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Full Length Research Paper

Prediction of metabolisable energy of poultry feeds by estimating *in vitro* organic matter digestibility

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The aim of this study was to develop equations to predict the *in vivo* apparent metabolisable energy (AME) of poultry feeds using an *in vitro* method for estimation of organic matter digestibility. In this study, a total of 57 samples of feedstuffs and 23 samples complete diets for poultry were used. Dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (CFat) and crude ash (CA) of the diets were determined. A modified method for estimating the enzymatic digestibility of organic matter (EDOM) was used. For the determination of *in vivo* ME, the rooster digestibility assay was followed. Obtained laboratory results, that is *in vitro* and proximate analysis values were regressed against the *in vivo* ME values and equations for predicting the *in vivo* ME of feeds for poultry have been derived. Using CA, CF, CFat and *in vitro* EDOM as predictors, the following equation for predicting the *in vivo* ME in poultry feeds was derived: ME (MJ/kg DM) = 5.46 – 0.2166 x CA – 0.0946 x CF + 0.2219 x CFat + 0.1054 x EDOM (R^2 = 0.844, RSD = 1.10). Using only EDOM as predictor generated the equation: ME (MJ/kg DM) = -0.41 + 0.1769 x EDOM (R^2 = 0.689; RSD = 1.63). Results show that using only EDOM as a predictor was not as accurate as when the other variables were included.

Key words: Metabolisable energy, prediction, poultry, feeds, organic matter digestibility.

INTRODUCTION

Quality control of animal feeds is commonly based on chemical analysis for determining the composition of the nutrients, example gross energy, protein, etc. It is questionable to what extent the results of chemical analysis of feed reflect its real quality, as these are only slightly influenced by physical and chemical treatments of feed, such as milling, heat treatment, enzyme treatment, etc., which all greatly influence the digestibility and thus availability of nutrients to the animal (Boisen, 2000; Palic et al., 2009). One of the most important parameters of feed quality is its energy, since it is needed for execution of metabolitic processes and animal activity. Not all energy of the feed (gross energy) will be utilized by the animal, but only a bio-available portion called metabolisable energy (ME). This parameter serves as an accurate indicator of feed quality, can be reliably used for feed quality control and is crucial for diet formulation (Farrel, 1999). Metabolisable energy is directly proportional to digestibility of nutrients, as it directly affects their availability and absorption (Čolović et al., 2011).The accepted method for direct determination of ME of feeds is by *in vivo* trials. These are often expensive and time-consuming. *In vitro* methods used for predicting ME are attractive because of rapidity and low cost (Farrel, 1999) and can be estimated directly from parameters accessible in the feeds (Noblet and Perez, 1993). Therefore, there has always been a need for reliable laboratory methods and related equations for

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Abbreviations: AME, Apparent metabolisable energy; **DM,** dry matter; **CP,** crude protein; **CF,** crude fibre; **CFat,** crude fat; **EDOM,** enzymatic digestibility of organic matter; **CA,** crude ash.

prediction of the *in vivo* ME values of feeds, in order to implement an adequate system of quality control (Alvarenga et al., 2011).

A number of methods have been developed for predicting the AME of feeds for monogastric animals, including method by Valdes and Leeson (1992) using a two step *in vitro* technique with pepsin, pancreatin, bile acids and enterokinase. The repeatebility of the method was similar to *in vivo* trials but the residual standard deviation of the prediction was high for some of the studied diets.

Boisen and Fernadiz (1997) modified the *in vitro* method by a three step enzymatic incubation of total tract energy digestibility in pigs. The authors investigated the relationship between the *in vitro* enzyme digestibility of organic matter (EDOM) and *in vivo* total tract digestibility of energy for 90 samples of 31 different feedstuffs. Their results showed a close relationship between predicted and determined digestibility of energy. *In vitro* metabolisable and digestible energy contents could also be predicted from chemical composition, employing either non-nutrients (ash and dietary fibre) or nutrients (starch, crude protein and crude fat) (Lijwgrex at al., 1992).

