Digestible indispensable amino acid scores of animal and plant ingredients potentially used in dog diet formulation: how this protein quality metric is affected by ingredient characteristics and reference amino acid profile

James R. Templeman,†,‡,1, and Anna K. Shoveller†,‡

1Department of Animal Biosciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada
‡Current address: Primal Pet Foods, Primal Pet Group, Fairfield, CA 94534, USA
†Corresponding author: jtemplem@uoguelph.ca

Abstract
The ability of a diet or an ingredient to satisfy the indispensable amino acid (IAA) requirements of an individual is a reflection of protein quality (PQ). The concept of PQ is gaining recognition in the pet food industry as a way to identify candidate ingredients for diet formulation. The objective of this report was to use IAA digestibility data from swine and ceccectomized rooster assays to generate digestible IAA scores (defined herein as DIAAS-like values) to predict the PQ of ingredients used in dog diets. However, as PQ equation development relies on a reference IAA profile, which is intended to be based on the physiological requirements of a specific population, we sought to generate DIAAS-like values using IAA requirements established by the National Research Council (NRC) as well as practical IAA recommendations presented by the Association of American Feed Control Officials (AAFCO) and European Pet Food Industry Federation (FEDIAF), to assess how these profiles may affect PQ. In total, 30 animal (75 unique inputs) and 27 plant ingredients (94 unique inputs) satisfied all inclusion criteria to be used in the final data set. Ingredients were initially categorized as animal or plant, and further categorized based on AAFCO Official Common and Usual Names and Definitions of Feed Ingredients to allow for additional, more distinct comparisons to be made. Data were analyzed using PROC GLIMMIX in SAS, with ingredient reference as a random effect, and ingredient category, regulatory body, and life stage as fixed effects. As expected, differences were observed in DIAAS-like values for nearly all ingredients and ingredient categories when determined using NRC, AAFCO, or FEDIAF IAA requirements or recommendations as the reference pattern. Moreover, applying reference patterns based on NRC adult maintenance IAA requirements consistently produced the lowest DIAAS-like values. Ultimately, while future studies assessing PQ should utilize NRC minimal requirements, individual ingredient and ingredient category differences in DIAAS-like values when using AAFCO and FEDIAF recommendations underpin the different regulatory approaches to establish dietary nutrient recommendations that exist globally and support the need for harmonization of dietary recommendations.

Lay Summary
Indispensable amino acids (IAA) are nutrients a dog requires, but that the dog cannot create naturally, so must be included in their diet. A concept known as protein quality (PQ) reflects how closely the IAA profile of an ingredient comes to meeting the dogs’ requirements. The focus of this report was to use IAA data from literature to predict PQ using equations to determine digestible indispensable amino acid scores (defined herein as DIAAS-like values) of ingredients used in dog diets. Additionally, as various resources exist that define dogs’ IAA needs differently, either based on physiological requirements or practical recommendations, we assessed how these differences may affect PQ. Ultimately, while PQ should be assessed utilizing physiological requirements of the population of interest, individual ingredient and ingredient category differences in DIAAS-like values when using practical recommendations highlight the different regulatory approaches to developing these recommendations.

Key words: amino acids, digestible indispensable amino acid score, pet food, protein quality

Abbreviations: AA, amino acid; AAA, aromatic amino acids (Phe + Tyr); AAFCO, Association of American Feed Control Officials; AM, adult maintenance; CP, crude protein; DIAAS, digestible indispensable amino acid score; DDGS, distillers dried grain with solubles; DM, dry matter; EG, early growth; FAO, Food and Agriculture Organization; FEDIAF, European Pet Food Industry Federation; GR, growth and reproduction; IAA, indispensable amino acid; LAA, limiting amino acid; LG, late growth; MR, minimal requirement; NRC, National Research Council; PDCAAS, protein digestibility-corrected amino acid score; PQ, protein quality; RA, recommended allowance; SAA, sulfur amino acids (Met + Cys)

Introduction
Dogs, like other mammals, do not have a requirement for protein per se, but rather have a requirement for indispensable amino acids (IAA) and sufficient dispensable amino acids (AA) that a dietary source of protein is comprised of. These IAA cannot be endogenously synthesized by the dog, and thus must be supplied in the diet to support protein synthesis as well as a variety of secondary metabolic functions. The ability of a diet (e.g., a mixture of protein sources) or an individual protein-containing ingredient to satisfy the IAA...
requirements of an individual is a reflection of protein quality (PQ; Nosworthy and House, 2017). A concept known as AA bioavailability is fundamental to the determination of PQ and is defined as the proportion of AA liberated from a protein source and absorbed in a metabolically available form for body protein maintenance and/or growth (Batterham, 1992; Stein et al., 2007a). However, to holistically evaluate the nutritional value of a diet or protein-containing ingredient, IAA bioavailability must be considered in combination with the concentration of IAA present in that diet or ingredient (Prolla et al., 2013). For any protein source, the IAA present in the lowest concentration relative to the subject’s biological requirements is defined as the limiting AA (LAA), which ultimately determines the rate of metabolic utilization of all other IAA present, and thus is directly related to the assessment of PQ (Fuller and Reeds, 1998).

In human nutrition, the concept of PQ is well recognized and widely applied to facilitate guidance on the extensive range of food items humans consume. As such, the Food and Agriculture Organization (FAO) has developed a criterion to collect and compile measures of PQ, which include mathematical equations such as the protein digestibility-corrected AA score (PDCAAS) and the digestible indispensable AA score (DIAAS). These equations allow for PQ of a protein-containing food or ingredient to be calculated using existing data in the literature on physiological IAA requirements of a reference population as well as the IAA content and true total tract protein digestibility (for the PDCAAS equation) or true ileal AA digestibility (for the DIAAS equation) of that ingredient (FAO, 2013). Indeed, it is important to note that measures of AA digestibility are used as a proxy for AA bioavailability. Furthermore, it is essential to recognize that true total tract protein digestibility does not account for differences in individual AA digestibility. Due to the inherent issues related to the use of total tract protein digestibility and potential for overestimation of AA bioavailability by using protein digestibility as an approximation of all AA bioavailability (Darragh et al., 1994; Murray et al., 1997; Stein et al., 2007a; Columbus et al., 2014), the DIAAS method (using ileal AA digestibility data) is considered to be a more accurate assessment of PQ (FAO, 2013).

In the pet food industry, the concept of PQ is gaining recognition as a way in which to identify and select ingredients for diet formulation. Though, while it is recognized that the bioavailability of AA should be estimated using true ileal AA digestibility data, due to the ethical and economical restraints surrounding the measurement of ileal digestibility in dogs, there remains a dearth of literature to support the generation of PQ equations such as DIAAS for dogs. Swine are acknowledged as excellent models for the digestive capacity and metabolism of AA in humans (Moughan and Rowan, 1989; Rowan et al., 1994; Deglaire et al., 2009), and in fact, data generated from swine ileal AA digestibility studies are used to supplement the published data available in humans for the generation of the DIAAS PQ equation. However, the pet food industry remains largely reliant on canine total tract protein digestibility data as well as estimations of dietary protein and AA bioavailability presented by the National Research Council (Nutrient Requirements for Dogs and Cats; NRC, 2006) as well as the North American (Association of American Feed Control Officials; AAFCO, 2016) and European (European Pet Food Industry Federation; FEDIAF, 2018) pet food regulatory bodies, even though these estimations have been criticized for a potential lack of scientific credibility and accuracy (Morris and Rogers, 1994; Hendriks et al., 2015). As such, the primary objective of this report was to use IAA digestibility data generated from comparative species that share similar digestive anatomy and protein/AA metabolism with the domestic dog, specifically swine (Harrison et al., 1990) and the cecectomized rooster (Johnson et al., 1998), to generate DIAAS-like values to predict the PQ of protein-containing ingredients used by the pet food industry. To accomplish this, the authors assessed the applicable literature related to swine ileal AA digestibility and cecectomized rooster model AA digestibility data for plant and animal protein-containing ingredients used for dog diet formulation.

It should be noted, though, that since the DIAAS equation requires the IAA content and ileal AA digestibility coefficients for a test diet or test ingredient along with the IAA requirements of a reference population to compare to (FAO, 2013), the PQ (DIAAS) of an ingredient may be affected by the reference population selected. Previously, as per a joint FAO/World Health Organization/United Nations University report (FAO/WHO/UNU, 1985), United States regulations stipulated that IAA requirements of preschool children aged 2 to 5 be used as a reference population for the evaluation of PQ for all age groups, aside from infants. However, following a more recent evaluation of these recommendations, the FAO now recommends that reference patterns based on IAA requirements of 0.5-yr-old infants and 3 to 10-yr-old children be used to calculate PQ metrics, such as DIAAS, for young children (0.5 to 3 yr of age) and older children/adolescents/adults (3+ yr of age), respectively (FAO, 2013). As dogs are typically fed a single complete diet that has been formulated to meet or exceed the IAA requirements of the physiological stage the diet is intended for (gestation/lactation/growth or adult maintenance), it is logical that steps are taken to ensure that estimations of PQ for dogs are made using life stage-specific IAA requirements as a reference pattern.

Nevertheless, for the pet food industry to appropriately adopt PQ metrics such as DIAAS, it is essential that the same procedures are followed when utilizing these equations as are in the human food industry (FAO, 2013). Specifically, when calculating DIAAS for individual protein sources, the most appropriate IAA reference patterns to apply are those based on physiological requirement estimates (e.g., children aged 3 to 10 yr; FAO, 2013). In the case of dogs, this would refer to IAA reference patterns based on minimal requirement (MR) estimates established by the NRC (2006) for adult dogs as well as for growing puppies. However, in recent years, a number of reports have been published wherein DIAAS for protein ingredients potentially used in commercial dog diet formulation have been calculated using reference patterns based on physiological IAA requirements from the NRC as well as practical dietary IAA recommendations from pet food regulatory bodies, such as AAFCO (Oba et al., 2019; Do et al., 2020; Reilly et al., 2020a, 2021; Gomez et al., 2021). It should be noted, though, that for each of these aforementioned reports, DIAAS values generated were referred to as “DIAAS-like” values, and the DIAAS equations utilized were adapted from the equation defined by the FAO (2013) so as to capture the fact that the IAA reference pattern utilized was generated using the “minimum protein recommendation” (Oba et al., 2019) rather than a “reference protein” (FAO, 2013).
Therefore, the secondary objective was to similarly generate DIAAS-like values for the ingredients of interest based on each reference population established by the NRC (adult maintenance, growing puppies 4 to 14 wk old, growing puppies 14 wk and older), as well as those defined by AAFCO (adult maintenance, growth, and reproduction) and FEDIAF (adult maintenance, early growth, and late growth). This was undertaken to establish how choice of regulatory body and/or life stage may affect the interpretation of an ingredient’s PQ while also offering the opportunity to provide quantitative data that DIAAS-like values generated using reference patterns based on NRC physiological requirement estimates will most closely reflect the DIAAS PQ metric recommended by the FAO (2013). We hypothesize that given the differences in crude protein (CP) and IAA requirements/recommendations from NRC, AAFCO, and FEDIAF, applying IAA reference patterns based on adult maintenance specifications will result in lower DIAAS-like values compared with those calculated using growth specifications, and that DIAAS-like values calculated using reference patterns based on NRC will be the lowest, followed by those calculated using AAFCO and then FEDIAF. Ultimately, the data generated will help to facilitate guidance on the formulation of dog diets with the intention of more precisely meeting the protein and IAA requirements or recommendations of the intended population by allowing for quantitative selection of protein-containing ingredients based on PQ predictions.

