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Non-ruminants

Prediction equations for energy values of animal meals obtained using meta-analysis

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ABSTRACT - The objective of this study was to determine prediction equations to estimate the nitrogen-corrected apparent metabolizable energy (AMEn) values of animal meals used in broiler diets through meta-analysis. A bibliographic review was undertaken with studies conducted in Brazil from 2000 to 2016 to catalogue information on AMEn values and the following chemical elements in the composition of the feedstuffs: crude protein (CP), ether extract (EE), gross energy (GE), mineral matter (MM), calcium (Ca), and phosphorus (P). Groups were also catalogued and formed according to sex and age of birds. Chemical correlations were analyzed, and a multiple linear regression model with the stepwise procedure was used to examine the association between the variables, which were included in the equation as a function of their importance. High and significant correlation coefficients between the independent (GE, MM, CP, EE, Ca, and P) and dependent variable (AMEn) contribute to the understanding of variations in the energy values of these feedstuffs. According to the coefficients of determination, the best equations to estimate AMEn of poultry offal meal and meat and bone meal are AMEn = 6139 – 45.5 CP + 0.356 GE –123.5 MM ($R^2 = 0.8302$) and AMEn = 2267 + 19.9 CP + 67.9 EE – 44.4 MM ($R^2 = 0.9021$), respectively.

Key Words: AMEn, broiler, meat and bone meal, metabolism, prediction

Introduction

Slaughterhouse byproducts like poultry offal meal and meat and bone meal are dietary sources of protein and phosphorus that can substitute costly ingredients such as soybean meal and dicalcium phosphate. This replacement also addresses environmental concerns, as it represents a proper destination for the waste generated by slaughterhouses.

The precise knowledge of the chemical composition and of nitrogen-corrected apparent metabolizable energy (AMEn) values of animal meal ingredients is necessary for the formulation of nutritionally and economically balanced diets

However, determining the AMEn content of feedstuffs involves the use of metabolic trials, which are costly and time-demanding. In this regard, feed composition tables

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are commonly consulted to obtain the energy values of the ingredients used in diets with greater practicality. Nevertheless, several factors can affect the values in those tables, e.g., the chemical composition of those feedstuffs, the age of birds, and the methodology applied to determine the energy value.

A rapid way to determine the metabolizable energy value is through prediction equations developed as a function of the chemical composition of feedstuffs, which is easily and quickly obtained in most cases. Many studies can be found in the literature establishing prediction equations for the energy values of a number of ingredients (NRC, 1994; Nascimento et al., 2009; Rostagno et al., 2017). However, the obtained results are only applicable to a group of feedstuffs, since one experiment alone reflects only the experimental conditions in which it was developed (Polycarpo et al., 2017).

Therefore, information originating from data collected under different conditions should be merged to generate more consistent results. A technique employed to integrate the quantitative knowledge of multiple studies is meta-analysis, which is based on the synthesis of data from several published studies and on the construction of a statistical model that better explains the observations, generating

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new results (St-Pierre, 2001; Lovatto et al., 2007). Metaanalysis can produce more-accurate prediction equations to calculate the AMEn values of feedstuffs.

The objective of this study was to develop equations to predict the AMEn content of poultry offal meal (POM) and meat and bone meal (MBM) through a meta-analysis of information on the chemical composition of these ingredients obtained by various authors and published in scientific papers.

Material and Methods

The database was developed from scientific papers published from 2000 to 2016 involving experiments conducted in Brazil, under the guidelines on the use of live animals for research purposes. All selected experiments were aimed at evaluating the chemical composition and AMEn of POM and MBM.

The scientific article search included studies published in journals in the following digital databases: Scielo, CAPES Journals, Scopus, Web of Science, and Google Scholar. This spectrum of mechanisms prevents a possible polarization in terms of articles found in only one database and broadens the search limits.

Once identified, the articles were evaluated critically as to their quality and relevance for the objectives of the meta-analysis. During this stage, the information contained in each selected study was evaluated, including factors pertaining to the experimental project, treatments, studied parameters, methodology of chemical analyses, and data analysis.

