2	27.5
Nitrogen-free extract	7.5	13.7	–
Crude fiber	1.1	1.6	0.3
Moisture %	71.8	70.7	78.5

*Ingredients: turkey, chicken, turkey broth, chicken broth, chicken meal, herring, carrots, whole egg, salmon meal, natural flavor, carrageenan, tomato flakes, cottage cheese, L-ascorbyl-2-polyphosphate, apples, guar gum, vitamin E supplement, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, thiamine mononitrate, niacin supplement, d-calcium pantothenate, pyridoxine hydrochloride, riboflavin supplement, folic acid, biotin, zinc amino acid chelate, cobalt amino acid chelate, copper amino acid chelate, manganese amino acid chelate, potassium iodide, inulin, herring oil, choline chloride, potassium chloride, salt, sunflower oil, taurine, sodium phosphate, beta carotene

†Ingredients: free-range organic chicken, apples, Atlantic clams, beets, broccoli, carrots, chicken hearts, chicken liver, cod liver oil, dried kelp, dried yeast, flax, flax seed, lecithin, mushrooms, water sufficient for processing, oysters, peas, rice bran, spinach, wheat germ, wheat germ oil

‡Ingredients: skinless chicken breast, supplement (freeze-dried bovine bone [New Zealand], egg yolk, whey protein isolate, beef liver, freeze-dried krill, taurine, cellulose, kelp, vitamin E, vitamin D3, vitamin A, vitamin B complex)

**ME (kcal/g) = $(FI \times GE_{food}) - (F \times GE_{feces}) - [(P_{food} - P_{feces}) \times c]/FI$, where FI = food intake, GE_{food} = gross energy of food (kcal/g), F = feces, GE_{feces} = gross energy of feces (kcal/g), P_{food} = amount of protein in food (g), P_{feces} = amount of protein in feces, c = correction factor of 0.86 for cats

Table 2 Apparent digestibility values of three diets in kittens (% mean ± SEM)

	Heat-processed	Commercial raw	Homemade raw
Dry matter	83.8 ± 0.6^c	90.6 ± 0.6^b	92.6 ± 0.6^a
Organic matter	88.4 ± 0.4^c	93.5 ± 0.4^b	96.5 ± 0.4^a
Crude protein	88.9 ± 0.4^c	94.7 ± 0.4^b	97.7 ± 0.4^a
Crude fat	94.2 ± 0.8^a	96.9 ± 0.8^a	93.9 ± 0.8^a
Nitrogen-free extract	40.5 ± 10.5^a	62.8 ± 10.5^a	–
Energy	90.2 ± 0.4^c	94.8 ± 0.4^b	96.7 ± 0.4^a

Different superscript letters within rows indicate statistical differences ($P \leqslant 0.05$)

Table 3 Kitten dry matter (DM) and caloric intake, DM fecal output (mean ± SEM) and fecal score (mean ± SEM)

	Heat-processed	Commercial raw	Homemade raw
Absolute			
Total intake (g/day)	54.7 ± 4.0^a	50.7 ± 4.0^a	60.5 ± 4.0^a
Fecal output (g/day)	8.6 ± 0.8^a	5.2 ± 0.8^b	4.4 ± 0.8^b
Energy (kcal/day)	333.3 ± 24.6^a	319.8 ± 24.6^a	330.3 ± 24.6^a
Fecal score	3.81 ± 0.08	4.00 ± 0.08	3.94 ± 0.08

Different superscript letters within rows indicate statistical differences ($P \leqslant 0.05$)

Table 3 lists the mean absolute kitten DM intake, kcal ingested, DM fecal output and fecal score during the 7 day collection periods. While there were no significant differences in fecal score, fecal output was significantly less ($P = 0.002$) on both raw diets compared with the heat-processed diet, despite similar DM intake.

Discussion

As previously stated, nutrient digestibility can be influenced by many factors, including differing macronutrient concentrations, ingredient differences, nutrient quality, presence of dietary fiber or phytate, particle size and processing techniques, as well as the age of the animal and alterations in gut flora. Fiber likely had minimal influence on digestibility as crude fiber levels were low; however, analysis of specific soluble and insoluble fiber fractions would be necessary to confirm this. Increased levels of soluble fiber will improve energy digestibility.[12] Owing to differences in protein, carbohydrate and fat levels, along with different dietary ingredients, generalities cannot be made. For the three diets examined in this study, apparent digestibility differences were most evident in crude protein, DM, OM and gross energy, with both raw diets having significantly higher digestibility than the heat-processed diet. The homemade raw diet was also significantly higher in DM, OM, crude protein and gross energy digestibility compared with the commercial raw diet. The homemade raw diet had the lowest crude fat digestibility. The reduced concentration of crude fat in this diet may have affected its apparent digestibility value owing to its low level in the diet and contributions from endogenous fecal fat. Similarly, differences in protein digestibility could be partly due to large differences in protein content between the homemade raw and other two diets because endogenous protein would provide proportionately more of the protein in the feces of kittens fed less protein. There was no significant difference in NFE apparent digestibility between the heat-processed and commercial raw diet. Low digestibility values in this category were probably related to the very low content of carbohydrate in the diets. The presence of small amounts of fiber (cellulose and rice bran) in the raw diets could also have affected the carbohydrate, fat and protein digestibilities.