Materials and Methods

Data collection

In order to be captured in the final data set, all ingredients had to fulfill the following requirements: 1) have accessible literature describing moisture or dry matter (DM) content, CP content, and IAA profile (including Tyr and Cys for assessment of total aromatic AA (AAA) and total sulfur AA (SAA), respectively) so as to present IAA data with units of mg/g CP; 2) have accessible literature describing the standardized ileal digestibility coefficients from swine or digestibility coefficients from cecectomized roosters for each IAA; 3) have a CP content of ≥8% on a DM basis; and 4) be considered as a source of animal or plant-derived protein, where they can then be further classified/categorized based on the Official Common and Usual Names and Definitions of Feed Ingredients as established by AAFCO (2016).

It is acknowledged that while the IAA digestibility coefficients included in this report from swine are based on standardized ileal digestibility measurements (Stein et al., 2007a), the cecectomized rooster assay data are based on true digestibility values determined using methodologies described by Sibbald (1979) and do not eliminate the minor contributions of colonic digestion, fermentation, and metabolism in the rooster. As such, the approach of determining true AA digestibility with the cecectomized rooster model differs from that determining true “ileal” digestibility commonly applied in swine and murine models (Stein et al., 2007b). These methodologies may yield marginally different results, yet both assays are considered to be comparable in nature to the ileally cannulated dog (Harrison et al., 1990; Johnson et al., 1998).

Ingredient classification

In total, 30 animal (75 unique inputs from literature) and 27 plant ingredients (94 unique inputs from literature) satisfied all inclusion criteria and were captured in the final data set. For clarity, “casein” would be considered a single animal ingredient with three unique inputs, as IAA data were obtained from three unique sources (NRC, 2012; Rojas and Stein, 2012; CVB Feed Table, 2016). After being initially identified as “animal” or “plant,” all ingredients were then further categorized based on “collective,” “broads,” and “specific” AAFCO classifications, as defined using AAFCO (2016) Official Common and Usual Names and Definitions of Feed Ingredients (Tables 1 and 2 for animal and plant ingredients, respectively). It should be noted that, when necessary, common names were utilized when “specifically” defining certain ingredients (e.g., “dried pea”; Table 2). For a more detailed breakdown of the ingredient classification, refer to Supplementary Material S1.

PQ calculations—reference IAA patterns

Nutritional data (e.g., CP content, IAA profiles, and IAA digestibility) were gathered for the purpose of generating ingredient-specific DIAAS-like values. This metric necessitates a “reference pattern” to compare the digestible IAA content of each ingredient to, and as such, DIAAS-like values were determined using estimations of physiological IAA requirements presented by the NRC (2006) as well as the practical dietary IAA recommendations presented by the North American (AAFCO, 2016) and European (FEDIAF, 2018) pet food regulatory bodies.

Moreover, given that these organizations have presented estimated IAA requirements (or practical dietary IAA recommendations) for a variety of canine life stages, discrete reference IAA patterns were compiled for each of these, including adult maintenance (AM; NRC, 2006; AAFCO, 2016; FEDIAF, 2018); early growth (EG; 4 to 14 wk of age, NRC, 2006; less than 14 wk of age, FEDIAF, 2018); late growth (LG; greater than 14 wk of age, NRC, 2006; FEDIAF, 2018); and growth and reproduction (GR; AAFCO, 2016). These IAA requirements/recommendations (presented on a g per 100 g DM basis) as well as the IIAA reference patterns (transformed to and presented on a mg/g CP basis) are outlined in Table 3 and the latter was used for the determination of DIAAS-like values.

However, as DIAAS is intended to be established by comparing the physiological requirement of an IAA to the bioavailability (digestibility, in this case) of that IAA in a particular dietary protein source (FAO, 2013), the model IAA reference patterns to be utilized for dogs are those based on MR estimates by the NRC (2006) for adult dogs and growing puppies. With regard to the dietary recommended allowance (RA) estimates proposed by the NRC (2006), these are determined by multiplying the MR estimates by a factor of 1.25, which accounts for an average protein/IAA digestibility in a commercial diet of 80%. As this factor is used to transform the MR estimates for both IIAA and CP (e.g., adult CP MR of 8%, adult CP RA of 10%; NRC, 2006), the net result is that the MR and RA estimates of the NRC (2006) will yield the same IAA reference patterns (see Table 3) and thus the same DIAAS.

For AAFCO and FEDIAF, recommendations for minimum levels at which IAA should be present in practical, commercial pet foods for dogs are essentially the RA estimates established by the NRC (e.g., account for average digestibility of 80%), aside from select instances where additional scaling factors have been implemented. For example, the
FEDIAF adult maintenance IAA recommendations are NRC RA estimates increased by 20% to account for lower estimated energy requirements of household dogs compared to the energy intake assumed by NRC (FEDIAF, 2018). However, while CP recommendations from AAFCO and FEDIAF do also account for digestibility of the dietary protein sources, they are determined differently from IAA. For example, both regulatory bodies adopted a CP recommendation for adult dogs of 18%, which is based on the estimated MR of 8% presented by the NRC scaled up to account for requirements of older/senior dogs (upwards of 50%), lower energy intakes for household dogs (difference of 20%), as well as the average/assumed CP digestibility in a commercial diet of 80% (AAFCO, 2016; FEDIAF, 2018).

In brief, what this suggests is that, yes, using reference IAA patterns based on these AAFCO or FEDIAF recommendations will generate different DIAAS-like values than those determined using NRC MR estimates; however, these data must be interpreted with caution as these reference patterns no longer represent the physiological requirements of the dog, but rather account for assumed digestibility of the protein source. That being acknowledged, with the propensity of the pet food industry to rely on the recommendations presented by AAFCO and FEDIAF for commercial formulation purposes, and with the recent literature assessing PQ of ingredients intended for dog diet formulation using reference IAA patterns based on these regulatory bodies (Oba et al., 2019; Do et al., 2020; Reilly et al., 2020a, 2020b, 2021; Gomez et al., 2021), it may be valuable to assess the extent to which using these reference patterns may affect the PQ interpretation.

### PQ calculations—DIAAS-like values

DIAAS-like values were calculated using equations adapted from the FAO (2013):

$$\text{Digestible IAA reference ratio} = \frac{\text{mg digestible IAA}}{\text{1 g test ingredient crude protein}}$$

$$\frac{\text{mg same IAA}}{\text{1 g crude protein requirement}}$$

(1)
This equation produced a digestible IAA reference ratio for each IAA for a given reference IAA pattern; however, the DIAAS-like values reported herein for each ingredient were determined using the following equation wherein the lowest calculated digestible IAA reference ratio, which corresponds to the first or most LAA in the protein, is expressed as a percentage:

\[
\text{DIAAS – like} \% = 100 \times \text{lowest value} \\
\text{[digestible IAA reference ratio]}, \text{(2)}
\]

Moreover, in accordance with the FAO, certain DIAAS-like thresholds were used to distinguish between ingredients that could be considered as an “excellent” quality protein source (DIAAS of ≥100), a “good” quality protein source (DIAAS of between 75 and 99), or ingredients that cannot carry a PQ claim at all (DIAAS of <75; FAO, 2013).

Statistical analysis

All statistical analyses were performed using SAS (v. 9.4; SAS Institute Inc., Cary, NC, USA). For CP content, data were analyzed using PROC GLIMMIX of SAS with study (literature reference) of the nutritional data treated as a random effect, while “collective” AAFCO classification, “broad” AAFCO classification, regulatory body, and regulatory life stage as fixed effects. DIAAS-like data were analyzed using PROC GLIMMIX of SAS. The study (literature reference) responsible for the nutritional data was treated as a random effect, while collective AAFCO classification, broad AAFCO classification, “specific” AAFCO classification, regulatory body, regulatory life stage, and regulatory body*regulatory life stage were treated as fixed effects. For each of the aforementioned GLIMMIX procedures, when fixed effects were significant, means were separated using a Tukey adjustment. Statistical significance was declared at \( P \leq 0.05 \).

Results

All ingredients

DIAAS-like values for all ingredients are presented in Table 4 (animal ingredients) and Table 5 (plant ingredients) along with the LAA associated with each DIAAS-like value (Tables 6 and 7, respectively).

All ingredients—NRC reference patterns

For animal ingredients, when using NRC estimated minimal requirements for AM, EG, and LG as reference patterns, there were 0, 25, and 27 respective cases wherein DIAAS-like classification, regulatory body, and regulatory life stage as fixed effects. DIAAS-like data were analyzed using PROC GLIMMIX of SAS. The study (literature reference) responsible for the nutritional data was treated as a random effect, while collective AAFCO classification, broad AAFCO classification, “specific” AAFCO classification, regulatory body, regulatory life stage, and regulatory body*regulatory life stage were treated as fixed effects. For each of the aforementioned GLIMMIX procedures, when fixed effects were significant, means were separated using a Tukey adjustment. Statistical significance was declared at \( P \leq 0.05 \).
Table 3. Crude protein content and indispensable amino acid requirements or recommendations as published by NRC, AAFCO, or FEDIAF for each discrete life stage presented on a percent of dry matter basis and the respective indispensable amino acid reference patterns presented on a mg/g crude protein basis

| NRC | AM | RA | MR | RM | RA | MD | MR | RM | RA | MD |
|-----|----|----|----|----|----|----|----|----|----|----|
|     | %  | mg/g | %  | mg/g | %  | mg/g | %  | mg/g | %  | mg/g |
| AM  | 18.00 | 10.00 | 22.50 | 14.00 | 17.50 | 18.00 | 22.50 | 18.00 | 25.00 | 20.00 |
| RA  | 0.28 | 0.35 | 0.79 | 0.63 | 0.66 | 0.51 | 0.70 | 0.54 | 0.45 | 0.59 |
| MR  | 0.30 | 0.38 | 0.65 | 0.52 | 0.50 | 0.38 | 0.52 | 0.38 | 0.52 | 0.59 |
| LG  | 0.54 | 0.68 | 0.73 | 0.52 | 0.65 | 0.68 | 0.73 | 0.68 | 0.73 | 0.73 |
|     | 0.28 | 0.35 | 0.79 | 0.63 | 0.66 | 0.51 | 0.70 | 0.54 | 0.45 | 0.59 |
|     | 0.30 | 0.38 | 0.65 | 0.52 | 0.50 | 0.38 | 0.52 | 0.38 | 0.52 | 0.59 |
|     | 0.54 | 0.68 | 0.73 | 0.52 | 0.65 | 0.68 | 0.73 | 0.68 | 0.73 | 0.73 |

| AAFCO | AM | GR | AM | EG | LG |
|-------|----|----|----|----|----|
| % DM  | 28.33 | 44.44 | 28.89 | 46.86 | 37.57 |
| mg/g CP | % DM | mg/g CP | % DM | mg/g CP | % DM |
| Arg   | 0.28 | 0.35 | 0.79 | 0.63 | 0.66 |
| His   | 0.30 | 0.38 | 0.65 | 0.52 | 0.50 |
| Leu   | 0.54 | 0.68 | 0.73 | 0.52 | 0.65 |
| Lys   | 0.28 | 0.35 | 0.79 | 0.63 | 0.66 |
| Phe   | 0.30 | 0.38 | 0.65 | 0.52 | 0.50 |
| Met   | 0.52 | 0.65 | 0.70 | 0.31 | 0.39 |
| Thr   | 0.34 | 0.43 | 0.65 | 0.81 | 0.63 |
| Trp   | 0.11 | 0.14 | 0.36 | 0.23 | 0.14 |
| Val   | 0.39 | 0.49 | 0.68 | 0.54 | 0.55 |

| FEDIAF | AM | EG | LG |
|--------|----|----|----|
| % DM  | 28.89 | 46.86 | 37.35 |
| mg/g CP | % DM | mg/g CP | % DM |
| Arg   | 0.28 | 0.35 | 0.79 |
| His   | 0.30 | 0.38 | 0.65 |
| Leu   | 0.54 | 0.68 | 0.73 |
| Lys   | 0.28 | 0.35 | 0.79 |
| Phe   | 0.30 | 0.38 | 0.65 |
| Met   | 0.52 | 0.65 | 0.70 |
| Thr   | 0.34 | 0.43 | 0.65 |
| Trp   | 0.11 | 0.14 | 0.36 |
| Val   | 0.39 | 0.49 | 0.68 |