The information obtained from the bibliographic review was tabulated according to feedstuff, sex, methodology of the metabolism trial, and age of the animal used in the experiments. The chemical and energy compositions of the feedstuffs, which included the variables crude protein (CP), ether extract (EE), gross energy (GE), mineral matter (MM), calcium (Ca), and phosphorus (P), were tabulated on the basis of the dry matter, which was adjusted at 920 g kg⁻¹, following Dale et al. (1993), to nullify the effects of the different moisture contents of the meals.

The database occupied a worksheet with 57 lines, representing the treatments, and 30 columns, representing the exploratory variables, containing 22 articles published between 2000 and early 2016. The studies included in the database amounted to 6,299 broilers, whose average initial age was 16 days (ranging from 1 to 41 days) and average final age was 25 days (ranging from 7 to 50 days). Most of the studies (57%) used male broilers, 7% used females, and 36% involved mixed batches. The total excreta collection

methodology was adopted in 86% of the articles, and the forced-feeding method with roosters was used in 14% of them. The level of 20% inclusion of the test feedstuff in control diet was indicated by most authors, in 40% of the papers.

The methodology used for coding the data, forming the groups, and weighting followed the proposals described by Lovatto et al. (2007) and Sauvant et al. (2008), considering the effects that influence the energy value of the feedstuffs directly, which do not alter the chemical composition, and which cause variability in the energy value of the feedstuffs, e.g., age and sex of the experimental animals.

Each article was coded to facilitate their identification in the database, with numbers used to form homogeneous groups with common traits to be included in statistical models as sources of variation. Codes were assigned to each effect, and groups were then formed. For the effect of sex, the codes 1, 2, and 3 were assigned to males, females, and mixed batches, respectively. For age, three codes were assigned: 1 for the pre-starter phase, 2 for the starter phase, and 3 for the grower phase. Therefore, the code of effects was 3×3 , with a total maximum of nine groups, which were subjected to analysis of weighted least squares. This weighting factor determines the existing variance for the dependent variable of the multiple linear regression model within each group; in this case, the AMEn of the feedstuffs used.

Assumptions of normal distribution and homoscedasticity of the data were checked, and then a descriptive analysis was carried out (Triola, 1999) to obtain the profile of the dataset based on the central-tendency and dispersion measures and observe the biological coherence of the data.

Pearson's correlation coefficient was used to measure the intensity of the linear correlation between AMEn and the other quantitative variables. Next, the data were subjected to multiple linear regression analysis employing the Stepwise method of indirect elimination, following Nunes et al. (2001) and Nascimento et al. (2011). The dependent variable was AMEn, whereas the independent variables were the CP, EE, GE, MM, Ca, and P contents.

Statistical analyses were performed using SAS statistical software (Statistical Analyses System, version 9.0), considering a significance level equal to or lower than 0.05.

Results

The average crude protein content in the samples of POM (634.6 g kg⁻¹) (Table 1) showed a visible variability in mineral content, in which the MM, Ca, and P percentages had the highest coefficients of variation: 25.73, 24.56, and

28.02%, respectively. The coefficient of variation for GE was 16.46%, while that of AMEn was 22.17%. Ether extract ranged from 101.4 to 201.8 g kg⁻¹.

For MBM (Table 2), the average CP content was 433.6 g kg⁻¹, ranging from 349.9 to 563.0 g kg⁻¹, with a coefficient of variation (CV) of 16.23%. The components that most varied were MM, Ca, and P, which averaged 19.35, 28.77, and 22.92%, respectively. Gross energy ranged from 3001 to 4668 kcal kg⁻¹, with a CV of 15.51%, whereas the CV of AMEn was 28.47%.

In the analysis of the correlations in POM (Table 3), CP was positively correlated (P<0.05) with GE, but negatively

with MM. A positive correlation was found between AMEn and CP and GE, whereas AMEn was negatively correlated with MM.

The analysis of the chemical components of MBM (Table 4) shows that the CP level is positively correlated with GE and establishes a high negative correlation with MM, Ca, and P. The AMEn in this feedstuff is highly correlated with CP and GE and negatively with MM, Ca, and P.

Using the information on chemical composition and the AMEn values obtained in the meta-analysis of the data, four prediction equations were generated for the AMEn of the animal meal (Table 5).

