Protein/Amino Acid Digestibility in Dogs and Cats

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Measurement of protein and amino acid digestibility in diets for cats and dogs is of paramount importance to ensure the formulation of diets that meet not only the minimum nutrient requirements of animals, but are also effective as veterinary therapeutic diets. In addition, bioavailability factors, which are often used in nutrient requirement tables to convert minimum physiological requirements[1] to practical allowance estimates, [2,3] rely largely on digestibility estimates. Finally, results of the testing of apparent total tract digestibility are commonly used in the marketing of pet foods to provide an indication of the quality to pet owners.

In pet nutrition, the apparent fecal digestibility (aFD) method is often used to derive such estimates for nitrogen (N) although a number of in vitro assays have been developed. The accuracy of the estimates generated using the aFD method depends on a number of factors. Estimation of amino acid digestibility is often erroneous using this method due to the large intestinal fermentation of dietary amino acid and the presences of endogenous amino acids. This contribution discusses the measurement of crude protein and amino acid digestibility in pet foods and highlights important considerations to increase the accuracy to ensure complete and balanced diets are formulated.

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Measurement of dietary protein digestibility

The most commonly used method to determine the digestibility of protein is the aFD method, where fecal N output and dietary N consumption are quantified. FEDIAF[2] and AAFCO[3] provide standardized protocols for determination of digestibility of energy and N which can be conducted within a specified time frame (e.g., 4-5 collection days) or by the use of an indigestible marker (e.g., Cr_2O_3, TiO_2, Y_2O_3.) The values obtained can be corrected for endogenous gut losses, and when these are determined using a protein-free diet, values are referred to as standardized fecal digestibility (sFD).[4] The latter is a more accurate measure of the dietary supply of N.

Average protein and amino acid digestibility values of commercial pet foods

In the scientific literature, there are a number of estimates of aFD of N for

commercial pet foods. More recent mean aFD estimates for commercial dry adult dog foods of 82.4 (range, 71.0-92.0, n=100)[5] and 81.9% (range, 78.1-83.9, n=5)[6] have been reported. Recently, an average aFD of N for 16 dry dog foods of 80.1% was reported[7] with the lowest values being ~67%. A more extensive evaluation[8] reported sFD values for 331 dry and moist canine foods of 89.7% and 88.0%, respectively. Conversion to aFD values would reduce these means to 83.7% and 82.4%, respectively. Much higher values were reported by the latter authors for the sFD of dry and moist commercial cat foods (93.0%, n=173 and 93.5%, n=54). Using the endogenous gut N losses used by the authors,[9] the corresponding aFD values are 88.0% and 89.3%.

Inaccuracy of the fecal N digestibility methodology

The canine colon has been reported to have the ability to transport amino acids against a concentration gradient

RELATIONSHIP BETWEEN APPARENT ILEAL AND APPARENT FECAL N DIGESTIBILITY IN ADULT DOGS FED VARIOUS DIET TYPES

$y = 0.5064 + 45.625$
$R2 = 0.5536$

$y = x$

Apparent ileal N digestibility (%)

