Chemical composition, true nutrient digestibility, and true metabolizable energy of chicken-based ingredients differing by processing method using the precision-fed cecectomized rooster assay[1]

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ABSTRACT: Chicken-based ingredients are commonly used in pet food products, but vary greatly in nutrient composition and processing conditions that may affect their protein quality and digestibility. Testing the quality of protein sources undergoing different processing conditions provides important information to pet food producers. The objective of this study was to determine the chemical composition, nutrient digestibility, protein, and AA digestibility scores, and nitrogen-corrected true metabolizable energy (**TMEn**) of chicken-based ingredients that had undergone different processing conditions (i.e., chicken meal, raw chicken, retorted chicken, and steamed chicken) using the precision-fed cecectomized rooster assay. True nutrient digestibility was variable among the protein sources (60% to 76% of DM, 66% to 81% of OM, 83% to 90% of AHF, 50% to 95% of AA and 73% to 85% of TMEn/GE). In general, the chicken meal had a lower ($P < 0.05$) nutrient digestibility than other ingredients tested, including DM, OM, and most indispensable and dispensable AA, with most having a true digestibility between 75% and 85%. The steamed chicken had the highest indispensable AA digestibilities, with all having a true digestibility greater than 88% and most being over 90%. TMEn value and digestible indispensable AA scores (**DIAAS**)-like values were higher ($P < 0.0001$) in the less processed chicken-based ingredients in comparison to chicken meal. Although animal proteins are often considered to be complete proteins, DIAAS-like values <100% suggest that ingredients like chicken meal may not provide all indispensable AA when included at levels to the meet minimal crude protein recommendation. Although raw protein sources are often touted as being the most digestible and of the highest quality, the steamed chicken had the highest ($P < 0.0001$) DIAAS-like values in this study. This study demonstrates the considerable variability that exists, not only in the chemical composition but also in the true nutrient digestibility among chicken-based ingredients undergoing different processing conditions. These data justify more in vivo testing and the use of DIAAS-like values that consider AA profile, in vivo digestibility, and species-specific recommendations, to evaluate protein-based ingredients intended for use in dog and cat foods.

Key words: animal model, cat, dog, nutrient digestion, pet food, protein source

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INTRODUCTION

Chicken-based ingredients are commonly used in pet food products and are considered to have high nutritional value (Faber et al., 2010; Deng

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et al., 2016). Cooking temperature and processing conditions may greatly affect the quality and digestibility of protein-based ingredients (Kondos and McClymont, 1974; Batterham et al., 1986; Johnson et al., 1998). Protein denaturation in meat begins at 70 °C, and at 100 °C the oxidation of protein forms aggregates that decrease enzyme activity, leading to reduced AA digestibility (Santé-Lhoutellier et al., 2008; Lund et al., 2011; Bax et al., 2012). Testing the quality of protein sources undergoing different processing conditions provides important information to pet food manufacturers. Traditionally, the pet food industry primarily used animal protein-based meals that had undergone the rendering process in their formulations. Recently, there has been an increased interest in less processed protein sources to satisfy marketing and consumer demands (Beaton, 2017; Food Processing, 2018; Wall, 2018a; Wall, 2018b). Recently, it was shown that true AA digestibility and digestible indispensable AA score (**DIAAS**) values of beef topside steak was affected by cooking conditions (Hodgkinson et al., 2018). In that study, DIAAS was greater for raw, boiled, and pan-fried meat treatments (97% to 99%) than for roasted meat (91%) or grilled meat (80%). To our knowledge, the effects of processing on the nutrient and AA composition, true nutrient and AA digestibility, DIAAS, and energy content of chicken-based ingredients have not been evaluated.

The cecectomized rooster assay (**CRA**) has been used frequently as a model for measuring true nutrient and AA digestibility of feed ingredients, including those intended for pet foods (Parsons et al., 1982). The CRA has been used to evaluate animal-based ingredients (Johnson et al., 1998; Folador et al., 2006; Faber et al., 2010; Deng et al., 2016), plant-based ingredients (Parsons et al., 1982; Knapp et al., 2008; de Godoy et al., 2009), and raw diets (Kerr et al., 2013). Data collected from the CRA has been shown to have similar AA digestibilities and response patterns to that of ileal-cannulated dogs (Johnson et al., 1998). Like ileal-cannulated animals, the CRA accurately estimates AA digestibility because it minimizes the bacterial fermentation of proteins in the hindgut that adds a source of error in the calculations (Gross et al., 2000). Another benefit of the CRA is the flexibility in type of ingredients that can be tested, varying from complete diets to most commonly using individual ingredients; this differs from most canine and feline studies that require the feeding of complete and balanced diets for longer-periods of time. Nutrient digestibility also impacts the overall energy content of an ingredient or diet, impacting food intake of animals and feeding guidelines determined for complete diets. Therefore, conventional roosters are often used to determine the nitrogen-corrected true metabolizable energy (**TMEn**) of novel ingredients for use in human and pet foods (Knapp et al., 2008; Deng et al., 2016).

Meat, meat and bone, pork, chicken, fish, calamari, lamb, venison, duck, and alligator meals were previously evaluated using the CRA (Folador et al., 2006; Faber et al., 2010; Deng et al., 2016), but chicken-based ingredients differing by processing method have not been included. Given the popularity of chicken-based ingredients and minimally processed and raw diets, research is needed to determine how the temperature and processing method affects nutrient digestibility and energy content. Therefore, the purpose of this study was to determine the chemical composition, nutrient digestibility, TMEn and DIAAS-like values of chicken-based ingredients that had undergone different processing conditions (i.e., chicken meal, raw chicken, retorted chicken, and steamed chicken) intended for use in dog and cat foods using the precision-fed CRA. We hypothesized that the digestibility would be highest for raw chicken, followed by steamed and retorted chicken, and lowest for chicken meal.