Table 1 - Descriptive statistics of the database with 32 samples (n) of poultry offal meal used in the meta-analysis¹

Variable	CP (g kg ⁻¹)	EE (g kg ⁻¹)	GE (kcal kg ⁻¹)	MM (g kg ⁻¹)	Ca (g kg ⁻¹)	P (g kg ⁻¹)	AMEn (kcal kg ⁻¹)
Minimum	467.8	101.4	3784	32.8	28.3	16.5	2384
Maximum	685.2	201.8	5622	210.7	61.9	39.2	4268
Average	634.6	157.8	5205	148.4	47.9	24.8	3330
Median	652.4	160.5	5577	147.9	51.2	22.6	3172
SEM	1.04	0.48	107	0.76	0.24	0.13	90.4
SD	5.61	6.46	912	6.82	7.17	4.69	678
CV%	10.85	15.59	16.46	25.73	24.56	28.02	22.17

CP - crude protein; EE - ether extract; GE - gross energy; MM - mineral matter; AMEn - nitrogen-corrected apparent metabolizable energy; SEM - standard error of the mean; SD - standard deviation; CV - coefficient of variation.

Table 2 - Descriptive statistics of the database with 25 samples (n) of meat and bone meal used in the meta-analysis¹

Variable	CP (g kg ⁻¹)	$EE (g kg^{-1})$	GE (kcal kg ⁻¹)	$MM (g kg^{-1})$	Ca (g kg ⁻¹)	$P(g kg^{-1})$	AMEn (kcal kg ⁻¹)
Minimum	349.9	98.7	3001	237.7	69.7	41.5	1183
Maximum	563.0	168.3	4668	498.9	177.6	87.1	2829
Average	433.6	123.4	3439	392.3	132.0	68.9	2251
Median	416.9	120.1	3571	416.4	132.3	73.8	1659
SEM	1.22	0.371	113	1.62	0.89	0.37	106
SD	5.73	1.73	505	7.59	3.80	1.57	499
CV%	16.23	14.07	15.51	19.35	28.77	22.92	28.47

CP - crude protein; EE - ether extract; GE - gross energy; MM - mineral matter; AMEn - nitrogen-corrected apparent metabolizable energy; SEM - standard error of the mean; SD - standard deviation; CV - coefficient of variation.

Table 3 - Pearson's correlation coefficient between AMEn and chemical components of poultry offal meal¹

Item	CP	EE	GE	MM	Ca	P
EE	0.144					
P*	0.483					
GE	0.801	0.720				
P*	0.002	0.022				
MM	-0.548	-0.150	-0.404			
P*	0.005	0.505	0.086			
Ca	0.231	0.334	0.578	0.386		
P*	0.278	0.218	0.009	0.076		
P	-0.218	-0.366	-0.441	0.352	-0.261	
P*	0.296	0.094	0.052	0.099	0.229	
AMEn	0.473	0.184	0.524	-0.545	-0.064	-0.380
P*	0.041	0.379	0.012	0.005	0.768	0.061

AMEn - nitrogen-corrected apparent metabolizable energy; CP - crude protein; EE - ether extract; GE - gross energy; MM - mineral matter.

Table 4 - Pearson's correlation coefficient between AMEn and chemical components of meat and bone meal¹

Item	CP	EE	GE	MM	Ca	P
EE	0.162					
P*	0.472					
GE	0.880	0.462				
P*	0.003	0.040				
MM	-0.776	-0.294	-0.856			
P*	0.005	0.184	0.007			
Ca	-0.469	-0.399	-0.761	0.520		
P*	0.049	0.101	0.001	0.027		
P	-0.733	-0.261	-0.811	0.866	0.780	
P*	0.001	0.295	0.002	0.003	0.005	
AMEn	0.787	0.416	0.836	-0.846	-0.505	-0.741
P*	0.000	0.054	0.006	0.004	0.032	0.003

AMEn - nitrogen-corrected apparent metabolizable energy; CP - crude protein; EE - ether extract; GE - gross energy; MM - mineral matter;

¹ Values adjusted for 920 g kg⁻¹ of dry matter.