The objective of this study was to develop equations to predict the *in vivo* ME of poultry feedstuffs from the digestibility of organic matter as determined by an *in vitro* method not used nowadays for poultry feeds, in order to mimic what actually happens in the gastro-intestinal tract of the animal.

MATERIALS AND METHODS

A total of 57 feedstuffs and 23 commercial complete diets for poultry was used in this study.

Laboratory analysis

Proximate analysis

Dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (CF) and ash were determined according to AOAC official methods (2000).

In vitro determination of enzyme digestible organic matter (EDOM)

The three-step procedure of Boisen and Fernadez (1997) was modified to 2-step thus using incubation of feed sample with pepsin for 75 min, followed by incubation with pancreatin for 18 h. Solubilised protein was precipitated with sulphosalicylic acid. Insolubilised and precipitated materials were collected after filtration and then dried and finally ashed. Based on the results from determined dry matter and ash in the sample and residue, respectively, EDOM was calculated.

In vivo determination of true metabolisable energy (TME_n)

The method used is a procedure for determining digestibility of nutrients (McNab and Fisher, 1982, 1984; Fisher and McNab, 1987). This is a rapid bioassay technique in which 50 g of the test feed is introduced into the crop of an adult rooster by means of a stainless steel funnel and tube. Each test feed is replicated among six roosters. Excreta are collected during 48-h period. These are dried, weighed and analysed. Endogenous energy or amino acid losses are determined in roosters kept under the same conditions, but glucose is fed in place of the test ingredient. These endogenous losses are used to calculate the true digestibility of the test nutrient.

Statistical analysis

Using statistical package STATISTICA (Data Analysis Software System), v.8.0. (2008), obtained EDOM and proximate analysis values were regressed against the *in vivo* ME results.

RESULTS

The results of the proximate analysis, *in vivo* TME_n and *in vitro* EDOM are shown in Tables 1 and 2. Obtained laboratory results, that is, *in vitro* and proximate analysis values were regressed against the *in vivo* ME values and equations for predicting the *in vivo* ME of feeds for poultry, was derived as follows:

Using both EDOM and proximate analysis results for regression analysis led to the following regresion parameters (Table 3). Using crude ash (CA), crude fibre (CF), crude fat (CFat) and *in vitro* EDOM as predictors, the following equation for predicting the *in vivo* ME in poultry feeds was derived:

ME (MJ/kg DM) = 5.46 – 0.2166 x CA – 0.0946 x CF + 0.2219 x CF + 0.1054 x EDOM

R^2 = 0.844, RSD = 1.10

On the other hand, using only EDOM values for regression analysis led to the following regression parameters (Table 4). Regression of the *in vitro* EDOM values against *in vivo* ME results generated the following equation for predicting the *in vivo* ME in poultry feeds:

ME (MJ/kg DM) = - 0.41 + 0.1769 x EDOM
R^2 = 0.689; RSD = 1.63
Results of the regression analysis showed that using only EDOM as a predictor is not as accurate as when the other variables were included.

DISCUSSION

Poultry, like other livestock species, eat to meet energy requirement. Therefore, food intake can be predicted accurately if the energy concentration of a diet is known precisely and no essential nutrients are limiting. This information is crucial for diet formulations (Farrel, 1999). Energy value of diets is of great importance for animal feed manufacturers and end users. The amount of

Table 1. Results of proximate analysis, *in vivo* true metabolisable energy (TMEn) and *in vitro* enzyme digestible organic matter (EDOM) determination in complete diets of poultry.