MATERIALS AND METHODS

Substrates

Four chicken-based ingredients, including raw chicken (frozen), steamed chicken (cooked to ~93 °C and held for 10 min at ~93 °C, cooled, and frozen), retorted chicken (retorted at ~121 °C for 30 min, cooled, and frozen), and rendered and dried chicken meal, were evaluated in this study. These ingredients were provided by Freshpet (Bethlehem, PA) and intended for use in commercial dog and cat foods. All ingredients were treated in a manner that is consistent with their typical pet food processing procedures. Before analysis, frozen ingredients were lyophilized (Dura-Dry MP microprocessor-controlled freeze-dryer; FTS Systems, Stone Ridge, NY) and ground through a 2-mm screen (Wiley mill model 4; Thomas Scientific, Swedesboro, NJ).

Cecectomized Rooster Assay

The protocol for the CRA, including all animal housing, handling, and surgical procedures, was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign prior to experimentation. Two precision-fed rooster assays utilizing cecectomized Single Comb White Leghorn roosters were conducted as described by Parsons (1985) to determine the true nutrient digestibility, standardized AA digestibility, and TMEn content of the four ingredients per diets tested. Prior to the study, cecectomy was performed on roosters under general anesthesia according to the procedures of Parsons (1985).

In the first rooster assay (to determine nutrient and AA digestibility), 16 cecectomized roosters were randomly assigned to the test ingredients (four roosters per test substrate evaluated). In the second rooster assay (to determine TMEn), 16 conventional roosters were randomly assigned to the test ingredients (four roosters per test substrate evaluated). In both assays, after 24 h of feed withdrawal, roosters were tube-fed 24 g of the test substrates. Following crop intubation, excreta were collected for 48 h on plastic trays placed under each individual cage. Excreta samples then were lyophilized, weighed, and ground through a 0.25-mm screen prior to analysis. Endogenous corrections for AA were made using five additional cecectomized roosters that had been fasted for 48 h. Standardized nutrient and AA digestibilities were calculated using the method described by Sibbald (1979).

Chemical Analyses

The substrates and rooster excreta were analyzed for DM (105 °C) and OM according to AOAC (2006). N and CP were measured using a Leco Nitrogen/Protein Determinator (Model FP-2000, Leco Corporation, St. Joseph, MI) according to the AOAC (2006; method 982.30E). Fat concentrations were determined by acid hydrolysis according to the AACC (1983) followed by diethyl ether extraction (Budde, 1952). Total dietary fiber (**TDF**) was determined according to Prosky et al. (1985). GE was measured using a bomb calorimeter (Model 1261; Parr Instrument Com., Moline, IL). AA was measured at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) according to the AOAC (2006; method 982.30E).

DIAAS-Like Calculations

Calculation of DIAAS-like values was performed according to Mathai et al. (2017). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation (FAO, 2011):

Digestible indispensable AA reference ratio = digestible indispensable AA content in 1 g protein of food (mg)/mg of the same dietary indispensable AA in 1 g of the reference protein.

The references used included the feline and canine nutrient recommendations suggested by AAFCO (2018) for (i) adult maintenance and (ii) growth and reproduction, and the recommended allowances suggested by the National Research Council (NRC, 2006) for (i) adult dogs at maintenance, (ii) adult cats at maintenance, (iii) growing puppies (4 to 14 wk old), and (iv) growing kittens. The DIAAS-like values were then calculated using the following equation adapted from FAO (2011):

DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein) / (mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

Nitrogen-Corrected TMEn Calculations

Calculation of TMEn was performed according to Parsons et al. (1992). The TMEn values, corrected for endogenous energy excretion using many fasted birds over many years, were calculated using the following equation:

$$TME_n\left(kcal/g\right) = EI_{fed} - (EE_{fed} \pm 8.22 * N_{fed}) \pm (EE_{fasted} \pm 8.22 * N_{fasted}) / FI$$

In that equation, EI_{fed} equals the GE intake of the test substrate consumed; EE_{fed} equals the energy in the excreta collected from fed birds; 8.22 is the correction factor for uric acid; N_{fed} equals the grams of N retained by the fed birds; EE_{fasted} equals the energy in the excreta collected from the fasted birds (16.74 kcal/g); N_{fasted} equals the g N retained by the fasted birds (1.1256 g); and FI equals the grams of dry test substrate consumed Parsons et al. (1982).

Statistical Analyses

All data were analyzed as a completely randomized design using the GLM procedure of Statistical Analysis Systems 9.3 (SAS Inst., Cary, NC). Substrates were considered to be a fixed effect. Tukey's multiple comparison analysis was used to separate the means when interaction effect was significant according to the procedures of SAS (SAS Inst. Inc., Cary, NC). Differences were considered significant with $P < 0.05$.

RESULTS

Chemical Composition

The chemical composition of tested ingredients is presented in Table 1. Of note, the DM content listed for the raw, steamed, and retorted chicken ingredients were the values present after the freeze-drying process, which was needed to properly conduct the chemical analyses and dosing for the rooster experiments. All other nutrients are represented on a DM basis (**DMB**). Ash content was fairly similar for raw chicken, steamed chicken, and retorted chicken (5.84% to 7.62% DMB), but higher in the chicken meal (16.29% DMB).