 $^{^{\}rm 1}$ Values adjusted for 920 g kg $^{\rm -1}$ of dry matter.

¹ Values adjusted for 920 g kg⁻¹ of dry matter.

 P^{\ast} - probability, significant when P<0.05.

¹ Values adjusted for 920 g kg⁻¹ of dry matter.

P* - probability, significant when P<0.05

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Table 5 - Nitrogen-corrected apparent metabolizable energy (kcal kg⁻¹) prediction equations of animal meal (AM) in function of the chemical composition¹

AM	Intercept	CP	EE	GE	MM	Ca	P	R ²
POM	6139	-45.5		0.356	-123.5			0.8302
	8375	-46.1		0.0951	-113.0			0.8051
MBM	2267	19.9	67.9		-44.4			0.9021
	3113		69.9		-57.00			0.8827

POM - poultry offal meal; MBM - meat and bone meal; CP - crude protein; EE - ether extract; GE - gross energy; MM - mineral matter; R^2 - coefficient of determination.

Values adjusted for 920g kg $^{-1}$ of dry matter.

The most representative variables to predict the AMEn values of POM were CP, GE, and MM. As such, they were those which best explained the metabolizable energy value. The equation that obtained the best coefficient of determination was AMEn = 6139 - 45.5 CP + 0.356 GE - 123.5 MM (R² = 0.8302).

For MBM, the equation that best represented the energy values was AMEn = 2267 + 19.9 CP + 67.9 EE - 44.4 MM (R² = 0.9021).

Discussion

The observed amplitude of values can be considered wide enough for the purposes of this study, ensuring a broad scope for use of the generated equations. This is a desirable feature, suggesting that the database used allows for representative projections of the AMEn values of these feedstuffs for broilers.

The fact that the coefficients of variation of GE and AMEn were different can be explained by differences in the methodologies applied, inclusion levels of the tested feedstuff, poultry line, among other factors. Martosiswoyo and Jensen (1988), Jensen (1991), and Dale (1997) considered that the AMEn values of MBM are routinely underestimated when determined by methodologies in which the MBM level in control diet ranges from 40 to 50%. This is possibly because the elevated Ca and P levels provided by the high inclusion of MBM compromised the utilization of the other nutrients. Furthermore, the most adequate level of MBM inclusion in control diet to determine energy values is 20% (Faria Filho et al., 2002).

For EE, the variation may be linked to how the meal is processed, how it is defatted, and even the environment where it is transported (chutes with water, screw press, or mechanical conveyors) (Silva et al., 2010). Butolo (2002) observed that elevated EE levels can reduce the storage time of MBM by increasing its susceptibility to rancidification.

Different byproduct sources can be included in the production of animal meal for the poultry industry (e.g.,

feathers, blood, and viscera), which contributes to the variation in nutritional levels between these ingredients. In addition to the proportion of raw material used, the concentrations of AMEn, CP, MM, and EE and the quality and digestibility of the amino acids in POM also depend on processing methods and methods of measuring digestibility (Dale et al., 1993; Cao and Adeola, 2016).

The main variation factor in the production of MBM is the percentage of bones in the mixture. Higher concentrations of bone mean lower percentages of protein and GE and, consequently, higher MM contents. According to Dale (1997), the MM content is in general inversely proportional to the amount of crude protein in MBM.

Meat and bone meals are classified according to their CP content. According to Rostagno et al. (2017), this fraction may vary from 38 to 63% (fresh matter basis). The average CP data found in the present study fall within the category of "43%" established by Rostagno et al. (2017), which includes meals with CP contents between 40 and 45%.

Results for POM reveal that low levels of these minerals mean a higher metabolizable energy value. These findings agree with Silva et al. (2010), who found that AMEn in poultry viscera meal was positively correlated with CP and GE and negatively with MM, Ca, and P. Pesti et al. (1986), likewise, reported a high negative correlation between AMEn and the MM and Ca contents and a high positive correlation between AMEn and GE.

The difference in the chemical composition of MBM explains the variation in results found for AMEn, since, according to Eyng et al. (2011), together with the Ca and sodium ions, the high MM value causes saponification of the fats present in the animal meal, reducing the energy utilization of MBM by the birds. This ultimately leads to a decrease in the energy utilization of the feedstuffs.