Because protein quality depends on the concentration and distribution of amino acids, proteins that are deficient in one or more indispensable amino acids are poorer in quality than proteins that contain these indispensable amino acids. A weakness of this study was that amino acid profiles were not measured and compared. On a rudimentary level, all three diets in this study used the same or similar primary protein source: chicken or turkey. The homemade diet used skinless boneless chicken breasts, while the other two diets did not specify which poultry components were used, which may have affected the quality of the diets owing to differing quantities of connective tissue. In addition, several other protein sources were used in each diet, each differing from the other. In general, crude protein digestibility in all three diets was high. The protein digestibility of both raw diets was significantly better than the heat-processed diet. Interestingly, the homemade raw diet crude protein content was significantly more digestible than the commercial raw diet. These differences in digestibility between the three diets may have been due to differences in protein concentration, protein quality and/or food particle size, but is unknown without knowledge of amino acid profiles. Chicken in the homemade raw diet was ground to a finer particle size (ie, consistency of oatmeal) compared with the commercial raw (consistency of hamburger). Finer particle size increases the surface area for exposure to digestive enzymes, thus potentially increasing digestibility.[13]

A known difference between the two raw diets and the heat-processed diet in our study was that the heat-processed diet went through commercial processing with application of at least 116°C over 60–90 mins. Previous studies have shown differences in crude protein digestibility between raw and processed proteins due to alterations in amino acid structure. Maillard reaction products, which result from chemical reactions between amino acids and sugars during the cooking process, reduce digestibility of crude protein by amino acid destruction and also through inhibition of digestive proteases.[14] Analysis for the presence of Maillard reaction products would be necessary to confirm this.

The differences in digestibility between raw and cooked diets in our study and a previous study by Kerr et al may be related to the quality of protein sources and differences in temperature and time processing.[10] The raw diets of Kerr et al contained meat by-products, fish meal and soybean meal as the second through fourth ingredients.[10] Our raw diets had no by-products or meals. In the study by Kerr et al, meat was cooked in a microwave for 45–60 s at 71°C, compared with conventional retorted or canned pet foods cooked to at least 116°C for 60–90 mins.[10] The higher temperature and longer time may have allowed for biochemical transformation of proteins/amino acids.

Digestibility is also influenced by gastrointestinal flora composition. Differences in gut flora have been shown to occur with different processing treatments and macronutrient proportions.[15,16] Backus et al found differing concentrations of hydrogen gas production in cats fed raw, canned and extruded diets, indicating differences in microflora.[15] Differences in macronutrient proportions (ie, high protein vs high carbohydrate) alter gut microbial metabolism,[16] and fecal microbial populations have been shown to be altered by altering dietary protein concentration.[17] The higher crude protein and lower crude fat content in the homemade diet could have resulted in gastrointestinal flora differences between the two raw diets. Further studies measuring concentration of Maillard compounds and differences in fecal bacteria between the same diet formula, raw and processed, would be needed to confirm these differences.

Improved digestibility should result in decreased fecal output. While there was no evidence of differences in fecal score, DM intake or kcal ingested, the kittens fed the raw diets had significantly less fecal output than kittens fed the heat-processed diet. Decreased feces removal from the litter box could be a significant benefit to some cat owners, as long as this decrease is not associated with constipation or alterations in colonic tissue health.

Limitations of this study include the small sample size, the large difference in crude protein and crude fat content between the homemade raw diet and the other two diets, differences in ingredients used in all diets without associated amino acid profiles and lack of measurement of dietary Maillard compounds. Ideally, a single diet ration would have been used, with commercial heat processing applied to one portion while the other remained unprocessed. This would have eliminated any potential differences in macronutrient content and protein quality.

Conclusions

Significantly higher digestibility of DM, OM, protein and energy were seen in both raw diets compared with a heat-processed canned diet in kittens. Improved digestibility seen in the raw diets resulted in significantly less fecal matter while normal fecal scores were maintained despite similar levels of intake and kcal ingested. These differences may have been due to differing macronutrient proportions, ingredient differences, changes in protein structure secondary to heat or commercial processing, alterations in gastrointestinal flora and/or protein quality itself, but further studies are needed to elicit these differences.

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