1AM, adult maintenance (using 110 kcal/kg⁰.⁷⁵ for FEDIAF); EG, early growth (NRC, 4 to 14 wk of age; FEDIAF, less than 14 wk of age); LG, late growth (NRC and FEDAF, greater than 14 wk of age), GR, growth and reproduction (AAFCO only).
2MR, minimal requirement; RA, recommended allowance.
3Crude protein content and indispensable amino acid requirements presented on a % dry matter basis.
4Indispensable amino acid reference patterns presented on a mg/g crude protein basis (used for DIAAS-like value calculations).
5CP, crude protein; AAA, aromatic amino acids (Phe + Tyr); SAA, sulfur amino acids (Met + Cys).
6In this report, reference values for Met and Phe were calculated as well as reference values for total SAA and AAA, while the FAO (2013) only calculates reference values for the latter. Bolded used for the calculation of DIAAS-like values.
| Ingredient                      | NRC | AM | GR | EG | LG | AM | EG | LG |
|--------------------------------|-----|----|----|----|----|----|----|----|
| Variety Sort Type Ref1 AM2 EG LG AM GR AM EG LG |
| Bovine Beef Lung meal I 39 SAA 82 SAA 85 SAA 70 SAA 67 SAA 61 SAA 14 ILE 11 ILE 14 ILE 13 ILE 14 ILE |
| Bovine Beef Blood meal G 9 ILE 16 ILE 12 ILE 12 ILE 11 ILE 16 ILE 16 ILE 13 ILE 14 ILE 14 ILE |
| Bovine Beef Loin R 49 SAA 102 TRP 93 TRP 92 TRP 93 TRP 92 TRP 93 TRP 92 TRP 93 TRP 92 TRP 93 TRP 92 TRP |
| Bovine Beef Ground U 54 SAA 98 AR 102 TRP 98 AR 102 TRP 98 AR 102 TRP 98 AR 102 TRP 98 AR 102 TRP |
| Bovine Milk Casein H 48 SAA 95 ARG 88 ARG 87 ARG 88 ARG 87 ARG 88 ARG 87 ARG 88 ARG 87 ARG 88 ARG |
| Bovine Milk Casein G 40 SAA 84 SAA 86 ARG 85 SAA 84 SAA 86 ARG 85 SAA 84 SAA 86 ARG 85 SAA 84 SAA 86 ARG |
| Bovine Milk Casein P 48 SAA 95 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG |
| Bovine Milk Whole P 48 SAA 95 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG |
| Bovine Milk Skimmed P 48 SAA 95 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG 90 ARG 93 ARG |
| Bovine Milk Whey protein concentrate O 68 MET 93 ARG 82 ARG 87 ARG 82 ARG 87 ARG 82 ARG 87 ARG 82 ARG 87 ARG 82 ARG |
| Marine N/A Meal H 46 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA 75 SAA |
| Marine N/A Meal G 45 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA |
| Marine N/A Meal K 45 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA |
| Marine N/A Meal P 45 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA 74 SAA |
| Poultry Chicken Meat M 37 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA 76 SAA |
| Poultry Chicken Meal M 30 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA |
| Poultry Chicken Neck meal M 30 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA 66 SAA |
| Poultry Chicken Breast R 47 SAA 98 THR 98 THR 98 THR 98 THR 98 THR 98 THR 98 THR 98 THR 98 THR 98 THR |
| Ingredient       | Variety | Sort     | Type       | Ref\(^1\) | AM\(^2\) | EG | LG   | AM  | GR  | AM  | EG  | LG  | NRC  | AAFCO | FEDIAF |
|------------------|---------|----------|------------|-----------|----------|----|------|-----|-----|-----|-----|-----|------|-------|--------|
| Poultry Egg Whole | S       | 50       | SAA        | 104       | SAA      | 106| THR  | 89  | SAA| 82 | THR | 77  | SAA | 115  | SAA | 118  | THR |
| Poultry N/A By-product meal I | 40      | SAA      | 83       | TRP       | 83       | TRP| 72   | SAA | 69 | THR | 62  | SAA | 90   | TRP  | 79   | TRP |
| Poultry N/A By-product meal I | 36      | SAA      | 74       | SAA       | 77       | SAA| 64   | SAA | 60 | THR | 55  | SAA | 83   | SAA | 87   | THR |
| Poultry N/A By-product meal V | 30      | SAA      | 53       | TRP       | 53       | TRP| 54   | SAA | 53 | THR | 47  | SAA | 57   | TRP  | 50   | TRP |
| Poultry N/A By-product meal G | 51      | TRP      | 70       | TRP       | 70       | TRP| 78   | TRP | 55 | THR | 73  | TRP | 76   | TRP  | 66   | TRP |
| Poultry N/A By-product meal V | 25      | SAA      | 37       | THR       | 37       | THR| 44   | SAA | 29 | THR | 38  | SAA | 41   | THR  | 40   | TRP |
| Poultry N/A Meal Y | 23      | SAA      | 48       | SAA       | 50       | SAA| 42   | SAA | 47 | THR | 36  | SAA | 54   | SAA | 49   | TRP |
| Poultry N/A Feather meal P | 12      | MET      | 26       | MET       | 27       | MET| 22   | MET | 26 | MET | 19  | MET | 29   | MET  | 31   | MET |
| Poultry N/A Feather meal K | 16      | MET      | 34       | MET       | 35       | MET| 29   | MET | 33 | HIS | 24  | MET | 38   | MET  | 41   | MET |
| Poultry N/A Feather meal Y | 16      | MET      | 23       | LYS       | 22       | LYS| 25   | LYS | 22 | LYS | 24  | MET | 25   | LYS  | 25   | LYS |
| Poultry N/A Feather meal H | 17      | MET      | 33       | LYS       | 35       | LYS| 29   | MET | 29 | HIS | 25  | MET | 36   | HIS  | 40   | LYS |
| Poultry N/A Feather meal V | 13      | MET      | 28       | MET       | 29       | MET| 24   | MET | 28 | MET | 20  | MET | 31   | MET  | 34   | MET |
| Poultry N/A Feather meal V | 16      | MET      | 34       | MET       | 35       | MET| 29   | MET | 34 | MET | 24  | MET | 37   | MET  | 40   | MET |
| Poultry N/A Feather meal J | 30      | TRP      | 41       | TRP       | 41       | TRP| 46   | TRP | 42 | HIS | 43  | TRP | 44   | TRP  | 39   | TRP |
| Poultry N/A Feather meal G | 14      | MET      | 29       | MET       | 31       | MET| 25   | MET | 29 | HIS | 21  | MET | 33   | MET  | 35   | MET |
| Poultry N/A Feather meal G | 17      | MET      | 35       | MET       | 36       | MET| 3    | MET | 35 | MET | 25  | MET | 39   | MET  | 42   | MET |
| Swine Pork Liver meal I | 56      | SAA      | 105      | THR       | 106      | THR| 101  | SAA | 82 | THR | 87  | SAA | 117  | THR  | 119  | THR |
| Swine Pork Lung meal I | 43      | SAA      | 90       | SAA       | 93       | SAA| 77   | SAA | 77 | THR | 67  | SAA | 99   | SAA  | 100  | TRP |
| Swine Pork Loin R | 40      | SAA      | 84       | SAA       | 87       | SAA| 73   | SAA | 70 | THR | 63  | SAA | 94   | SAA  | 86   | TRP |
| Ovine Lamb Meal I | 29      | SAA      | 60       | SAA       | 62       | SAA| 52   | SAA | 54 | THR | 45  | SAA | 67   | SAA  | 70   | SAA |
| Ovine Lamb Lung meal I | 36      | SAA      | 67       | TRP       | 67       | TRP| 64   | SAA | 70 | THR | 55  | SAA | 73   | TRP  | 64   | TRP |
| N/A N/A Meat and bone meal V | 31      | SAA      | 64       | SAA       | 66       | SAA| 55   | SAA | 58 | THR | 47  | SAA | 71   | SAA  | 63   | TRP |
| N/A N/A Meat and bone meal V | 20      | SAA      | 41       | SAA       | 43       | SAA| 36   | SAA | 40 | THR | 31  | SAA | 46   | SAA  | 42   | TRP |
| N/A N/A Meat and bone meal V | 26      | SAA      | 55       | SAA       | 57       | SAA| 47   | SAA | 52 | THR | 40  | SAA | 61   | SAA  | 58   | TRP |
| N/A N/A Meat and bone meal Y | 21      | SAA      | 43       | SAA       | 45       | SAA| 37   | SAA | 43 | SAA | 32  | SAA | 48   | SAA  | 51   | SAA |
| N/A N/A Meat and bone meal H | 26      | SAA      | 38       | TRP       | 38       | TRP| 42   | TRP | 42 | TRP | 40  | TRP | 41   | TRP  | 36   | TRP |
| N/A N/A Meat and bone meal G | 27      | SAA      | 57       | SAA       | 59       | SAA| 49   | SAA | 57 | SAA | 42  | SAA | 63   | SAA  | 60   | TRP |
| N/A N/A Meat and bone meal K | 28      | SAA      | 48       | TRP       | 48       | TRP| 50   | SAA | 54 | SAA | 43  | SAA | 52   | TRP  | 46   | TRP |
| N/A N/A Meat and bone meal P | 14      | TRP      | 19       | TRP       | 19       | TRP| 21   | TRP | 21 | TRP | 20  | TRP | 21   | TRP  | 18   | TRP |
| N/A N/A Bone meal P | 19      | TRP      | 25       | TRP       | 25       | TRP| 29   | TRP | 29 | TRP | 27  | TRP | 28   | TRP  | 24   | TRP |
| N/A N/A Meat meal P | 24      | SAA      | 49       | SAA       | 51       | SAA| 42   | SAA | 49 | SAA | 36  | SAA | 54   | SAA  | 49   | TRP |
| N/A N/A Meat meal H | 28      | SAA      | 54       | TRP       | 54       | TRP| 50   | SAA | 54 | THR | 43  | SAA | 59   | TRP  | 52   | TRP |
| N/A N/A Meat meal W | 27      | SAA      | 57       | SAA       | 58       | SAA| 49   | SAA | 44 | THR | 42  | SAA | 63   | SAA  | 64   | THR |
| N/A N/A Meat meal V | 30      | SAA      | 62       | SAA       | 64       | SAA| 53   | SAA | 56 | THR | 46  | SAA | 69   | SAA  | 61   | TRP |
| N/A N/A Meat meal V | 22      | SAA      | 46       | SAA       | 48       | SAA| 40   | SAA | 40 | THR | 34  | SAA | 51   | SAA  | 52   | TRP |
| N/A N/A Blood meal P | 24      | ILE      | 31       | ILE       | 32       | ILE| 43   | ILE | 29 | ILE | 36  | ILE | 35   | ILE  | 36   | ILE |
values of between 75 and 99 ("good" quality protein source) were achieved, and there were 0, 3, and 4 respective cases wherein DIAAS-like values of ≥100 ("excellent" quality protein source) were achieved (Table 4). The most prevalent LAA when using NRC requirements for AM, EG, and LG as reference IAA patterns were SAA (53 instances), SAA (31 instances), and Trp (19 instances), respectively (Table 6).