Dale (1997), Wang and Parsons (1998), and Shirley and Parsons (2001) found that CP and GE decrease when the concentration of MM increases, and as the latter component increases, so do the Ca and P contents.

As the MM content increases, the concentration of digestible amino acids declines, resulting in decreased digestibility of the meal and a negative effect on the protein efficiency value, which in turn leads to a lower body weight gain of the birds (Shirley and Parsons, 2001).

Karakas et al. (2001) used bovine and swine MBM with different MM values in the feeding of broilers and observed that there was no significant difference between the MBM of different origins in the determination of AMEn. However, the authors noted that high levels of MM (above 43%) at high dietary inclusion levels (above 20%) reduced AMEn values.

The high and significant correlation coefficients between the independent variables (GE, MM, CP, EE, Ca, and P) and the dependent variable (AMEn) corroborate the literature results and provide a better understanding of the variations in the energy values of these feedstuffs.

Because AMEn is influenced by various factors, the choice of variables that might be part of the AMEn prediction model should respect the highest correlation coefficient that will exert an influence upon AMEn, but the ease of using this equation should also be considered. Additionally, models comprising a large number of variables may become complex, since some chemical analyses, which are not easily available, may often prevent the use of equations (Nascimento et al., 2011).

Equations determined by Rodrigues et al. (2002), composed of four variables in the model, explained 94% or more of the variation in the AMEn values of the soy-based feedstuffs. However, the equation composed of only two variables, EE and GE, explained 93% of the variations. This proves that the fit of a model with two independent variables can be well-applied in the estimate of AMEn of the feedstuffs.

Dolz and Blas (1992) studied MBM in poultry diets and obtained better predictions when they used two variables (CP and EE), which accounted for more than 96% of the total variation in the estimates of AMEn values. However, according to NRC (1994), in the case of MBM, the MM variable is important and should be included in the prediction equation.

Moreover, considering that determining MM is a practical procedure, it can be applied as an instrument for estimating the chemical composition, since, as stated by Najafabadi et al. (2007), the MM content is a good indicator of the chemical composition of animal-derived meals.

The NRC (1994) suggests an AMEn prediction equation developed by Janssen (1989) for MBM, equal to $33.94 \times DM - 45.77 \times MM + 59.99 \times EE$. For the prediction of AMEn for POM, the NRC (1994) indicates the equations developed by Pesti et al. (1986): $561 - 154 \times Ca - 622 \times P$ and $556 - 63 \times MM - 506 \times P$, both with $R^2 = 0.9300$.

To obtain a single prediction equation to estimate the AMEn values of protein vegetable feedstuffs commonly used in broiler diets, Nascimento et al. (2011) conducted a bibliographic review with Brazilian articles cataloguing information on the AMEn values and chemical composition of ingredients. In their review, the best fitting equation to estimate AMEn of protein feedstuffs was AMEn = 2,707.71 + 58.63 EE - 16.06 NDF ($R^2 = 0.8100$). Based on these results, the correlations among chemical components, the

variability of animal-derived feedstuffs, and the criterion adopted in the choice of mathematical models to determine the energy values of animal-derived meals are critical factors for a successful diet formulation.

Conclusions

The accuracy of fit of the prediction equation for nitrogen-corrected apparent metabolizable energy values, obtained via meta-analysis, is directly related to the variability in the chemical composition of the feedstuffs. The models indicated as adequate, based on the coefficient of determination, to estimate the nitrogen-corrected apparent metabolizable energy values of poultry offal meal and meat and bone meals are AMEn = 6139 - 45.5 CP + 0.356 GE - 123.5 MM (R² = 0.8302) and AMEn = 2267 + 19.9 CP + 67.9 EE - 44.4 MM (R² = 0.9021), respectively.

The chemical composition and energy values of animal-derived meals and their interaction can be used as data for the development of equations to predict the nitrogen-corrected apparent metabolizable energy contents of these feedstuffs. Considering the wide use of these ingredients in poultry diets, it is appropriate to determine and validate these equations.

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