Apparent fecal N digestibility (%)
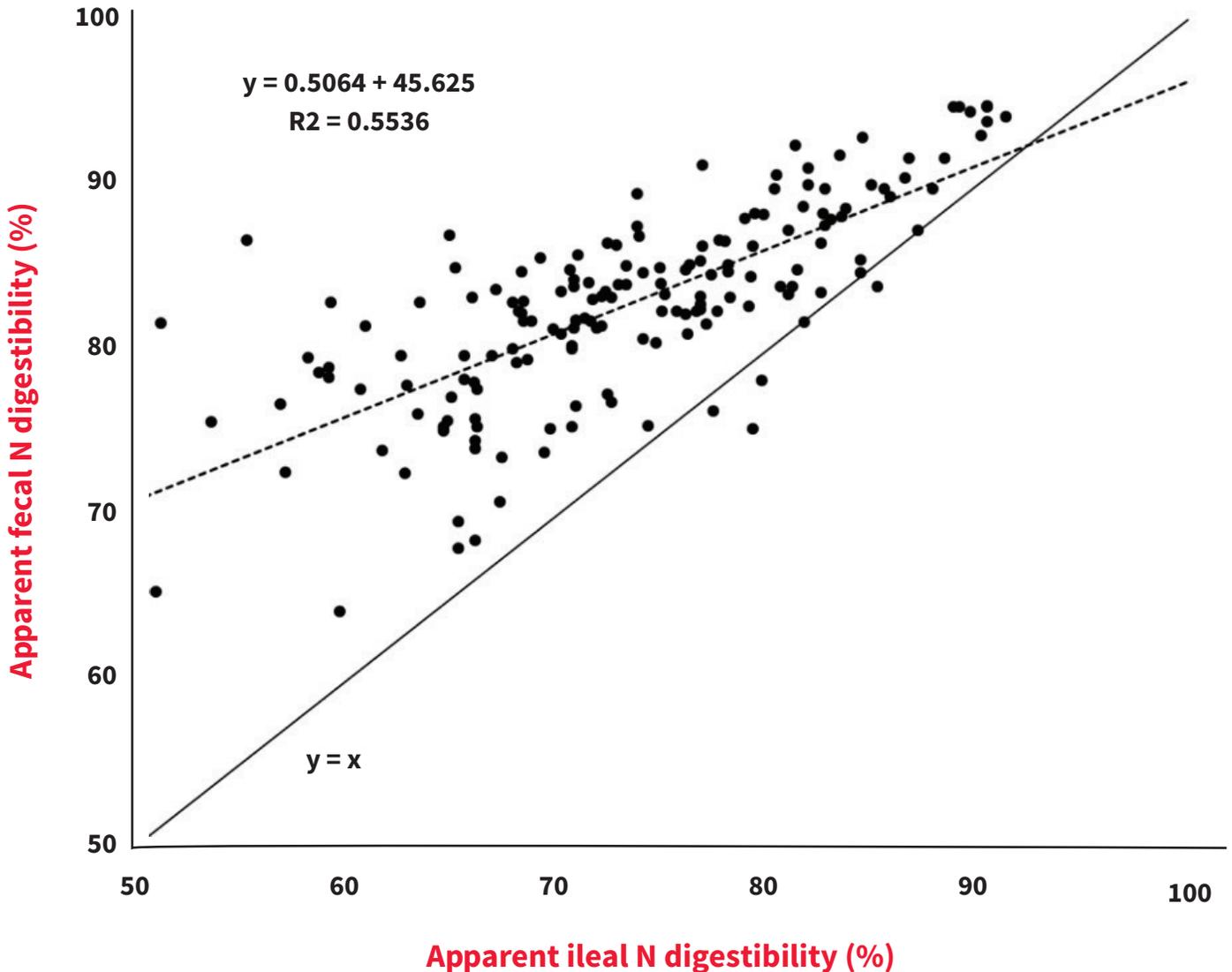
in vitro.[10] In several animal species, amino acid transporters have been identified in the large intestine. However, infusion studies using free amino acid into the cecum and colon of horses[11] and pigs[12] have not shown significant amino acid absorption in vivo. Although no such studies have been performed in dogs, it is the current consensus that, although some absorption of amino acids may occur, the large intestine does not contribute significantly to the amino acid supply. In cats it is also unlikely that significant amino acid absorption occurs in the large intestine, although, to the author's knowledge, there are also no data available to substantiate this statement.