CP concentration increased as the processing of chicken products increased, most likely due to losses in fat. The chicken meal had the highest CP (67.42% DMB) and AA of all chicken-based ingredients, with raw chicken having the lowest, and retorted and steamed chicken being intermediate. The retorted chicken had the lowest TDF concentration (0.11% DMB), while chicken meal had the highest (6.65% DMB). Raw chicken, steamed chicken, and retorted chicken had similar acid-hydrolyzed fat (AHF) content (41.80% to 52.44% DMB) but was lower in the chicken meal (15.73% DMB).

The chicken meal had the lowest GE (5.1 kcal/g DM), with the raw, steamed, and retorted chicken-based ingredients being higher (6.6 to 7.0 kcal/g DM) due to greater AHF and CP concentrations. Concentrations of indispensable and dispensable AA are presented in Table 2. AA profile was similar among the protein sources. The chicken meal had higher AA concentrations, with the exception of histidine, lysine, methionine, and tryptophan, and raw chicken had lower AA composition. Retorted and steamed chicken had similar and higher concentrations of histidine, lysine, methionine, and tryptophan than raw chicken and chicken meal.

Cecectomized Rooster Assay

The nutrient digestibility for DM and OM was similar among raw chicken, steamed chicken, and retorted chicken (73.49% to 76.46% DM and 77.78% to 80.56% OM), and greater ($P < 0.01$) than chicken meal (60.05% DM and 65.87% OM; Table 1). TMEn values were higher for raw chicken, steamed chicken, and retorted chicken (5.34 to 5.92 kcal/g) than chicken meal and were not statistically different from each other. The chicken meal had the lowest caloric value (3.72 kcal/g) of all chicken-based ingredients ($P < 0.0001$). The TMEn expressed as a percentage of GE was higher ($P = 0.0158$) for raw chicken and retorted chicken than chicken meal, with steamed chicken being intermediate.

Standardized AA digestibility data are presented in Table 3. For all indispensable and dispensable AA, the steamed chicken had the highest digestibilities. In that ingredient, all indispensable AA had a digestibility greater than 88%. For all indispensable AA and the majority of the dispensable AA, raw and retorted chicken digestibilities were similar to one another and higher than that of chicken meal. Lysine, valine, histidine, and

Table 1. Chemical composition (%, DM basis), true macronutrient digestibility and nitrogen-corrected true ME (**TMEn**) of chicken-based ingredients using the precision-fed cecectomized rooster assay[1]

Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	*P*-value
Chemical composition						
CP, %	67.42	55.56	52.97	41.72	—	—
N, %	10.79	8.89	8.48	6.68	—	—
AHF, %	15.73	41.80	44.77	52.44	—	—
TDF, %	6.65	0.11	1.06	3.07	—	—
GE, kcal/g	5.09	6.68	6.59	6.98	—	—
Nutrient digestibility						
DM, %	60.05^{b}	73.49^{a}	76.46^{a}	75.91^{a}	2.329	0.0012
OM, %	65.87^{b}	77.78^{a}	80.56^{a}	80.51^{a}	1.927	0.0006
AHF, %	90.34	83.45	86.46	88.25	2.185	0.2711
Nitrogen-corrected true ME						
TMEn, kcal/g	3.72^{b}	5.52^{a}	5.34^{a}	5.92^{a}	0.126	<0.0001
TMEn/GE, %	73.14^{b}	82.68^{a}	81.05^{ab}	84.83^{a}	1.880	0.0158

[a,b]Means with different superscripts within a row differ ($P < 0.05$).

[1]$n = 4$ roosters per treatment.

Table 2. Concentrations (%, DM basis) of indispensable AA and select dispensable AA in chicken-based ingredients intended for pet foods

Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken
Indispensable AA				
Arginine, %	4.19	3.31	3.41	2.22
Histidine, %	1.36	1.66	1.55	1.02
Isoleucine, %	2.33	2.34	2.35	1.75
Leucine, %	4.17	3.91	3.94	2.87
Lysine, %	3.94	4.20	4.29	2.94
Methionine, %	1.13	1.26	1.29	0.87
Phenylalanine, %	2.25	1.95	2.00	1.42
Threonine, %	2.30	2.11	2.16	1.59
Tryptophan, %	0.53	0.56	0.58	0.44
Valine, %	2.88	2.51	2.53	1.88
Selected dispensable AA				
Alanine, %	4.23	3.12	3.08	2.01
Aspartic acid, %	4.86	4.40	4.49	3.22
Cysteine, %	0.69	0.42	0.52	0.36
Glutamic acid, %	7.68	6.89	6.88	4.57
Glycine, %	6.57	3.38	3.30	1.70
Proline, %	3.98	2.37	2.23	1.29
Serine, %	2.11	1.69	1.76	1.27
Tyrosine, %	1.41	2.09	1.89	1.24
Taurine, %	0.39	0.15	0.12	0.11

Table 3. Standardized digestibility (%) of indispensable AA and select dispensable AA of chicken-based ingredients using the precision-fed cecectomized rooster assay[1]

Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	*P*-value
Indispensable AA						
Arginine, %	85.56[b]	89.29[ab]	92.62[a]	88.89[ab]	1.166	0.0120
Histidine, %	76.94[c]	83.34[ab]	87.83[a]	79.75[bc]	1.355	0.0013
Isoleucine, %	82.30[b]	88.33[a]	91.90[a]	90.76[a]	0.885	0.0001
Leucine, %	82.90[b]	88.31[a]	92.10[a]	90.74[a]	0.917	0.0004
Lysine, %	78.78[b]	84.68[ab]	91.02[a]	86.57[ab]	2.038	0.0133
Methionine, %	85.95[c]	90.63[b]	94.79[a]	93.35[ab]	0.649	<0.0001
Phenylalanine, %	80.95[b]	86.68[a]	90.63[a]	88.84[a]	1.088	0.0016
Threonine, %	75.32[b]	82.94[a]	88.02[a]	84.64[a]	1.492	0.0011
Tryptophan, %	89.60[b]	93.78[a]	95.36[a]	94.24[a]	0.569	<0.0001
Valine, %	78.60[b]	84.97[a]	89.09[a]	86.27[a]	1.261	0.0017
Selected dispensable AA						
Alanine, %	82.34[b]	88.15[a]	91.73[a]	88.82[a]	1.124	0.0023
Aspartic acid, %	66.14[b]	73.30[b]	90.36[a]	88.85[a]	2.038	<0.0001
Cysteine, %	52.44[b]	52.90[ab]	68.37[a]	50.22[b]	3.443	0.0178
Glutamic acid, %	80.24[b]	87.20[a]	90.80[a]	87.41[a]	1.201	0.0006
Glycine, %	72.89[a]	73.56[a]	73.78[a]	57.58[b]	2.626	0.0040
Proline, %	77.09[b]	85.61[ab]	86.66[a]	77.64[b]	1.853	0.0117
Serine, %	71.02[b]	78.70[ab]	84.38[a]	79.67[ab]	1.896	0.0059
Tyrosine, %	78.20[c]	86.49[ab]	90.42[a]	84.18[b]	0.965	<0.0001

[a–c]Means with different superscripts within a row differ ($P < 0.05$).

[1]$n = 4$ roosters per treatment.

threonine had digestibilities <80% for chicken meal. For proline, the steamed chicken had a higher ($P = 0.0117$) digestibility than that of raw chicken and chicken meal, which were similar to one another. For glycine, the raw chicken had a lower ($P = 0.004$) digestibility than all other chicken-based ingredients. For aspartic acid, chicken meal and retorted chicken had a lower ($P < 0.0001$) digestibility than that of steamed chicken and raw chicken. Raw chicken had a higher digestibility of alanine (P = 0.0023), aspartic acid (P < 0.0001), and glutamic acid (P = 0.0006), but lower digestibility of cysteine (P = 0.0178), glycine (P = 0.004), proline (P = 0.0117), and tyrosine (P < 0.0001) compared to steamed chicken.

DIAAS-Like Calculations

DIAAS-like values for adult dogs and cats at maintenance are presented in Tables 4 and 6, respectively. DIAAS-like values for growing puppies and kittens are presented in Tables 5 and 7, respectively. Using these calculations, the first-limiting AA for adult dogs was methionine or tryptophan, depending on the ingredient.

Using the AAFCO recommended allowances for adult dogs, chicken meal was the only protein source that did not meet 100% DIAAS-like values for all AA (methionine, tryptophan, and threonine). Using the NRC recommended allowances for adult dogs, all protein sources had some DIAAS-like values <100%. Steamed chicken had the most DIAAS-like values for indispensable AA over 100% (arginine, histidine, isoleucine, leucine, and lysine), followed by raw chicken (arginine, histidine, isoleucine, and lysine), retorted chicken (arginine, histidine, and lysine), and chicken meal (arginine and lysine).

Using the AAFCO recommended allowances for canine growth and reproduction and NRC recommended allowances for growing puppies, the first-limiting AA was threonine for almost all protein sources. The exception was chicken meal when using the NRC recommended allowances a reference, where tryptophan was the first-limiting AA. Using AAFCO recommendations as a reference, steamed chicken and retorted chicken had DIAAS-like values below 100% for only threonine and phenylalanine, while raw chicken had DIAAS-like values below 100% for threonine, phenylalanine, and histidine. Chicken meal, however, had DIAAS-like values below 100% for seven indispensable AA, with only arginine, lysine, and valine being sufficient. Similar comparisons were observed when using NRC recommended allowances as a reference, with steamed chicken (threonine), raw chicken (threonine; tryptophan), and retorted chicken (threonine; tryptophan) having DIAAS-like values <100% for only one or two indispensable AA. Similar to using AAFCO references, chicken meal had DIAAS-like values below 100% for seven indispensable AA (only arginine, lysine, and valine were sufficient) when using NRC as a reference.

For adult cat AAFCO and NRC recommended allowance references, threonine from the chicken meal was the only DIAAS-like value <100%. Using the AAFCO recommended allowances for feline growth and reproduction, methionine was the first-limiting AA and had a DIAAS-like value <100% for chicken meal, retorted chicken, and raw chicken. When using AAFCO as a reference, tryptophan also had a DIAAS-like value <100% for chicken meal. Using NRC recommended allowances of growing kittens as a reference, methionine was the first-limiting AA and had a DIAAS-like value <100% for chicken meal. When using NRC as

Table 4. Digestible indispensable AA scores[1] values of chicken-based ingredients for adult dogs at maintenance[2]

	AAFCO					NRC				
Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM
Arginine, %	187.66[b]	187.75[b]	210.44[a]	166.94[c]	2.500	151.92[b]	151.99[b]	170.36[a]	135.14[c]	2.023
Histidine, %	147.03[c]	235.90[a]	243.47[a]	184.71[b]	3.255	81.68[c]	131.06[a]	135.26[a]	102.62[b]	1.808
Isoleucine, %	134.73[c]	176.21[b]	193.13[a]	180.34[b]	1.645	74.85[c]	97.89[b]	107.30[a]	100.19[b]	0.914
Leucine, %	135.73[c]	164.50[b]	181.35[a]	165.24[b]	1.636	75.41[c]	91.39[b]	100.75[a]	91.80[b]	0.909
Lysine, %	131.54[c]	182.90[b]	210.62[a]	174.30[b]	4.143	131.54[c]	182.90[b]	210.62[a]	174.30[b]	4.143
Methionine, %	78.58[d]	112.10[b]	125.91[a]	106.17[c]	0.721	43.65[d]	62.28[b]	69.95[a]	58.98[c]	0.401
Phenylalanine, %	108.06[c]	121.69[b]	136.87[a]	120.94[b]	1.518	60.03[c]	67.61[b]	76.04[a]	67.19[b]	0.843
Threonine, %	96.36[c]	118.12[b]	134.59[a]	120.97[b]	2.083	59.76[c]	73.25[b]	83.47[a]	75.01[b]	1.292
Tryptophan, %	79.24[d]	106.34[c]	117.46[a]	111.82[b]	0.633	50.31[d]	67.51[c]	74.58[a]	70.99[b]	0.401
Valine, %	123.34[c]	141.01[b]	156.32[a]	142.81[b]	2.082	68.52[c]	78.34[b]	86.84[a]	79.34[b]	1.157