For plant ingredients, when using NRC requirements for AM, EG, and LG as reference IAA patterns, there were 0, 3, and 3 respective cases wherein DIAAS-like values of between 75 and 99 were achieved, and there were 0, 3, and 3 respective cases wherein DIAAS-like values of ≥100 were achieved (Table 5). The most prevalent LAA when using NRC requirements for AM, EG, and LG as reference IAA patterns were Met (49 instances), Thr (25 instances), and Lys (23 instances), respectively (Table 7).

All ingredients—AAFCO reference patterns

For animal ingredients, when using AAFCO recommendations for AM and GR as reference IAA patterns, there were 24 and 17 respective cases wherein DIAAS-like values of 75 to 99 were achieved, and there were 2 and 0 respective cases wherein DIAAS-like values of ≥100 were achieved (Table 4). The most prevalent LAA when using AAFCO (2016) requirements for AM and GR as reference IAA patterns were SAA (50 instances) and Thr (38 instances), respectively (Table 6).

For plant ingredients, when using AAFCO recommendations for AM and GR as reference IAA patterns, there were 16 and 5 respective cases wherein DIAAS-like values of 75 to 99 were achieved, and there were 0 and 0 respective cases wherein DIAAS-like values of ≥100 were achieved (Table 5). The most prevalent LAA when using AAFCO recommendations for AM and GR as reference IAA patterns were Met (45 instances) and Thr (46 instances), respectively (Table 7).

All ingredients—FEDIAF reference patterns

For animal ingredients, when using FEDIAF recommendations for AM, EG, and LG as reference IAA patterns, there were 13, 18, and 27 respective cases wherein DIAAS-like values of between 75 and 99 were achieved, and there were 1, 14, and 4 respective cases wherein DIAAS-like values of ≥100 were achieved (Table 4). The most prevalent LAA when using FEDIAF recommendations for AM, EG, and LG as reference IAA patterns were SAA (50 instances), SAA (23 instances), and Trp (38 instances), respectively (Table 6). For plant ingredients, when using FEDIAF recommendations for AM, EG, and LG as reference IAA patterns, there were 8, 24, and 24 respective cases wherein DIAAS-like values of between 75 and 99 were achieved, and there were 0, 4, and 4 respective cases wherein DIAAS-like values of ≥100 were achieved (Table 5). The most prevalent LAA when using FEDIAF recommendations for AM, EG, and LG as reference IAA patterns were Met (52 instances), Thr (23 instances), and Trp (34 instances), respectively (Table 7).

All ingredients—reference pattern comparison

Table 8 summarizes the DIAAS-like values for all ingredients combined as determined using NRC, AAFCO, and FEDIAF IAA requirements or recommendations at each life stage. When considering all ingredients together, regardless of plant or animal origin, the greatest DIAAS-like value was achieved using the FEDIAF EG reference pattern, while the
Table 5. Digestible indispensable amino acid scores (DIAAS)-like values and associated limiting amino acids (LAA) for plant ingredients (27 ingredients, 94 unique inputs) as determined using NRC, AAFCO, or FEDIAF amino acid requirements or recommendations at each discrete life stage as a reference pattern

| Ingredient          | Variety | Sort | Type | Ref² | AM² | EG  | LG  | AM¹  | EG  | LG  |
|---------------------|---------|------|------|------|-----|-----|-----|------|-----|-----|
| Grain Barley Whole  | H       | 44   | MET  | 67   | THR | 66  | LYS | 75   | LYS | 52  |
| Grain Barley Whole  | B       | 45   | MET  | 79   | THR | 79  | TRP | 80   | MET | 61  |
| Grain Barley Whole  | K       | 44   | MET  | 71   | THR | 71  | LYS | 79   | MET | 56  |
| Grain Barley Whole  | C       | 35   | MET  | 57   | THR | 57  | THR | 62   | MET | 44  |
| Grain Canola Meal   | H       | 33   | PHE  | 51   | PHE | 51  | PHE | 59   | PHE | 40  |
| Grain Canola Meal   | D       | 47   | MET  | 79   | THR | 80  | THR | 83   | MET | 62  |
| Grain Cottonseed Meal| H  | 22   | MET  | 35   | THR | 35  | THR | 39   | MET | 27  |
| Grain Cottonseed Meal| H  | 29   | MET  | 61   | MET | 60  | LYS | 52   | MET | 51  |
| Grain Canola Meal   | E       | 47   | MET  | 79   | THR | 80  | THR | 83   | MET | 62  |
| Grain Canola Meal   | B       | 23   | TRP  | 31   | TRP | 31  | TRP | 35   | TRP | 35  |
| Grain Canola Meal   | K       | 29   | TRP  | 40   | TRP | 39  | LYS | 45   | TRP | 39  |
| Grain Canola Meal   | P       | 32   | TRP  | 40   | LYS | 38  | LYS | 44   | LYS | 38  |
| Grain Corn Whole    | H       | 44   | TRP  | 57   | LYS | 56  | LYS | 64   | LYS | 56  |
| Grain Corn Whole    | J       | 48   | TRP  | 66   | LYS | 56  | LYS | 64   | LYS | 56  |
| Grain Corn Whole    | E       | 38   | TRP  | 53   | TRP | 53  | TRP | 59   | TRP | 57  |
| Grain Corn Whole    | C       | 36   | TRP  | 49   | TRP | 49  | TRP | 55   | TRP | 46  |
| Grain Corn Whole    | K       | 37   | TRP  | 51   | TRP | 51  | TRP | 58   | TRP | 58  |
| Grain Corn Whole    | P       | 38   | TRP  | 52   | TRP | 52  | TRP | 59   | TRP | 54  |
| Grain Corn DDGS     | E       | 39   | LYS  | 35   | LYS | 34  | LYS | 39   | LYS | 34  |
| Grain Corn DDGS     | H       | 41   | TRP  | 44   | LYS | 43  | LYS | 49   | LYS | 43  |
| Grain Flaxseed Meal | H       | 40   | PHE  | 55   | AAA | 56  | AAA | 73   | LYS | 62  |
| Grain Flaxseed Meal | D       | 26   | VAL  | 43   | VAL | 40  | VAL | 47   | VAL | 43  |
| Grain Flaxseed Meal | P       | 49   | SAA  | 59   | TRP | 59  | TRP | 59   | TRP | 59  |
| Grain Oat Whole     | H       | 45   | MET  | 74   | THR | 75  | THR | 80   | MET | 58  |
| Grain Oat Whole     | K       | 47   | MET  | 67   | THR | 68  | THR | 82   | MET | 52  |
| Grain Oat Whole     | P       | 42   | MET  | 73   | THR | 74  | THR | 75   | MET | 57  |
| Grain Oat Groats    | C       | 44   | TRP  | 60   | TRP | 60  | TRP | 68   | TRP | 58  |
| Grain Oat Groats    | H       | 36   | MET  | 65   | LYS | 63  | LYS | 63   | MET | 51  |
| Grain Oat Groats    | K       | 47   | MET  | 77   | THR | 78  | THR | 82   | MET | 60  |
| Grain Peanut Meal    | H       | 28   | MET  | 45   | LEU | 55  | LEU | 50   | MET | 43  |
| Grain Peanut Meal    | J       | 37   | MET  | 75   | THR | 76  | THR | 66   | MET | 58  |
| Ingredient                  | Variety  | Sort       | Type   | Ref1 | AM2 | EG | LG | AM3 | GR  | AM | EG | LG |
|----------------------------|----------|------------|--------|------|-----|----|----|-----|-----|----|----|----|
| Grain Rice Bran            | H        | 42         | SAA    | 73   | THR | 74 | THR| 76  | SAA | 57 | THR| 82 | THR|
| Grain Rice Bran            | B        | 44         | TRP    | 60   | TRP | 60 | TRP| 68  | TRP | 54 | THR| 86 | TRP|
| Grain Rice Whole           | H        | 58         | THR    | 68   | THR | 69 | THR| 92  | THR | 53 | THR| 86 | THR|
| Grain Rice Whole           | P        | 52         | SAA    | 75   | THR | 75 | THR| 93  | SAA | 58 | THR| 79 | THR|
| Grain Rice Whole           | P        | 60         | MET    | 97   | THR | 98 | THR| 106 | MET | 75 | THR| 89 | THR|
| Grain Rice Whole           | C        | 5          | TRP    | 73   | TRP | 73 | TRP| 82  | TRP | 58 | THR| 77 | THR|
| Grain Rice Whole           | H        | 25         | PHE    | 39   | PHE | 40 | PHE| 45  | PHE | 31 | PHE| 38 | PHE|
| Grain Rice Whole           | C        | 34         | MET    | 48   | THR | 49 | THR| 61  | MET | 38 | THR| 51 | THR|
| Grain Rice Whole           | K        | 39         | MET    | 69   | THR | 69 | THR| 69  | MET | 54 | THR| 57 | THR|
| Grain Rye Whole            | P        | 40         | MET    | 68   | THR | 69 | THR| 71  | MET | 53 | THR| 60 | THR|
| Grain Sorghum Whole        | H        | 41         | SAA    | 40   | LYS | 39 | LYS| 44  | LYS | 39 | LYS| 59 | LYS|
| Grain Sorghum Whole        | C        | 29         | TRP    | 37   | LYS | 35 | LYS| 41  | LYS | 35 | LYS| 41 | LYS|
| Grain Sorghum Whole        | K        | 41         | MET    | 44   | LYS | 43 | LYS| 49  | LYS | 43 | LYS| 61 | LYS|
| Grain Sorghum Whole        | P        | 49         | SAA    | 50   | LYS | 48 | LYS| 55  | LYS | 48 | LYS| 73 | LYS|
| Grain Sunflower Meal       | D        | 41         | SAA    | 68   | LYS | 66 | LYS| 74  | SAA | 58 | THR| 64 | SAA|
| Grain Sunflower Meal       | K        | 54         | SAA    | 74   | LYS | 72 | LYS| 83  | SAA | 64 | THR| 84 | SAA|
| Grain Wheat Bran           | H        | 32         | MET    | 65   | LYS | 63 | LYS| 57  | MET | 55 | THR| 48 | MET|
| Grain Wheat Bran           | J        | 37         | MET    | 67   | THR | 68 | THR| 65  | MET | 53 | THR| 54 | MET|
| Grain Wheat Bran           | B        | 33         | MET    | 53   | TRP | 53 | TRP| 59  | MET | 48 | THR| 49 | MET|
| Grain Wheat Bran           | K        | 36         | MET    | 57   | THR | 58 | THR| 63  | MET | 45 | THR| 53 | MET|
| Grain Wheat Middlings      | H        | 40         | MET    | 67   | THR | 68 | THR| 71  | MET | 53 | THR| 59 | MET|
| Grain Wheat Middlings      | K        | 39         | MET    | 64   | THR | 64 | THR| 69  | MET | 30 | THR| 58 | MET|
| Grain Wheat Middlings      | P        | 42         | MET    | 67   | THR | 67 | THR| 74  | MET | 52 | THR| 62 | MET|
| Grain Wheat DDGS           | H        | 29         | LYS    | 26   | LYS | 26 | LYS| 29  | LYS | 26 | LYS| 47 | LYS|
| Grain Wheat DDGS           | K        | 37         | LYS    | 33   | LYS | 32 | LYS| 37  | LYS | 32 | LYS| 59 | LYS|
| Grain Wheat Whole          | O        | 42         | MET    | 74   | THR | 75 | THR| 74  | MET | 58 | THR| 62 | MET|
| Grain Wheat Whole          | H        | 42         | MET    | 57   | LYS | 55 | LYS| 63  | LYS | 50 | THR| 62 | MET|
| Grain Wheat Whole          | B        | 50         | MET    | 69   | TRP | 69 | TRP| 77  | TRP | 57 | THR| 72 | TRP|
| Grain Wheat Whole          | C        | 42         | THR    | 49   | THR | 50 | THR| 60  | LYS | 38 | THR| 62 | THR|
| Grain Wheat Whole          | P        | 45         | MET    | 60   | LYS | 58 | LYS| 66  | LYS | 53 | THR| 66 | MET|
| Legume Soybean Meal        | H        | 38         | SAA    | 80   | SAA | 82 | SAA| 68  | MET | 72 | THR| 57 | MET|
| Legume Soybean Meal        | W        | 44         | TRP    | 60   | TRP | 60 | TRP| 68  | TRP | 68 | TRP| 63 | TRP|
| Legume Soybean Meal        | J        | 34         | MET    | 71   | MET | 73 | MET| 6   | MET | 71 | MET| 50 | MET|
| Legume Soybean Meal        | B        | 41         | MET    | 85   | MET | 88 | MET| 72  | MET | 81 | THR| 60 | MET|
| Legume Soybean Meal        | K        | 40         | MET    | 83   | MET | 86 | MET| 71  | MET | 75 | THR| 59 | MET|
| Legume Soybean Protein concentrate | H | 36         | SAA    | 75   | SAA | 77 | SAA| 64  | SAA | 72 | THR| 55 | SAA|