The intestinal tract of adult cats and dogs has a very low coefficient of fermentation (large intestinal/ small intestinal volume × 10) of 2.9 and 4.1, respectively,[13] indicating that hindgut fermentation makes a relatively unimportant contribution to absorbing nutrients from the gastrointestinal tract. However, despite this low coefficient, there is significant fermentation in the large intestine of dogs. **Figure 1** shows the relationship between ileal and fecal digestibility of N in adult dogs compiled from data available in the literature. The linear regression line shows that the apparent ileal N digestibility decreases approximately twice when aFD is decreased by 1 percentage unit. The relatively large disappearance of N from the large intestine is due to the deamination of amino acids (dietary or endogenous) and/or catabolism of other N-containing compounds such as urea to NH_3 by the microbiota, with subsequent diffusion across the intestinal wall and excretion in the urine. Differences between ileal and fecal amino acid digestibility

values of dry dog foods have been reported to be as large as 19% for Cys and 17% for His and Thr, with all amino acids being overestimated by the aFD methodology. Digestibility measurement at an ileal level, as is common in production animals, is ethically questionable in the case of companion animals, although such studies have been performed in dogs and cats for nutritional research.

Bioavailability factors

Bioavailability of a dietary amino acid is defined as the proportion of the ingested dietary nutrient that is absorbed in a chemical form that renders this amino acid potentially suitable for metabolism or protein synthesis.[4] Several reference tables[1-3] make use of factors in order to convert minimal requirement estimates for crude protein and amino acid to minimum allowance estimates. The NRC[1] assumes that the availability of N and amino acids in commercial feline and canine foods is equal to or above 80% and uses this value to derive recommended allowance estimates. This practice is in contrast to FEDIAF[2] and AAFCO[3] where crude protein and amino acid specific factors are used to account for dietary bioavailability. These factors need to account for the use of ingredients in pet food formulation that differ in digestibility compared to the highly digestible ingredients used to derive the minimal requirements estimates. Additionally, several of the minimum requirements estimates for amino acids were determined using an amino acid mixture or protein-free diet and did not account for the processing effects on availability of amino acids and increased gut endogenous amino acid losses associated with commercial

diets. It is known that diets containing amino acid mixtures and protein free diets result in lower endogenous ileal amino acid losses compared to diets containing peptides or protein in cats and dogs[14,15].

Bioavailability factors for dietary crude protein and amino acids for adult dogs using existing literature reporting ileal and fecal digestibility data in adult dogs have recently been reported. As for the majority of amino acids, bioavailability is equal to the absorption of that amino acid; determination of the ileal digestibility of amino acids provides a good estimate of bioavailability. Through a set of regression equations, bioavailability estimates for N and amino acids for ten diets varying in crude protein content (18% to 42%) and aFD of N (70% and 80%) for an adult dog were generated.[16] Using an average aFD of N of 80% (see above) for commercial dog foods, the bioavailability factor for a number of amino acids as utilized by FEDIAF[2] and AAFCO[3] appears too low.

For some amino acids, determination of amino acid digestibility underestimates the bioavailability of that amino acid.

For some amino acids, determination of amino acid digestibility underestimates the bioavailability of that amino acid. The most affected amino acids in pet foods are lysine (Lys), methionine (Met) and cysteine (Cys), which may be absorbed in a form that is not fully utilized by metabolism. During heat treatment and storage, Lys can be involved in early and advanced

Maillard reaction products. Under the hydrolysis conditions with strong acid, as is commonly employed with the quantification of amino acids, Lys bound to other components can revert back to Lys, a reversion which does not occur in vivo. Met can be oxidized to methionine sulfoxide (MetO) and methionine sulfone (MetO$_2$) and Cys to cysteic acid. MetO$_2$ and cysteic acid are nutritionally unavailable in many animal species.[17] In cats, cysteic acid can be utilized to synthesize Tau.[18] However, the oxidation state of Met and Cys in commercial dog foods, relative absorption of oxidized Met and Cys to unoxidized forms, and whether oxidized Met and Cys can be utilized by the metabolism of dogs and, to a lesser extent, cats remains to be determined.

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