[a–d]Means with different superscripts within a row and guidelines (AAFCO or NRC) differ (P < 0.05); n = 4 roosters per treatment.

[1]DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

[2]DIAAS-like values were calculated from the ileal digestibility of AA in cecectomized roosters and Association of American Feed Control Officials (AAFCO, 2018) recommended allowances and National Research Council (NRC, 2006) minimal requirements of AA for adult dogs at maintenance. The indispensable AA reference patterns are expressed as gram AA per kilogram DM.

Table 5. Digestible indispensable AA scores[1] values of chicken-based ingredients for growing puppies after weaning[2]

	AAFCO					NRC				
Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM
Arginine, %	119.65[b]	119.70[b]	134.17[a]	106.43[c]	1.595	151.44[b]	151.51[b]	169.82[a]	134.72[c]	2.018
Histidine, %	79.35[c]	127.30[a]	131.39[a]	99.67[b]	1.757	89.55[c]	143.69[a]	148.30[a]	112.50[b]	1.983
Isoleucine, %	90.12[c]	117.87[b]	129.19[a]	120.63[b]	1.102	98.46[c]	128.77[b]	141.14[a]	131.78[b]	1.203
Leucine, %	89.44[c]	108.40[b]	119.50[a]	108.89[b]	1.078	89.44[c]	108.40[b]	119.50[a]	108.89[b]	1.078
Lysine, %	115.09[c]	160.04[b]	184.29[a]	152.51[b]	3.625	117.71[c]	163.68[b]	188.49[a]	155.98[b]	3.709
Methionine, %	92.58[d]	132.09[b]	148.35[a]	125.10[c]	0.849	98.20[d]	140.10[b]	157.35[a]	132.69[c]	0.901
Phenylalanine, %	73.23[c]	82.46[b]	92.76[a]	81.96[b]	1.029	93.51[c]	105.30[b]	118.44[a]	104.66[b]	1.314
Threonine, %	55.59[c]	68.15[b]	77.65[a]	69.79[b]	1.202	71.38[c]	87.50[b]	99.70[a]	89.60[b]	1.543
Tryptophan, %	79.24[d]	106.34[c]	117.46[a]	111.82[b]	0.633	68.92[d]	92.49[c]	102.16[a]	97.25[b]	0.550
Valine, %	111.10[c]	127.02[b]	140.81[a]	128.64[b]	1.876	111.10[c]	127.02[b]	140.81[a]	128.64[b]	1.876

[a–d]Means with different superscripts within a row and guidelines (AAFCO or NRC) differ ($P < 0.05$); $n = 4$ roosters per treatment.

[1]DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

[2]DIAAS-like values were calculated from the ileal digestibility of AA in cecectomized roosters and Association of American Feed Control Officials (AAFCO, 2018) recommended allowances of AA for canine growth and reproduction and National Research Council (NRC, 2006) minimal requirements of AA for growing puppies after weaning. The indispensable AA reference patterns are expressed as gram AA per kilogram DM.

Table 6. Digestible indispensable amino acid scores[1] values of chicken-based ingredients for adult cats at maintenance[2]

	AAFCO					NRC				
Item	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM
Arginine, %	132.92[b]	132.99[b]	149.07[a]	118.24[c]	1.772	138.11[b]	138.17[b]	154.87[a]	122.85[c]	1.840
Histidine, %	130.17[c]	208.84[a]	215.55[a]	163.52[b]	2.882	119.38[c]	191.54[a]	197.69[a]	149.98[b]	2.642
Isoleucine, %	142.21[c]	186.00[b]	203.86[a]	190.36[b]	1.737	132.29[c]	173.02[b]	189.64[a]	177.08[b]	1.616
Leucine, %	107.52[c]	130.30[b]	143.64[a]	130.89[b]	1.296	100.54[c]	121.85[b]	134.33[a]	122.40[b]	1.212
Lysine, %	144.21[c]	200.53[b]	230.92[a]	191.10[b]	4.543	270.81[c]	376.57[b]	433.64[a]	358.85[b]	8.530
Methionine, %	187.28[d]	267.18[b]	300.09[a]	253.06[c]	1.718	169.48[d]	241.79[b]	271.57[a]	229.01[c]	1.554
Phenylalanine, %	175.59[c]	197.74[b]	222.41[a]	196.54[b]	2.467	135.07[c]	152.11[b]	171.09[a]	151.18[b]	1.898
Threonine, %	91.52[c]	112.18[b]	127.83[a]	114.89[b]	1.978	98.83[c]	121.15[b]	138.04[a]	124.07[b]	2.136
Tryptophan, %	114.45[d]	153.60[c]	169.67[a]	161.51[b]	0.913	108.36[d]	145.42[c]	160.63[a]	152.91[b]	0.865
Valine, %	140.80[c]	160.98[b]	178.45[a]	163.03[b]	2.377	131.67[c]	150.54[b]	166.88[a]	152.45[b]	2.223

[a–d]Means with different superscripts within a row and guidelines (AAFCO or NRC) differ ($P < 0.05$); $n = 4$ roosters per treatment.

