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# Letter to the Editor and Rebuttal: Protein and amino acid bioavailability estimates for canine foods

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Dear Editor-in-Chief of the *Journal of Animal Science*,

With great interest, we, the members of the Nutrition and Analytical Sciences working group of the European Pet Food Industry Federation (**FEDIAF**), have read the article *Protein and Amino Acid Bioavailability Estimates for Canine Foods* (Hendriks et al., 2015). We agree with the authors that the bioavailability of dietary AA is an important aspect for the determination of the nutritional quality of animal diets. For this reason bioavailability estimates are applied to convert minimal requirements to inclusion estimates in pet food formulation. The study aimed to generate estimates for the bioavailability of dietary CP and AA for adult dogs using existing literature data and to evaluate the accuracy of estimates currently used in authoritative publications, specifically the FEDIAF *Guidelines for Complete and Complementary Pet Foods for Cats and Dogs* (FEDIAF, 2014) and the Association of American Feed Control Officials (**AAFCO**) in the United States.

These publications have the primary objective to contribute to the production of nutritionally balanced pet food while complying with relevant legislation on animal nutrition. They incorporate up-to-date scientific knowledge on cat and dog nutrition, because scientific knowledge regarding nutrient requirements, digestion of feed, and metabolism of nutrients are the principles that guide the formulation of appropriate diets for dogs and cats.

The study published by Hendriks et al. (2015) and the methodology applied in this work are of a high interest as they can provide valuable insights toward setting the recommended standards for the formulation of complete and balanced pet food, practically, as animal experimentations are very scarce for ethical reasons. The authors conclude the study with deriving bioavailability estimates for CP and AA, to convert minimum physiological requirements to practical allowance estimates of these nutrients in maintenance dog foods, and compared them with estimates currently used in the authoritative publications. As the FEDIAF guidelines (FEDIAF, 2014) recommended values are based on scientific principles, we have a high surveillance of the latest research results, and we would, therefore, like to ask the authors further insights regarding their conclusions.

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### *1. Deriving Bioavailability Estimates for CP and AA*

The authors describe the data sets used to derive the equations for standardized ileal digestibility/outflow of N (**$sID_N$** and **$sIO_N$**, respectively) and standardized ileal digestibility of AA (**$sID_{AA}$**). A first data set of 158 diets was used to generate regression equations between apparent fecal outflow of N (**$aFO_N$**) and $sIO_N$, and a second data set of 24 diets was used to generate regression equations between $sIO_N$ and $sID_{AA}$. These relationships were used to predict $sID_N$ and $sID_{AA}$ of 10 hypothetical diets of varying CP content. The bioavailability estimates are defined as $1/sID_N$, in which $sID_N$ is the lower 0.95 confidence limit of the predicted value.

The origin of the 158 diets in the first data set is unclear, as this data set originates from a previously reported data set supplemented with data from 5 studies. When references are verified, the 5 studies include the data of 24 diets, and the data set of the remaining 134 diets is referenced in a review article without further details (Hendriks et al., 2012). We would like to request that the authors provide additional information on the data set used, particularly information relating to diet formulation, breed, age, BW and BCS, and energy intake and for the authors to comment on how such variables were considered by the modeling.

### *2. Converting to Allowance Estimates in Maintenance Dog Foods*

The authors describe the first data set of 158 diets "to contain data on 158 diets used in fundamental nutritional research, formulated to evaluate specific ingredients or investigate specific technological treatments." Indeed, at least for the 24 diets from the 5 studies added to the previously described data set,

13 diets are experimental (Zentek, 1995; Gajda et al., 2005; Tjernsbekk et al., 2014), and 8 of them used in 2 studies have extremely high CP levels (Zentek, 1995; Tjernsbekk et al., 2014). The second data set of 24 diets consists of "24 diets ranging from commercial dry foods to experimental diets containing 2 or more protein sources." At least 3 diets are experimental with extremely high protein levels (Tjernsbekk et al., 2014). We would like to ask the authors to comment on the extrapolation of the bioavailability estimates generated from these 2 data sets including experimental diets to application in maintenance diets, which have more standard levels of nutrients in general and of CP specifically.

### *3. Comparison with Estimates in European Pet Food Industry Federation Guidelines*

One of the 10 hypothetical diets has a protein digestibility of 80% and an 18% CP level, comparable to the minimum recommendations in the FEDIAF guidelines (FEDIAF, 2014). Taking into consideration the requested insights regarding content of the data sets and extrapolation to standard maintenance diets, we additionally would like to request that the authors comment on the robustness of applying the derived bioavailability estimates generated by these data sets to 1 hypothetical diet and concluding that the bioavailability estimate is too low. We are also interested in their view on the bioavailability estimates of the separate AA using the high bioavailability estimate for CP in the FEDIAF guidelines (FEDIAF, 2014) rather than the $sID_N$ generated from the data sets.

We look forward to a response from the authors.