Table 5. Continued
Table 5. Continued

| Ingredient       | Sort | Type          | Ref | AM | EG | LG | AM | GR | AM | EG | LG |
|------------------|------|---------------|-----|----|----|----|----|----|----|----|----|
| Legume Soybean   | D    | Protein       |     | 26 | 42 | 39 | 46 | 41 | 39 | 46 | 45 |
| Legume Soybean   | J    | Protein       |     | 35 | 73 | 75 | 62 | 73 | 52 | 81 | 87 |
| Legume Lupin bean| B    | Whole         |     | 19 | 39 | 40 | 33 | 39 | 27 | 43 | 46 |
| Legume Fava bean | H    | Whole         |     | 16 | 34 | 35 | 28 | 34 | 24 | 37 | 40 |
| Legume Fava bean | K    | Whole         |     | 18 | 38 | 39 | 32 | 38 | 27 | 42 | 45 |
| Legume Fava bean | P    | Whole         |     | 16 | 34 | 35 | 29 | 34 | 24 | 38 | 40 |
| Legume Fava bean | P    | Whole         |     | 21 | 44 | 46 | 38 | 44 | 31 | 49 | 53 |
| Legume Pea       | K    | Whole         |     | 24 | 49 | 51 | 42 | 50 | 35 | 55 | 59 |
| Legume Pea       | P    | Whole         |     | 22 | 47 | 48 | 40 | 47 | 33 | 52 | 56 |
| Legume Pea       | H    | Whole         |     | 23 | 47 | 49 | 40 | 47 | 34 | 53 | 57 |
| Legume Pea       | F    | Whole         |     | 18 | 37 | 39 | 32 | 37 | 27 | 42 | 45 |
| Tuber Potato     | H    | Protein       |     | 58 | 109| TRP| 102| MET| 86 | 119| TRP|
| Tuber Potato     | D    | Protein       |     | 33 | 53 | VAL| 38 | VAL| 49 | 59 | 57 |
| Tuber Potato     | K    | Protein       |     | 47 | 92 | TRP| 85 | SAA| 73 | 99 | 88 |
| Tuber Potato     | P    | Protein       |     | 50 | 104| SAA| 90 | SAA| 77 | 1156| TRP|
| Tuber Yeast      | P    | Brewers       |     | 32 | 66 | SAA| 57 | SAA| 49 | 73 | 78 |
| Tuber Yeast      | K    | Brewers       |     | 32 | 59 | TRP| 57 | MET| 47 | 64 | 56 |
| Yeast Yeast      | H    | Brewers       |     | 25 | 32 | AAA| 45 | AAA| 39 | 33 | 37 |

1Reference of nutritional data (amino acid profile, crude protein content, amino acid digestibility) for each ingredient; H, NRC, 2012; B, Lee et al., 2020; K, Sauvant et al., 2004; C, Cervantes-Pahn et al., 2014; D, Cotten et al., 2016; E, Almeida et al., 2011; J, Leme et al., 2019; P, CVB Feed Table, 2016; Q, Mathai et al., 2017; W, Kong and Adeola, 2014; F, Grosjean et al., 2000.

2AM, adult maintenance (using 110 kcal/kg0.75 for FEDIAF); EG, early growth (NRC, 4 to 14 wk of age; FEDIAF, less than 14 wk of age); LG, late growth (NRC and FEDAF, greater than 14 wk of age); GR, growth and reproduction (AAFCO only).

3AAA, aromatic amino acids (Phe + Tyr); SAA, sulfur amino acids (Met + Cys). Bolded limiting amino acids are not necessarily limiting.
| AA     | NRC               | AAFCO          | FEDIAF          | Total |
|--------|------------------|----------------|-----------------|-------|
|        | AM   | EG   | LG   | AM   | GR   | AM   | EG   | LG   |       |
| Arg    | 0    | 12   | 14   | 4    | 14   | 2    | 12   | 14   | 72    |
| His    | 0    | 1    | 0    | 0    | 4    | 0    | 1    | 0    | 6     |
| Ile    | 5    | 7    | 7    | 5    | 7    | 5    | 7    | 7    | 30    |
| Leu    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     |
| Lys    | 0    | 1    | 2    | 1    | 1    | 0    | 1    | 2    | 8     |
| Phe    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     |
| AAA    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     |
| Met    | 13   | 6    | 6    | 10   | 4    | 13   | 6    | 6    | 64    |
| SAA    | 53   | 23   | 19   | 50   | 3    | 50   | 23   | 3    | 224   |
| Thr    | 0    | 7    | 8    | 0    | 38   | 0    | 5    | 5    | 63    |
| Trp    | 4    | 18   | 19   | 5    | 4    | 5    | 20   | 38   | 113   |
| Val    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     |

1NRC, National Research Council Nutrient Requirements of Dogs and Cats (2006); AAFCO, Association of American Feed Control Officials (2016); FEDIAF, European Pet Food Industry Federation Nutritional Guidelines for Complete and Complementary Pet Foods for Cats and Dogs (2018).

2AA, amino acid; AAA, aromatic amino acids (Phe + Tyr); SAA, sulfur amino acids (Met + Cys).

3AM, adult maintenance (using 110 kcal/kg<sup>0.75</sup> for FEDIAF); EG, early growth (NRC, 4 to 14 wk of age; FEDIAF, less than 14 wk of age); LG, late growth (NRC and FEDIAF, greater than 14 wk of age), GR, growth and reproduction (AAFCO only).
The lowest DIAAS-like value was achieved using NRC AM (P ≤ 0.05).

Animal ingredients—specific AAFCO classification
Table 9 summarizes the DIAAS-like values for the animal ingredients determined using NRC, AAFCO, and FEDIAF IAA requirements or recommendations at each life stage for the specific AAFCO ingredient category. No differences were observed for DIAAS-like values between any life stage (P > 0.05) for “blood meal”, “fish meal”, or “poultry meal”. For “animal by-product meal”, the greatest DIAAS-like values were achieved using NRC and FEDIAF EG and LG reference patterns, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). The FEDIAF EG reference pattern generated the greatest DIAAS-like values for “casein”, “dried milk”, “dried skimmed milk”, “meat”, “meat and bone meal”, and “meat meal”, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). For “egg product” and “poultry by-product meal”, the greatest DIAAS-like values were achieved using FEDIAF EG and LG reference patterns, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). The AAFCO and FEDIAF AM reference patterns produced the greatest DIAAS-like values for “dried whey concentrate”, while the lowest DIAAS-like values were achieved using AAFCO GR (P ≤ 0.05). For “dried whey”, the greatest DIAAS-like values were achieved using the AAFCO AM reference pattern, while the lowest DIAAS-like value was achieved using NRC AM (P ≤ 0.05). The greatest DIAAS-like value was achieved using NRC AM (P ≤ 0.05).

Plant ingredients—specific AAFCO classification
Table 10 summarizes the DIAAS-like values for the plant ingredients determined using NRC, AAFCO, and FEDIAF IAA requirements or recommendations at each life stage for the specific AAFCO ingredient category. For “barley grain” and “potato protein”, the greatest DIAAS-like values were achieved using FEDIAF EG reference patterns, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). For “brewers dried yeast”, the greatest DIAAS-like values were achieved using NRC EG and LG, AAFCO AM and GR, and FEDIAF EG and LG reference patterns, while the lowest DIAAS-like value was achieved using NRC AM (P ≤ 0.05). The greatest DIAAS-like values for “canola meal”, “linseed meal”, “oat grain”, “rice bran”, and “sunflower meal”,

| AA | NRC | AAFCO | FEDIAF | Total |
|----|-----|-------|--------|-------|
|    | AM  | EG    | LG     | AM    | GR    | AM    | EG    | LG     |
| Arg | 0   | 0     | 0      | 0     | 0     | 0     | 0     | 0      |
| His | 0   | 0     | 0      | 0     | 3     | 0     | 0     | 3      |
| Ile | 0   | 0     | 0      | 0     | 0     | 0     | 0     | 0      |
| Leu | 0   | 2     | 1      | 0     | 0     | 1     | 0     | 1      |
| Lys | 4   | 18    | 23     | 18    | 15    | 3     | 20    | 18     | 119    |
| Phe | 3   | 2     | 2      | 3     | 4     | 3     | 2     | 2      | 21     |
| AAA | 1   | 2     | 2      | 1     | 0     | 1     | 2     | 2      | 11     |
| Met | 49  | 18    | 17     | 45    | 15    | 52    | 18    | 14     | 228    |
| SAA | 15  | 5     | 5      | 9     | 2     | 9     | 5     | 4      | 54     |
| Thr | 2   | 25    | 22     | 1     | 46    | 2     | 23    | 16     | 137    |
| Trp | 17  | 19    | 19     | 14    | 5     | 21    | 20    | 34     | 149    |
| Val | 3   | 3     | 3      | 3     | 3     | 3     | 3     | 3      | 24     |

1NRC, National Research Council Nutrient Requirements of Dogs and Cats (2006); AAFCO, Association of American Feed Control Officials (2016); FEDIAF, European Pet Food Industry Federation Nutritional Guidelines for Complete and Complementary Pet Foods for Cats and Dogs (2018).

2AA, amino acid; AAA, aromatic amino acids (Phe + Tyr); SAA, sulfur amino acids (Met + Cys).

3AM, adult maintenance (using 110 kcal/kg0.75 for FEDIAF); EG, early growth (NRC, 4 to 14 wk of age; FEDIAF, less than 14 wk of age); LG, late growth (NRC and FEDAF, greater than 14 wk of age), GR, growth and reproduction (AAFCO only).
were achieved using AAFCO AM and FEDIAF EG and LG reference patterns, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). The greatest DIAAS-like values for “corn germ meal”, “corn gluten meal”, and “rice grain” were generated using AAFCO AM and FEDIAF AM and EG reference patterns, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). For “corn grain”, the greatest DIAAS-like values were produced using the AAFCO AM reference pattern, while the lowest DIAAS-like values were achieved using NRC AM (P ≤ 0.05). For “cottonseed meal”, “oat groats”, “soy protein concentrate”, “soybean meal”, and “wheat bran”, the greatest DIAAS-like values were achieved using FEDIAF EG and LG reference patterns, while the lowest DIAAS-like values were generated using NRC AM (P ≤ 0.05). For “DDGS”, the greatest DIAAS-like value was produced using NRC AM (P ≤ 0.05). In addition, “animal products” and “marine products” had greater CP (% DM basis) than “milk products” (P ≤ 0.05).