[1]DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1g of the minimum protein recommendation)].

[2]DIAAS-like values were calculated from the ileal digestibility of AA in cecectomized roosters and Association of American Feed Control Officials (AAFCO, 2018) recommended allowances and National Research Council (NRC, 2006) minimal requirements of AA for adult cats at maintenance. The indispensable AA reference patterns are expressed as gram AA per kilogram DM.

a reference, threonine also had a DIAAS-like value <100% for chicken meal. All other DIAAS-like values were above 100%.

In general, steamed chicken had the highest ($P < 0.0001$) and chicken meal had the lowest ($P < 0.0001$) DIAAS-like values for all indispensable AA, while retorted chicken and raw chicken had intermediate values. The one exception was arginine, whereby raw chicken typically had the lowest ($P < 0.0001$) DIAAS-like value, but was still above 100% in all protein sources.

DISCUSSION

Pet owners have become more interested in raw and less-processed ingredients and diets recently (Schlesinger and Joffe, 2011; Freeman et al., 2013; Parr and Remillard, 2014; Wall, 2018b; Wall, 2018a). As a result, pet food companies have started the commercialization of raw foods and diets using mild processing methods (Parr and Remillard, 2014). It is known that ash content and processing temperature can affect AA digestibility (Kondos and

Table 7. Digestible indispensable AA scores values[1] of chicken-based ingredients for growing kittens after weaning[2]

Item	AAFCO					NRC				
	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM	Chicken meal	Retorted chicken	Steamed chicken	Raw chicken	SEM
Arginine, %	128.65[b]	128.71[b]	144.27[a]	114.44[c]	1.714	138.47[b]	138.53[b]	155.28[a]	123.17[c]	1.845
Histidine, %	141.09[c]	226.37[a]	233.63[a]	177.25[b]	3.123	117.57[c]	188.64[a]	194.70[a]	147.70[b]	2.602
Isoleucine, %	152.35[c]	199.25[b]	218.38[a]	203.92[b]	1.861	131.68[c]	172.22[b]	188.76[a]	176.26[b]	1.609
Leucine, %	120.17[c]	145.64[b]	160.55[a]	146.30[b]	1.448	100.15[c]	121.38[b]	133.80[a]	121.92[b]	1.206
Lysine, %	115.09[c]	160.04[b]	184.29[a]	152.51[b]	3.625	135.40[c]	188.28[b]	216.81[a]	179.43[b]	4.266
Methionine, %	69.69[d]	99.43[b]	111.68[a]	94.17[c]	0.640	81.85[d]	116.78[b]	131.15[a]	110.60[c]	0.750
Phenylalanine, %	155.88[c]	175.54[b]	197.45[a]	174.48[b]	2.189	135.07[c]	152.11[b]	171.09[a]	151.18[b]	1.898
Threonine, %	105.61[c]	129.46[b]	147.52[a]	132.58[b]	2.283	98.83[c]	121.15[b]	138.04[a]	124.07[b]	2.136
Tryptophan, %	84.52[d]	113.43[c]	125.30[a]	119.27[b]	0.675	110.05[d]	147.69[c]	163.14[a]	155.30[b]	0.878
Valine, %	157.41[c]	179.97[b]	199.50[a]	182.26[b]	2.658	131.15[c]	149.95[b]	166.22[a]	151.86[b]	2.214

[a–d]Means with different superscripts within a row and guidelines (AAFCO or NRC) differ ($P < 0.05$); $n = 4$ roosters per treatment.

[1]DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

[2]DIAAS-like values were calculated from the ileal digestibility of AA in cecectomized roosters and Association of American Feed Control Officials (AAFCO, 2018) recommended allowances of AA for feline growth and reproduction and National Research Council (NRC, 2006) minimal requirements of AA for growing kittens after weaning. The indispensable AA reference patterns are expressed as gram AA per kilogram DM.

McClymont, 1974; Batterham et al., 1986; Johnson et al., 1998; Hodgkinson et al., 2018). Processing temperature has been shown to greatly affect AA digestibility in animal meals, with high temperatures (≥150 °C) reducing digestibility (Kondos and McClymont, 1974; Batterham et al., 1986; Wang et al., 1997; Johnson et al., 1998; Hodgkinson et al., 2018). Protein denaturation in meat begins at 70 °C and, at 100 °C the oxidation of protein forms aggregates, leading to reduced AA digestibility (Bax et al., 2012). This occurs because the myofibrillar proteins bound with the AA form carbonyl groups on the side chains of arginine, lysine, and proline (Santé-Lhoutellier et al., 2008; Lund et al., 2011), disulfide cross-linkages in S-containing AA (cysteine and methionine), and di-tyrosine cross linkages (Lund et al., 2011). These changes are thought to have negative effects on enzyme action, decreasing AA digestibility. Additionally, cooking meat at 90 °C for 30 min was shown to have lower true ileal digestibility of protein than cooking at 55 °C for 5 min (90.1% vs. 94.1%; $P = 0.08$) (Oberli et al., 2015), which demonstrates that even with lower cooking temperatures (<150 °C), it is possible to have losses in protein digestibility. The response to ash content appears to be more variable; however, it did not have a negative effect on AA digestibility in roosters (Johnson et al., 1998) or protein digestibility in pigs (Partanen, 1994). Because of differences in raw materials and processing method, the AA composition may be variable among animal protein sources, within and across animal species and ingredient categories.