Kind regards,
**The Nutrition and Analytical Science Working Group**
The European Pet Food Industry Federation,
Brussels, Belgium

## REBUTTAL

We thank the FEDIAF for their interest in our article *Protein and Amino Acid Bioavailability Estimates for Canine Foods*. We hope that the answers below address the questions raised by the members of the Nutrition and Analytical Sciences working group.

1. The 34 peer reviewed journal articles in the data set are provided (Appendix I). Detailed information on diet formulation is provided in each article but information on breed, age, and BCS are often lacking. For example, age is provided in some publications, whereas in other publications, dogs are characterized as "mature" or "adult." The average age (when reported) and BW across studies was 4.1 yr ($n$ = 38; 2–7 yr) and 23.0 kg ($n$ = 158; 10.4–31.0 kg), respectively. The average ME intake of the dogs was 125 kcal/$kg^{0.75}$ ($n$ = 100; 63–183 kcal/$kg^{0.75}$).

The modeling did not specifically account for variation in $sIO_N$ or $aFO_N$ due to diet formulation, breed, age, BW and BCS, and energy intake. A number of the abovementioned factors (e.g., ME intake, diet type, and age), however, greatly varied between studies and, as such, can be expected to be representative of what can be observed in the pet dog population and commercial foods on offer. Other factors in the data set were not as variable (e.g., BW, breed) as the studies were conducted under controlled laboratory conditions. It is likely that measurement within the pet dog population would yield a greater variation in $sID_N$ and apparent fecal digestibility of N (**$aFD_N$**) values compared with these controlled studies. The latter would increase the variation of the difference between $aFO_N$ and $sIO_N$ and yield lower bioavailability factors compared with the values calculated using our model. As a result, the difference compared with the factors used by the NRC (2006), FEDIAF (2012), and AAFCO (2011) will be larger.

2. The number of studies using experimental diets is low. Of the 34 studies, the majority ($n$ = 22) were extruded foods; in 6 studies, commercial prepared (dry and moist) foods were fed; 2 used a liquid formula; 1 used a dry mixture; and 3 studies did not report information on postformulation treatment of the diet. The CP content of the diets used by Tjernsbekk et al. (2014) ranged from 27.0 to 27.3% in the DM. This is close to the mean (±SEM) protein content of the 158 foods (28.6 ± 0.72%) and can be considered to be normal for a commercial maintenance food for dogs. Of the 5 diets fed by Zentek (1995), only 2 can be considered to have "extremely high" CP levels (i.e., 76.9 and 79.7% in the DM). The remaining 3 diets (39.7, 50.4, and 51.0% in the DM) are high in protein but comparable to commercial diets. The average CP content of the 24 diets in the data set is 29.5 ± 0.75% (24.3–33.0%) in the DM. The total number of diets with an "extremely high" CP level is, therefore, 2 out of 158. The $aFO_N$ for the 2 diets are 0.269 and 0.305 g/(kg $BW^{0.75}$·d) with a corresponding $sIO_N$ of 0.315 and 0.601 g/(kg $BW^{0.75}$·d). These values cannot be considered outliers (see Figure 1 in Hendriks et al., 2015). The second relationship between $sID_{AA}$ or sID of the sum of nonessential AA and $sID_N$ used in the model does not contain any diets

that can be considered to have "extremely high" CP levels.

As the relationship between $aFO_N$ and $sIO_N$ is used in the model, dietary protein content is less important than values for $sIO_N$. It is this colonic N (dis) appearance that is estimated by the model. The composition and postformulation treatment of diets may affect this difference between $aFO_N$ and $sIO_N$, as ileal undigested components influence colonic fermentation characteristics. The latter can affect microbial protein breakdown or synthesis resulting in N disappearance or fixation in the large intestine.

Overall, the model should provide a good representation of the physiological response of dogs ingesting commercial diets of various types having a wide range in protein concentrations.

3. The article states, in the conclusion section, "In general, the estimates used by the European Pet Food Industry Federation (FEDIAF, 2012) and the Association of American Feed Control Officials (2011) are too small as well, with the exception of N, Arg, and Lys." We therefore did not conclude that the bioavailability estimate for N (CP) is too low.

A higher bioavailability factor for CP as used by the FEDIAF (2012) and the AAFCO (2011) increases the confidence that commercial foods for adult dogs, when formulated to the minimum CP level, provide sufficient protein to meet the minimum physiological requirements of the animal. Whether the requirements for individual essential AA are met depends on the AA pattern of the dietary protein. The use of poorly digestible protein sources combined with a low content of one of the essential AA in the protein and severe heat treatment could result in a deficiency. The latter, however, is unlikely when a higher bioavailability factor for CP is used. The most critical AA would be Lys (Maillard reaction) and Met + Cys (oxidation state), as these AA can be present in the diet in a form that cannot be or is poorly utilized by the animal. Especially when processed ingredients are used in combination with the heat treatments commonly used to manufacture pet foods, chemical analysis of these AA can yield inaccurate results.

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## APPENDIX I

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