Supplementary Table S2 summarizes the DIAAS-like values for all broad AAFCO ingredient categories of plant ingredients determined using NRC, AAFCO, and FEDIAF IAA requirements or recommendations at each life stage. Briefly, differences were observed for DIAAS-like values and CP content for each broad AAFCO category, regardless of the reference pattern utilized. Refer to Supplementary Material S2 for a more detailed overview of the collective AAFCO ingredient category results.

Collective AAFCO ingredient classification

Supplementary Table S3 summarizes the DIAAS-like values for each collective AAFCO ingredient category of plant ingredients (“plant protein products”, “processed grain by-products”, “grain products”). In addition, this table summarizes the comparisons between the collective AAFCO ingredient categories of “animal protein products” and “plant protein products”. In brief, no differences were observed between the mean DIAAS-like values of “animal protein products” and “plant protein products”, regardless of the reference pattern (P > 0.05). Refer to Supplementary Material S3 for a more detailed breakdown of the collective AAFCO ingredient category results.

Discussion

The main objective of this report was to generate DIAAS-like values to predict the PQ of a variety of plant and animal...
Table 10. Least squares means (± SEM) for digestible indispensable amino acid score (DIAAS)-like values as determined using NRC, AAFCO, or FEDIAF amino acid requirements or recommendations at each discrete life stage as a reference pattern for plant ingredients categorized based on specific AAFCO Official Common and Usual Names and Definitions of Feed Ingredients

| Specific AAFCO classification | NRC² | EG | LG | AAFCO | AM | EG | LG | FEDIAF | AM | EG | LG | SEM | P-value |
|------------------------------|------|----|----|--------|----|----|----|---------|----|----|----|------|---------|
|                              | AM²  |    |    |        |    |    |    |         |    |    |    |      |         |
| Barley grain                 | 42.2³ | 68.3⁴ |     | 68.1³ |    |    |    | 74.0⁴ | 53.4³ |    |    | 3.92 | <0.01 |
| Brewers dried yeast          | 29.4³ | 52.1⁴ | 53.0⁴ | 74.0³ | 53.4³ |    |    | 44.6³ | 57.4³ | 56.6³ | 9.05 | <0.01 |
| Canola meal                  | 39.7³ | 64.9⁴ | 65.6⁴ | 70.9³ | 50.7⁴ |    |    | 59.2³ | 72.3³ | 73.9³ | 13.01| <0.01 |
| Corn germ meal               | 29.1³ | 41.5⁴ | 41.1⁴ | 44.4³ | 39.7³ |    |    | 43.2³ | 45.3³ | 41.7³ | 9.29 | 0.03  |
| Corn gluten meal             | 29.5³ | 35.8⁴ | 35.3⁴ | 40.0³ | 36.8⁴ |    |    | 43.9³ | 39.2³ | 36.1³ | 3.19 | 0.03  |
| Corn grain                   | 40.0³ | 54.7⁴ | 54.4⁴ | 61.4³ | 55.6³ |    |    | 57.9³ | 59.6³ | 52.5³ | 2.60 | <0.01 |
| Cottonseed meal              | 25.5³ | 47.6⁴ | 47.5⁴ | 45.3³ | 39.0³ |    |    | 37.8³ | 53.0³ | 53.8³ | 11.10| 0.04  |
| DDGS¹                        | 36.3³ | 34.6⁴ | 33.6⁴ | 38.5³ | 33.6³ |    |    | 38.5³ | 34.6³ | 33.6³ | 38.5³ | 3.66  |
| Dried bean                   | 17.7³ | 37.1³ | 38.5³ | 31.5³ | 37.1³ |    |    | 26.3³ | 41.3³ | 44.5³ | 1.68 | <0.01 |
| Dried pea                    | 22.7³ | 46.7³ | 47.8³ | 39.3³ | 46.7³ |    |    | 33.3³ | 51.2³ | 54.6³ | 2.15 | <0.01 |
| Linseed meal                 | 38.5³ | 58.5³ | 56.7³ | 68.7³ | 51.0³ |    |    | 58.0³ | 64.7³ | 65.3³ | 9.85 | <0.01 |
| Oat grain                    | 44.6³ | 71.6³ | 72.3³ | 79.1³ | 55.9³ |    |    | 66.2³ | 79.1³ | 80.7³ | 2.16 | <0.01 |
| Oat groats                   | 41.9³ | 67.4³ | 67.1³ | 71.0³ | 56.4³ |    |    | 61.1³ | 74.3³ | 72.1³ | 5.44 | <0.01 |
| Peanut meal                  | 32.5³ | 59.7³ | 65.3³ | 57.6³ | 51.4³ |    |    | 48.2³ | 66.4³ | 72.6³ | 10.34| <0.01 |
| Potato protein               | 45.0³ | 90.1³ | 90.9³ | 82.6³ | 86.8³ |    |    | 70.8³ | 99.2³ | 89.9³ | 10.93| <0.01 |
| Rice bran                    | 42.9³ | 66.5³ | 66.9³ | 71.8³ | 55.3³ |    |    | 64.3³ | 73.2³ | 69.7³ | 6.24 | 0.02  |
| Rice grain                   | 53.9³ | 76.4³ | 77.0³ | 91.6³ | 59.7³ |    |    | 80.9³ | 84.8³ | 80.1³ | 5.77 | <0.01 |
| Rye grain                    | 34.5³ | 55.9³ | 56.6³ | 61.4³ | 43.7³ |    |    | 51.4³ | 62.3³ | 59.7³ | 6.36 | <0.01 |
| Sorghum grain                | 39.6³ | 42.4³ | 41.2³ | 47.1³ | 41.2³ |    |    | 58.6³ | 46.8³ | 39.6³ | 3.80 | <0.01 |
| Soy protein concentrate      | 32.0³ | 62.9³ | 63.8³ | 57.2³ | 61.9³ |    |    | 48.5³ | 69.8³ | 73.0³ | 9.91 | <0.01 |
| Soybean meal                 | 39.2³ | 75.7³ | 78.0³ | 67.6³ | 73.1³ |    |    | 57.9³ | 83.8³ | 84.7³ | 4.23 | <0.01 |
| Sunflower meal               | 47.6³ | 71.3³ | 69.3³ | 78.2³ | 61.2³ |    |    | 73.8³ | 78.2³ | 79.2³ | 5.22 | <0.01 |
| Wheat bran                   | 34.4³ | 60.7³ | 60.5³ | 61.0³ | 50.0³ |    |    | 51.0³ | 67.1³ | 65.8³ | 3.21 | <0.01 |
| Wheat grain                  | 43.8³ | 61.6³ | 61.3³ | 68.0³ | 51.1³ |    |    | 64.7³ | 68.1³ | 65.0³ | 3.58 | <0.01 |
| Wheat middlings              | 40.0³ | 65.8³ | 66.5³ | 71.0³ | 54.2³ |    |    | 59.4³ | 73.3³ | 74.2³ | 1.20 | <0.01 |

¹NRC, National Research Council Nutrient Requirements of Dogs and Cats (2006); AAFCO, Association of American Feed Control Officials (2016); FEDIAF, European Pet Food Industry Federation Nutritional Guidelines for Complete and Complementary Pet Foods for Cats and Dogs (2018).

²AM, adult maintenance; EG, early growth (NRC, 4 to 14 wk of age; FEDIAF, less than 14 wk of age); LG, late growth (NRC and FEDIAF, greater than 14 wk of age), GR, growth and reproduction (AAFCO only).

³DDGS, distillers grain with solubles.

Values in a row with different superscript are significantly different (P ≤ 0.05).
protein-containing ingredients potentially used for dog diet formulation. However, as DIAAS development in the human food industry relies on a reference IAA pattern based on the physiological IAA requirements of a specific population of interest (FAO, 2013), the secondary objective was to similarly assess PQ for dogs using a DIAAS equation adapted from the FAO (2013) wherein life stage-specific reference IAA patterns are applied. Fundamentally, the most appropriate IAA reference patterns to apply for dogs are based on MR estimates established by the NRC (2006) for adult dogs and growing puppies, as these represent physiological IAA requirements. Though, based on the pet food industry’s reliance on practical dietary IAA recommendations presented by AAFCO and FEDIAF as well as the recent literature reporting “DIAAS-like” values for dog food ingredients calculated using reference patterns based on IAA recommendations from these regulatory bodies (Oba et al., 2019; Do et al., 2020; Reilly et al., 2020a, 2020b, 2021; Gomez et al., 2021), we sought to assess the degree to which using these reference patterns may affect the PQ interpretation.

As expected, the choice of IAA reference pattern can greatly affect the resulting DIAAS-like value, and thus the subsequent interpretation of PQ. Although, while it appears as though a substantial amount of variability within these values stem from the selection of regulatory body, it has to be acknowledged that for the pet food industry to validly adopt PQ metrics such as DIAAS, it is critical that the same procedures are followed when utilizing these equations as are in the human food industry (FAO, 2013). Essentially, only reference patterns based on MR estimates established by the NRC for adult dogs and growing puppies should be applied considering they are the only IAA reference patterns based on physiological IAA requirements. While recommendations presented by AAFCO or FEDIAF are widely recognized and utilized throughout the pet food industry for commercial diet formulation purposes, these recommendations all, to an extent, take into account an assumed digestibility of mixed protein sources. Ultimately, while data presented herein should be interpreted prudently, particularly the DIAAS-like values generated using reference patterns associated with AAFCO or FEDIAF recommendations, this report should also be used to initiate a deeper conversation around: 1) how these recommendations are developed, 2) how they relate to the estimated physiological requirements presented by the NRC, 3) why they may differ between North American and European regulatory bodies and whether global synchronization is a necessary next step, and 4) whether the estimated physiological requirements and/or scaling factors used to account for factors like digestibility are indeed accurate.

The NRC is an advisory board organized by the United States National Academy of Sciences that has established an ad hoc nutrition committee designated to compile literature pertaining to the nutrient requirements for both dogs and cats. In 2000, this committee was tasked with revising the respective 1985 and 1986 publications on Nutrient Requirements of Dogs and Nutrient Requirements of Cats into one single report, while also thoroughly examining any relevant literature published since those editions were made available. The new report, Nutrient Requirements of Dogs and Cats, was published in 2006 and included updated estimations for requirements for all nutrients, including the IAA. In North America, regulatory recommendations for pet food formulation are defined by AAFCO, while FEDIAF establishes the regulatory guidelines in Europe. As discussed earlier, the AAFCO and FEDIAF dietary recommendations for IAA are essentially based on RA estimations from the NRC (2006) that have been scaled up by a factor of 1.25 to account for an assumed protein digestibility in a practical diet of 80%. Moreover, in the case of FEDIAF (2018) recommendations for adult dogs, an additional increase of 20% is applied to account for lower estimated energy requirements of household dogs compared to the average energy intake assumed by NRC (2006). Considering the multitude of factors that may affect diet protein digestibility and/or energy requirements of adult dogs, it brings into question the validity and accuracy of these scaling factors. Moreover, while NRC estimated requirements of IAA for growing dogs are based on extrapolated dose-response data from nitrogen balance and/or growth performance studies, the NRC states that “no individual dose-response peer-reviewed reports could be found for the minimal requirements of any of these amino acids (His, Ile, Leu, Lys, Phe, Thr, Trp, and Val) for dogs for maintenance” (2006). In fact, the estimated requirements of those aforementioned IAA for adult dogs at maintenance are based only on the lowest concentrations reported in one doctoral dissertation and one peer-reviewed report wherein dogs were fed low CP diets for an extended period of time and displayed no observable clinical signs of IAA deficiency (Ward, 1976; Sanderson et al., 2001). So, essentially, these MR estimates for adult dogs do not account for gut endogenous IAA losses, while those established for growing dogs do. Nevertheless, the NRC (2006) has indicated that no diet that meets the MR for CP and that is based on cereal grains, animal by-products, and plant proteins have been shown to be deficient in any of the IAA mentioned above. While that declaration may be true, that manner of thinking does not necessarily facilitate the advancement of our understanding of precise and sustainable pet nutrition. Furthermore, there has been a recent inundation of data generated using more advanced methodologies, such as indicator AA oxidation techniques, that challenge the accuracy of current regulatory recommendations with regard to dietary Phe, Trp, Met, Thr, and Lys requirements for adult dogs (Shoveller et al., 2017; Mansilla et al., 2018, 2020a, 2020b; Templeman et al., 2019; Harrison et al., 2020; Sutherland et al., 2020). Based on the progression of research in canine nutrition since 2006, it is increasingly clear that amendments to companion animal nutrient requirements are overdue, which would undoubtedly affect the estimation of ingredient PQ and the interpretation of this PQ metric as it relates the adequacy of protein ingredients and application of these data to develop complementary protein combinations.