In the current study, the chicken meal had the highest ash, CP, and TDF content and the lowest AHF. This was probably due to the processing of this ingredient, where the fat is extracted to be sold as chicken fat. Compared with the chicken meal evaluated by Deng et al. (2016), this protein source had a similar chemical composition, but the ash content was higher in the present study (16.29% vs. 11.8% DMB), perhaps because the animal flesh of the chicken meal in the present study had more pieces of bone compared with that tested in the Deng study. Additionally, while the nutrient digestibility was similar, DM (64.5% vs. 60.05%) and OM (75.7% vs. 65.87%) digestibilities were slightly higher in the Deng et al. (2016) study. The chemical composition of chicken breast evaluated by Faber et al. (2010) had lower ash and AHF, but higher CP than all of the chicken-based products tested in the present study. However, it is important to note that the chicken breast used on Faber et al. (2010) was a prime meat cut, used for human grade, and the ingredients used in the present study may add other parts of the chicken carcass or meat cuts besides the chicken breast, with could explain the differences in the chemical composition.

The TMEn value of the chicken meal (3.72 kcal/g) in the present study was similar to that of the chicken meal (3.49 kcal/g) reported by Deng et al. (2016) and low-ash poultry byproduct meal (PBP) (3.805 kcal/g) reported by Johnson et al. (1998). Furthermore, the retorted chicken (5.52 kcal/g) in the present study had similar TMEn values to the ground chicken (5.53 kcal/g) reported by Kerr et al.

(2014). The variation in energy content among protein sources may result from the processing procedures of producing meals, including cooking, pressing, drying, and milling (Deng et al., 2016). High-energy and high-quality protein sources are important when formulating diets because it allows for a lower amount of protein compared to those containing low energy and/or AA concentrations.

Compared with the concentrations of all indispensable AA reported by Deng et al. (2016) for chicken meal and by Johnson et al. (1998) for high- and low-ash PBP, the chicken meal tested in the present study was similar. However, the chicken breast from the Faber et al. (2010) study had higher indispensable AA concentrations than all of the chicken-based ingredients tested in the present study. The ground chicken reported by Kerr et al. (2014) had similar concentrations of indispensable AA with the chicken meal, retorted chicken, and steamed chicken tested in the present study. For the dispensable AA, the chicken meal tested by Deng et al. (2016) had similar concentrations of aspartic acid, glutamic acid, and glycine to the chicken meal tested in the present study. The PBP with high-ash content tested by Johnson et al. (1998) study had a similar concentration of alanine, glycine, and tyrosine to the chicken meal tested in the present study, and the low-ash PBP had similar concentrations of alanine and proline compared to the chicken meal tested in the present study. The chicken breast tested by Faber et al., (2010) had higher concentrations of the majority of the dispensable indispensable AA, but lower taurine than all of the chicken-based ingredients tested in the present study. Additionally, glycine and proline concentrations were similar to the retorted and steamed chicken, but lower than the chicken meal tested in the present study.

Donadelli et al. (2018) used chicks and the protein efficiency ratio (**PER**) assay, which ranks protein source based on AA composition (Cramer et al., 2007), to evaluate protein-based ingredients intended for pet foods. In that study, diets contained 10% CP from a novel protein source and demonstrated that spray-dried egg (**SDEG**), spray-dried inedible whole egg and low-temperature and pressure fluid bed dried chicken (**LTPC**) had the highest PER, low-temperature fluid bed air-dried chicken (**LTCK**) and spray-dried chicken (**SDCK**) were intermediate, and the chicken by-product meal (**CBPM**) and chicken meal (**CKML**) were the lowest. Additionally, CBPM had lower methionine than SDEG and CKML had lower tryptophan. Phenylalanine was the limiting AA for LTCK and SDCK, and while valine was limiting for LTPC (Donadelli et al., 2018). Similar to our study, chicken meal had the lowest performance of all animal protein sources tested, which suggests that the processing by which the chicken meal is subjected to negatively affects the protein quality of this ingredient.

Nutrient composition does not correlate with in vivo digestibility (Moughan, 1999; Ravindran and Bryden, 1999; Butts et al., 2012), stressing the need for animal testing. Testing nutrient digestibility of protein sources is needed to verify adequacy and improve diet quality. The CRA or ileal-cannulated dogs may be used to evaluate the quality of proteins without the influence of gut microbiota in the large intestine. Ileal cannulation in cats is not recommended due to complications with cannulation, including displacement and leakage, with subsequent abscess and skin inflammation, and difficulty in obtaining sufficient sample size (0.5 mL sample of ileal fluid requires ~3 h) (Mawby et al., 1999). Because of the issues with cannulation in cats and the cost and animal welfare concerns pertaining to ileal-cannulated dogs, the cecectomized rooster is a popular model and appropriate alternative. Additionally, a previous study reported a high correlation between cecectomized rooster and ileal-cannulated dog data (Johnson et al., 1998).

For all AA in the current study, the digestibility was highest for steamed chicken. Each AA had a digestibility >90% in steamed chicken, except for two dispensable AA (cysteine and glycine). Therefore, it appears that steamed chicken was the most easily hydrolyzed and absorbed. In contrast, standardized indispensable AA digestibility data were lowest for chicken meal.