With each DIAAS-like value, there is an associated LAA, unless the DIAAS-like value is greater than 100, in which case the LAA may not actually limit protein synthesis if fed in adequate amounts. As expected, SAA, Met, and Lys (plant ingredients in particular for the latter) were commonly identified LAA, which is potentially less problematic with regard to diet formulation considering that dietary supplementation of anhydrous DL-Met and Lys is commonplace in the pet food industry. However, attention should be drawn to the frequency at which Trp and Thr were identified as LAA, as these IAA are rarely supplemented in their anhydrous forms to commercial pet food, even though they are often considered for supplementation in animal agriculture. It is well known that complementary protein sources can be included in the diet to counteract potential IAA deficiencies and improve the
PQ of the complete diet (Herreman et al., 2020). In fact, the FAO (2013) states that documentation of protein sources of greater PQ (higher DIAAS) with the intention of complementing proteins of lesser quality in mixed diets is one of the primary uses of this PQ model. This concept of complementary protein is vital in the human food industry, as humans typically consume a varied diet; however, ingredient complementarity is also fundamental in diet formulation for companion animals. With regard to diet formulation and/or production, the use of complementary protein sources helps to resolve difficulties related to protein ingredient sourcing and supply, a problem that has hampered the pet food industry globally, particularly in recent years (Hill, 2022). Moreover, as the trend of humanization continues to influence the pet food industry, the demand for products utilizing novel and alternative protein sources is increasing (Simonsen et al., 2014; Dodd et al., 2019; Schleicher et al., 2019), presenting an additional opportunity for the application of protein ingredient complementarity so as to ensure that diets formulated with less traditional protein sources provide sufficient levels of dietary nitrogen and all IAA. Nevertheless, it should be acknowledged that when using complementary sources to increase the inclusion of LAA such as Trp, which is often present in much lower concentrations relative to its requirement than other IAA (Templeman et al., 2020), the attempt to meet the Trp requirement may result in other IAA being supplied in excess. Ultimately, if we are to ensure that the industry remains sustainable, resources must be directed toward identifying ways we can approach diet formulation from an “ideal protein” or “precision protein” perspective, whether it be with anhydrous AA supplementation and/or complementary protein sources.

Ultimately, it was hypothesized that applying IAA reference patterns based on IAA requirements or recommendations for growth would result in DIAAS-like values that would exceed those generated when applying IAA patterns for maintenance; however, while this was observed when NRC and FEDIAF reference patterns were applied, it was not always the case when applying AAFCO reference patterns. This outcome was a reflection of the DIAAS-like values presented herein being generated using reference IAA patterns on an mg IAA/g CP basis. The practical IAA recommendations proposed by AAFCO to support GR were, in most cases, nearly 2-fold that of maintenance, yet the CP recommendations only marginally different between the two life stages. Essentially, these data underpin the stark differences in proposed nutrient guidelines across scientific and regulatory bodies, even with a dearth of actual empirical estimates of biological requirements. This clearly highlights the need for research on protein and IAA requirements under different conditions as well as a unified regulatory approach to facilitate the global pet food market.

The authors do acknowledge that the present study has limitations aside from those previously discussed regarding the use of AAFCO/FEDIAF IAA reference patterns, including that there were characteristics of these ingredients (e.g., degree of processing, presence of anti-nutritional factors) that could not be captured in this report, but that could affect the interpretation of PQ. However, due to the sheer quantity of data, some definitive boundaries had to be set. Moreover, it is assumed that some of these effects were inherently captured in the digestibility coefficients used for the PQ equations. There has been an increased interest in less processed ingredients as well as plant protein ingredients in the pet food industry, largely driven by the need to satisfy consumer demand (Simonsen et al., 2014; Dodd et al., 2019; Schleicher et al., 2019). As such, future research is necessary to investigate how various commonly used methods of ingredient (e.g., rendering, drying) or diet processing (e.g., extrusion and freeze-drying) as well as the presence/activity of anti-nutritional factors (e.g., protease inhibitors, lectins, and tannins) may influence these metrics of PQ. This is of importance as processing conditions (e.g., cooking temperature) and anti-nutritional factor content have been shown to affect the quality of protein-containing ingredients, with the latter predominantly affecting plant ingredients (Johnson et al., 1998; Opstvedt et al., 2003; Sarwar et al., 2012; Hodgkinson et al., 2018). Furthermore, while processing conditions, such as heat treatment, can induce deleterious molecular alterations to proteins (e.g., Maillard reaction; González-Vega et al., 2011) rendering them less digestible, these same conditions may be necessary to reduce the concentrations of anti-nutritional factors (Purushotham et al., 2007). By improving our knowledge of how these factors may influence nutrient availability/ingredient quality, we can help to meet the demands of the pet owners for diets formulated with less processed ingredients or with a greater contribution from plant ingredients while also ensuring that changes in PQ and nutrient availability are accounted for when considering the processing conditions of diet production. Finally, it should be acknowledged that this report is based solely upon publicly available literature and, as such, the outcomes presented herein are only a reflection of the amount and type of ingredient data available.

Conclusions and Implications

The current work provides DIAAS-like values for numerous animal and plant ingredients potentially used in the pet food industry. The authors acknowledge that for the pet food industry to align with the human food industry with regards to the assessment of ingredient PQ, that only reference patterns based on MR estimate established by the NRC should be applied as these are based on an adult or growing dog’s physiological IAA requirements. However, the reliance of the pet food industry on IAA and CP recommendations presented by AAFCO and FEDIAF as well as the recent arrival of literature assessing PQ using reference patterns based on these regulatory body recommendations encouraged us to present and discuss how the selection of reference pattern may affect PQ interpretation. Ultimately, while future studies assessing PQ of ingredients or complete commercial diets should utilize MR estimates presented by the NRC, difference in DIAAS-like values for ingredients when using AAFCO and FEDIAF recommendations underpin the different regulatory approaches to establishing dietary nutrient recommendations that exist globally and suggest that harmonization these recommendations may be valuable, especially with consideration of the globalization of pet food companies.

Supplementary Data

Supplementary data are available at Journal of Animal Science online.

Acknowledgments

We would like to thank and acknowledge Dr. Christopher P.F. Marinangeli for his valuable insights and contributions. The
work was funded by Champion Petfoods LT (Morinville, AB) and Mitacs (Toronto, ON).

**Authors’ Contributions**

J.R.T. and A.K.S. conceptualized the project. J.R.T. conducted the research and analyzed the data, and all authors contributed to the writing of the manuscript. J.R.T. had primary responsibility for the final content. All authors read and approved the final manuscript.

**Conflict of Interest Statement**

The work was funded by Champion Petfoods LT (Morinville, AB) and Mitacs (Toronto, ON). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. A.K.S. is the Champion Petfoods Chair in Canine and Feline Nutrition, Physiology and Metabolism, a Champion Petfoods consultant, receives research funding from private industry, and was a former employee of P&G Petcare and Mars Petcare. J.R.T. is currently employed by Primal Pet Foods (Fairfield, CA).

**LITERATURE CITED**

Almeida, F. N., G. I. Petersen, and H. H. Stein. 2011. Digestibility of amino acids in corn, corn coproducts, and bakery meal fed to growing pigs. *J. Anim. Sci.* 89:4109–4115. doi:10.2527/jas.2011-4143

Association of American Feed Control Officials. 2016. AAFCO manual. West Lafayette (IN): AAFCO Inc.

Bailey, H. M., J. K. Mathai, E. P. Berg, and H. H. Stein. 2020. Pork products have digestible indispensible amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. *J. Nutr.* 150:475–482. doi:10.1093/ jn/nzx284

Batterham, E. S. 1992. Availability and utilization of amino acids for growing pigs. *Nutr. Res. Rev.* 5:1–18. doi:10.1079/NRR199200004

Cervantes-Pahn, S. K., Y. Liu, and H. H. Stein. 2014. Digestible indispensible amino acid score and digestible amino acids in eight cereal grains. *Br. J. Nutr.* 111:1663–1672. doi:10.1017/ S0007114513004273

Columbus, D. A., H. Lapiere, J. K. Htoo, and C. F. de Lange. 2014. Nonprotein nitrogen is absorbed from the large intestine and increases nitrogen balance in growing pigs fed a valine-limiting diet. *J. Nutr.* 144:614–620. doi:10.3945/jn.113.187070

Cotten, B., D. Ragland, J. E. Thomson, and O. Adebola. 2016. Amino acid digestibility of plant protein feed ingredients for growing pigs. *J. Anim. Sci.* 94:1073–1082. doi:10.2527/jas.2015-9662

Cramer, K. R., M. W. Greenwood, J. S. Moritz, R. S. Beyer, and C. M. Parsons. 2007. Protein quality of various raw and rendered by-product meals commonly incorporated into companion animal diets. *J. Anim. Sci.* 85:3285–3293. doi:10.2527/jas.2006-225

CVB Feed Table. 2016. Chemical composition and nutritional values of feedstuffs. Wageningen (The Netherlands): Federatie Nederlandse Diervoederketen.

Darragh, A. J., P. D. Cranwell, and P. J. Moughan. 1994. Absorption of lysine and methionine from the proximal colon of the piglet. *Br. J. Nutr.* 71:739–752. doi:10.1079/bsj19940181

Deglaire, A., C. Bos, D. Tomé, and P. J. Moughan. 2009. Ileal digestibility of dietary protein in the growing pig and adult human. *Br. J. Nutr.* 102:1752–1759. doi:10.1017/s0007114509991267

Do, S., L. Koutsos, P. L. Utterback, C. M. Parsons, M. de Godoy, and K. S. Swanson. 2020. Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecec-tomized rooster assay. *J. Anim. Sci.* 98:skz363. doi:10.1093/jas/skz363

Dodd, S. A. S., N. J. Caje, J. L. Adolphe, A. K. Shoveller, and A. Verbrugghe. 2019. Plant-based (vegan) diets for pets: a survey of pet owner attitudes and feeding practices. *PLoS One* 14:e0210806. doi:10.1371/journal.pone.0210806

European Pet Food Industry Federation. 2018. Nutritional guidelines for complete and complementary pet food for cats and dogs. Bruxelles (Belgium): FEEDIAF Inc.

Faber, T. A., P. J. Bechtel, D. C. Hernott, C. M. Parsons, K. S. Swanson, S. Smiley, and G. C. Fahey Jr. 2010. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. *J. Anim. Sci.* 88:1421–1432. doi:10.2527/jas.2009-2140

Food and Agriculture Organization. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. FAO Food Nutrition Paper; p. 92.

Food and Agriculture Organization/World Health Organization-United Nations. 1985. *Energy and protein requirements*. Report of a joint FAO/WHO/UNU expert consultation, WHO Tech. Rep. Ser. Pap; p. 724.

Fuller, M. E., and P. J. Reeds. 1998. Nitrogen cycling in the gut. *Annu. Rev. Nutr.* 18:385–411. doi:10.1146/annurev.nutr.18.1.385

Gomez, V. A., P. L. Utterback, C. M. Parsons, and M. de Godoy, and H. Russell. 2021. Evaluation of macronutrient composition and amino acid digestibility of select novel dietary proteins for use in canine and feline nutrition. *J. Anim. Sci.* 99:57 (Abstr.). doi:10.1093/j a/skab233.102

González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. *J. Anim. Sci.* 89:3617–3625. doi:10.2527/jas.2010-3465

Gottlob, R. O., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. L. Nelssen, C. W. Hatstad, and D. A. Knabe. 2006. Amino acid and energy digestibility of protein sources for growing pigs. *J. Anim. Sci.* 84:1396–1402. doi:10.2527/2006.8461396X

Grosjean, F., C. Jondreville, I. Williatte-Hazour, F. Skiba, B. Carroué, and F. Gätel. 2000. Ileal digestibility of protein and amino acids of feed peas with different trypsin inhibitor activity in pigs. *Can. J. Anim. Sci.* 80:643–652. doi:10.4141/a99-075

Harrison, M. D., M. E. Jackson, D. G. McLaren, C. M. Parsons, and D. A. Knabe. 1990. A direct comparison of true amino acid digestibility determined with poultry and apparent amino acid digestibility determined with swine. *Poult. Sci.* 69:38.

Harrison, M. C., G. Thomas, M. Gilham, K. Gray, A. Colyer, and D. Al-laway. 2020. Short-term amino acid evaluation and long-term evaluation of the dietary methionine requirement in adult dogs. *Br. J. Nutr.* 26:1–12. doi:10.1017/S0007114520000869

Hendriks, W. H., E. J. Baker, and G. Bosch. 2015. Protein and amino acid bioavailability estimates for canine foods. *J. Anim. Sci.* 93:4788–4795. doi:10.2527/jas.2015-9231

Herreman, L., P. Nommensen, B. Pennings, and M. C. Laos. 2020. Comprehensive overview of the quality of plant- and animal-sourced proteins based on the digestible indispensable amino acid score. *Food Sci. Nutr.* 8:5379–5391. doi:10.1002/fsn3.1809

Hill, M. R. 2012. Evaluating the supply chain of animal protein-based pet food ingredients and international trade of pet food. *MSc Diss. Manhattan (NY): Kansas State University.

Hodgkinson, A. J., O. A. M. Wallace, I. Boggs, M. Broadhurts, and C. G. Prosser. 2018. Gastric digestion of cow and goat milk: impact of infant and young child in vitro digestion conditions. *Food Chem.* 245:275–281. doi:10.1016/j.foodchem.2017.10.028

Johnson, M. L., C. M. Parsons, G. C. Fahey Jr., N. R. Merchen, and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals ceccectomized roosters and ileally cannulated dogs. *J. Anim. Sci.* 76:1112–1122. doi:10.25271998.761112x

Kerr, B. J., P. E. Urriola, R. Jha, J. E. Thomson, S. M. Curry, and G. C. Shurson. 2019. Amino acid composition and digestible amino acid content in animal protein by-product meals fed to growing pigs. *J. Anim. Sci.* 97:4540–4547. doi:10.1093/jas/skz294
Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. *Asian-Australas. J. Anim. Sci.* 27:197. doi:10.5713/ajas.2014.133

Lee, S. A., J. Y. Ahn, A. R. Son, and B. G. Kim. 2020. Standardized ileal digestibility of amino acids in cereal grains and co-products in growing pigs. *Asian-Australas. J. Anim. Sci.* 33:1148–1155. doi:10.5713/ajas.19.0449

Leme, B. B., N. Sakomura, G. S. Viana, L. Soares, M. C. Melar, J. K. Oliveira, and C. F. M. Mansano. 2019. Amino acid digestibility of feed ingredients in cecotomized adult roosters. *Brazil. J. Poult. Sci.* 21. doi:10.1590/1806-9061-2018-0924

Mansilla, W. D., L. Fortener, A. Gorman, and A. K. Shoveller. 2018. Dietary phenylalanine requirements are similar in small, medium, and large breed adult dogs using the indicator amino acid oxidation technique. *J. Anim. Sci.* 96:3112–3120. doi:10.1093/jas/sky208

Mansilla, W. D., J. R. Templeman, L. Fortener, and A. K. Shoveller. 2020a. Minimum dietary methionine requirements in Miniature Dachshund, Beagle, and Labrador Retriever adult dogs using the indicator amino acid oxidation technique. *J. Anim. Sci.* 98:skaa324. doi:10.1093/jas/skaa324

Mansilla, W. D., J. R. Templeman, L. Fortener, and A. K. Shoveller. 2020b. Adult dogs of different breed sizes have similar threonine requirements as determined by the indicator amino acid oxidation technique. *J. Anim. Sci.* 98:skaa066. doi:10.1093/jas/skaa066

Mathai, J. K., Y. Liu, and H. H. Stein. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). *Br. J. Nutr.* 117:490–499. doi:10.1017/S0007114517001225

Morris, J. G., and Q. R. Rogers. 1994. Assessment of the nutritional adequacy of pet foods through the life cycle. *J. Nutr.* 124:2520S–2534S. doi:10.1093/jn/124.suppl_12.2520S

Oba, P. M., P. L. Utterback, C. M. Parsons, and K. S. Swanson. 2019. True nutrient and amino acid digestibility of dog foods made with human-grade ingredients using the precision-fed cecotomized rooster assay. *Transl. Anim. Sci.* 4:444–451. doi:10.1093/tas/txz215

Opstvedt, J., E. Nygård, T. A. Samuelsen, G. Venturini, U. Luzzana, and H. Mundheim. 2003. Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. *Transl. Anim. Sci.* 4:442–451. doi:10.1093/tas/txz175

Park, C. S., V. D. Naranjo, J. K. Htoo, and O. Adeola. 2020. Comparative amino acid digestibility between broiler chickens and pigs fed different poultry by-products and meat and bone meal. *J. Anim. Sci.* 98:skaa223. doi:10.1093/jas/skaa223

Prolla, I. R. D., M. Rafai, G. Courtneymartin, R. Elango, L. P. da Silva, R. O. Ball, and P. B. Pencharz. 2013. Lysine from cooked white rice consumed by healthy young men is highly metabolically available when assessed using the indicator amino acid oxidation technique. *J. Nutr.* 143:302–306. doi:10.3945/jn.112.166728

Rajesh, P., B. M. Radhakrishna, and B. S. Sherigara. 2007. Effects of steam conditioning and extrusion temperature on some antinutritional factors of soyabean (Glycine max) for pet food applications. *Am. J. Anim. Vet. Sci.* 2:1–5. doi:10.3844/ajavsp.2007.1.5

Reilly, L. M., P. C. von Schaumburg, J. M. Hoke, G. M. Davenport, P. L. Utterback, C. M. Parsons, and M. de Godoy. 2020b. Macronutrient composition, true metabolizable energy and amino acid digestibility, and indispensable amino acid scoring of pulse ingredients for use in canine and feline diets. *J. Anim. Sci.* 98:1–8. doi:10.1093/jas/skaa149

Reilly, L. M., P. C. von Schaumburg, J. M. Hoke, G. M. Davenport, P. L. Utterback, C. M. Parsons, and M. de Godoy. 2020a. Use of precision-fed cecotomized rooster assay and digestible indispensable amino acid scores to characterize plant- and yeast-concentrated proteins for inclusion in canine and feline diets. *Transl. Anim. Sci.* 4:txaa082. doi:10.1093/tas/txaa082

Rojas, O. J. and H. H. Stein. 2012. *Nutritional value of animal proteins fed to pigs*. Proc. Midwest Swine Nutr. Conf., Indianapolis, IN. p. 9–24.

Rowan, A. M., P. J. Moughan, M. Wilson, K. Maher, and C. Tasman-Jones. 1994. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *Br. J. Nutr.* 71:29–42. doi:10.1079/bjn19940108

Sanderson, S. I., K. L. Gross, P. N. Ogburn, C. Calvert, G. Jacobs, S. R. Lowry, K. A. Bird, L. A. Koehler, and L. L. Swanson. 2001. Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. *Am. J. Vet. Res.* 62:1616–1623. doi:10.2460/ajvr.2001.62.1616

Sarwar, G. X., X. C. Wu, and K. A. Cockell. 2012. Impact of antinutritional factors in foods on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br. J. Nutr.* 108:S315–S332. doi:10.1017/S0007114512002371

Sanderson, S. I., K. L. Gross, P. N. Ogburn, C. Calvert, G. Jacobs, S. R. Lowry, K. A. Bird, L. A. Koehler, and L. L. Swanson. 2001. Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. *Am. J. Vet. Res.* 62:1616–1623. doi:10.2460/ajvr.2001.62.1616

Schleicher, M., S. B. Cash, and L. M. Freeman. 2019. Determinants of pet food purchasing decisions. *Can. Vet. J.* 60:644–650.

Shoveller, A. K., J. J. Danelon, J. L. Atkinson, G. M. Davenport, R. O. Ball, and P. B. Pencharz. 2017. Calibration and validation of carbohydrate digestion in the human gut. *AJP—Liver and GI.* 1:401–410. doi:10.3382/ajplg/2017.401

Simonsen, J. E., G. M. Faskeno, and J. M. Lillywhite. 2014. The value-added dog food market: do dog owners prefer natural or organic dog foods? *J. Agric. Sci.* 6:86–97. doi:10.5539/JAS.V6N6P86

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenhinz, A. J. M. Jansman, J. A. Fernandez, and C. F. M. de Lange. 2007a. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. *Livest. Sci.* 109:282–285. doi:10.1016/j.livsci.2007.01.019

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007b. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *J. Anim. Sci.* 85:172–180. doi:10.2527/jas.2005-742

Sutherland, K., W. D. Mansilla, L. Fortener, and A. K. Shoveller. 2020. Lysine requirements in small, medium, and large breed adult dogs using the indicator amino acid oxidation technique. *Transl. Anim. Sci.* 4:txaa082. doi:10.1093/tas/txaa082

Templeman, J. R., W. D. Mansilla, L. Fortener, and A. K. Shoveller. 2019. Tryptophan requirements in small, medium, and large breed adult dogs using the indicator amino acid oxidation technique. *J. Anim. Sci.* 97:3274–3285. doi:10.1093/jas/skr142
Templeman, J. R., E. Thornton, C. Cargo-Froom, E. J. Squires, K. S. Swanson, and A. K. Shoveller. 2020. Effects of incremental exercise and dietary tryptophan supplementation on the amino acid metabolism, serotonin status, stool quality, fecal metabolites, and body composition of mid-distance training sled dogs. *J. Anim. Sci.* 98:skaa128. doi:10.1093/jas/skaa128

Ward, J. 1976. *The amino acid requirements of the adult dog* [Ph.D. dissertation]. Cambridge (UK): Wolfson College, University of Cambridge.

Woyengo, T. A., J. M. Heo, Y. L. Yin, and C. M. Nyachoti. 2015. Standardized and true ileal amino acid digestibilities in field pea and pea protein isolate fed to growing pigs. *Anim. Feed Sci. Technol.* 207:1986–203. doi:10.1016/j.anifeedsci.2015.06.008

Zhang, C. X., K. K. Huang, L. Wang, K. Song, L. Zhang, and P. Li. 2015. Apparent digestibility coefficients and amino acid availability of common protein ingredients in the diets of bullfrog, *Rana (Lithobates) catesbeiana*. *Aquaculture* 437:38–45. doi:10.1016/j.aquaculture.2014.11.015