When compared with the digestibility data of chicken meal reported by Deng et al. (2016), the chicken meal in the current study had similar responses, but higher isoleucine, lysine, methionine, tryptophan, and glutamic acid digestibilities. The ground chicken reported by Kerr et al. (2014) had similar AA digestibilities to the steamed chicken in the present study, with the exceptions of histidine and threonine that were more digestible in the ground chicken. Compared with the high- and low-ash PBP reported by Johnson et al. (1998), all AA digestibilities with the exception of cysteine, glycine, and serine were higher in chicken meal from the present study, but similar to PBP with high-ash content. It was reported that increased ash content had a negative effect on AA digestibility and protein efficient ratio (Cramer et al., 2007). In agreement with that study, the chicken meal in the current study, which had the greatest ash content among all ingredients, had the lowest indispensable and dispensable AA digestibilities. The variability of digestibility among different chicken meals can be due to the different tissues included in the meals, processing methods, variation in analytical procedures, experimental design, and animal models.

The digestibility of the chicken breast reported by Faber et al. (2010) was similar to the retorted and raw chicken digestibility of the present study. However, cysteine had a much lower digestibility value in the present study (50.2% raw chicken and 52.9% retorted chicken) compared to that of Faber et al. (2010; 82.0% chicken breast). Cysteine is a component of keratin and other fibrous proteins, and typically has a lower digestibility (Kerr et al., 2014). One must consider the low digestibility of cysteine (range from 50.22% to 68.37% in present study) and low methionine DIAAS-like value for chicken meal (78.10) when formulating diets, especially when using meals that have undergone rendering and drying processes due to the fact that cysteine is supported by the metabolism of methionine (Weichselbaum et al., 1932). If an ingredient has a low cysteine digestibility plus a methionine DIAAS-like value that does not meet 100%, supplementation of methionine or cysteine may be required to prevent deficiency.

Recent studies have supported the use of DIAAS-like values to estimate protein quality of ingredients and diets for humans (Mathai et al., (2017) and we believe that this an appropriate method to score AA and determine protein quality of ingredients used in pet foods. Using the DIAAS-like values, our data suggest that if chicken meal is used as the only source of protein in a diet formulation, it may not provide sufficient methionine (DIAAS-like value = 78.58%), tryptophan (DIAAS-like value = 79.24%), and threonine (DIAAS-like value = 96.36%) when adult dogs, and threonine (DIAAS-like value = 91.52%) for adult cats if diets are formulated to meet the AAFCO recommendations. If NRC recommendations are used as a reference, chicken meal only provides sufficient arginine (DIAAS-like value = 151.92%) and lysine (DIAAS-like value = 131.54%) for adult dogs, and does not provide sufficient threonine (DIAAS-like value = 98.83%) for adult cats. For puppies, chicken meal was also the lowest quality protein source, only providing sufficient (over 100% DIAAS-like value) for arginine (DIAAS-like value = 119.65% and 151.44% for AAFCO and NRC references, respectively), lysine (DIAAS-like value = 115.09% and 117.71% for AAFCO and NRC references, respectively), and valine (DIAAS-like value = 111.10% for AAFCO and NRC). Similar data were observed when using growing kittens as a reference, with chicken meal having DIAAS-like values below 100% for methionine (81.85%) and threonine (98.83%) when compared to NRC recommendations. When compared to AAFCO recommendations, chicken meal (69.69%), raw chicken (94.17%), and retorted chicken (99.43%) did not meet sufficient (DIAAS-like values of at least 100%) for methionine, and chicken meal did not meet 100% for tryptophan (DIAAS-like value = 84.52%). Stated another way, our data indicate that if the chicken meal has a low digestibility, it may not meet the minimal recommendations for indispensable AA without supplementation, especially if the diet is formulated to meet the minimal protein requirement for the dog or cat. Although many consider animal-based proteins to be complete proteins not requiring additional supplementation, our data demonstrate that supplementation may be required when formulating with meals that have undergone extensive heat processing.

Our results corroborate those of a recent study evaluating how cooking conditions affect the protein quality of beef topside steak, as indicated by true ileal digestible AA and DIAAS determined using a pig model (Hodgkinson et al., 2018). Similar to our study, the less processed protein (boiled: ≤80 °C intermediate cooking duration; pain-fried: 186 °C and shortest duration compared with the other meat protein sources) had greater ileal digestible AA concentrations than the more processed proteins (roasted: 160 °C and longest cooking duration; grilled: 225 °C and intermediate cooking duration). In that study, valine was the limiting AA, with DIAAS values for boiled (99%), pan-fried (98%) and raw meat (97%) being higher than roasted meat (91%) and grilled meat (80%).

Steamed chicken appears to be the best option of those tested in the current study, followed by raw chicken and retorted chicken. This evidence suggests that cooking provides benefits over raw chicken because of greater AA availability. Even though the raw chicken tested had intermediate digestibilities, it was a good source of indispensable AA.

In conclusion, this study provides the true nutrient digestibility data of four chicken-based ingredients intended for use in dog and cat foods. To our knowledge, it is also the first study to use DIAAS-like values to predict protein quality for use in pet food. The protein sources varied greatly in ash, CP, N, and fat content. According to our data, the chicken meal has the lowest nutrient and AA digestibilities and may not be sufficient if used as the sole protein source, when the cat or dog is fed to meet the minimum protein recommendations of AAFCO or NRC. In contrast, AA digestibility was highest for steamed chicken, out-performing all other protein sources tested. AA digestibilities of raw and retorted chicken-based proteins were slightly lower than that of steamed chicken, but still high-quality proteins. This study demonstrates the importance of in vivo testing to evaluate protein-based ingredients, as raw material and processing methods can greatly affect their protein quality and energy content. Our study also justifies the use of DIAAS-like calculations to evaluate protein-based ingredients for use in pet foods and complete diets because they not only consider AA profile but also digestibility data and species-specific nutrient recommendations. Future studies should evaluate the nutrient digestibility of these ingredients as the main protein ingredients in complete and balanced diets for dogs and cats.

Conflict of interest statement. None declared.

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