Diet	No		DM (%)	CP (%)	CF (%)	CFat (%)	Ash (%)	OM (%)	TMEn (MJ/Kg DM)	*In vitro* EDOM (%)
Starter	13	Min	87.99	19.97	1.90	2.79	5.21	93.75	14.71	77.85
		Max	88.77	26.77	4.24	8.82	6.25	94.79	16.51	82.68
Grower	5	Min	85.30	17.19	2.06	4.32	4.28	88.61	12.60	71.50
		Max	89.84	25.99	7.25	9.74	11.39	96.00	17.18	86.76
Finisher	3	Min	88.90	16.63	3.68	7.50	6.43	91.71	14.63	83.31
		Max	89.85	27.37	8.87	11.06	8.29	93.57	16.92	84.94

No = Number of samples; DM = Dry matter; CP = Crude protein; CF = Crude fibre; CFat = Crude fat; OM = Organic matter; min = Lowest value; max = Highest value.

Table 2. Results of proximate analysis, *in vivo* true metabolisable energy (TMEn) and *in vitro* enzyme digestible organic matter (EDOM) determination in feedstuffs for poultry.

Feedstuff	No		DM (%)	CP (%)	CF (%)	CFat (%)	Ash (%)	OM (%)	TMEn (MJ/Kg DM)	*In vitro* EDOM (%)
Fishmeal	7	Min	88.70	69.01	0.19	7.10	13.32	84.14	13.53	87.27
		Max	91.22	75.45	0.75	15.11	15.86	86.68	17.58	95.49
Full fat soya	6	Min	90.61	38.20	5.50	15.86	4.85	94.41	17.31	70.76
		Max	93.82	41.49	9.98	20.96	5.59	95.15	18.17	82.73
Alfalfa hay	3	Min	88.09	17.45	33.16	1.04	7.08	89.01	4.68	28.42
		Max	90.55	20.05	34.70	3.54	10.99	92.92	6.13	36.82
Soya oilcake	8	Min	88.44	52.25	2.66	0.78	6.81	92.52	12.36	73.36
		Max	90.21	53.85	5.78	4.45	7.48	93.19	14.69	79.12
Sunflower oilcake	8	Min	88.87	37.74	15.68	0.92	5.57	91.17	7.14	53.00
		Max	92.00	41.51	23.69	6.79	8.83	94.23	11.97	60.72
Wheat bran	6	Min	87.16	16.45	8.85	3.34	4.38	94.39	9.58	51.72
		Max	89.68	17.85	10.21	4.23	5.61	95.62	11.23	60.21
White maize	2	Min	87.20	8.62	1.46	3.82	1.58	98.41	15.82	74.52
		Max	87.28	8.68	4.13	4.22	1.59	98.42	17.92	79.72
Yellow maize	15	Min	87.15	8.07	2.46	2.69	0.99	98.11	15.51	82.36
		Max	88.53	9.95	3.22	6.79	1.89	99.01	17.55	85.57

No = Number of samples; DM = Dry matter; CP = Crude protein; CF = Crude fibre; CFat = Crude fat; OM = Organic matter; min = Lowest value; max = Highest value.

available energy in feeds is described either by its metabolizable energy (ME) or by organic matter digestibility (OMD) (Pojić et al., 2008). Metabolisable energy is the most widely accepted value when expressing feed energy for poultry (Nwokolo, 1986; Farrell et al., 1991), however, its capability to estimate feed energy contents must be validated with *in vivo* determined values (Losanda et al., 2009, 2010).

In *vitro* methods used for predicting ME are rapid and not expensive (Farrel, 1999) as compared to *in vivo*

Table 3. Regression parameters using EDOM and proximate analysis results.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	4	553.36	138.341	114.77	<0.001
Residual	80	96.43	1.205		
Total	84	649.79	7.736		

Response variate: TMEn; fitted terms: constant, ash, crude fibre, crude fat and EDOM. Df, Degree of freedom; ss, sum of square; ms, mean of square; Fpr, final probability; vr, variance.

Table 4. Regression parameters using EDOM values.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	432.0	432.028	162.50	<0.001
Residual	72	191.4	2.659		
Total	73	623.5	8.540		

Response variate: TMEn; fitted terms: constant, EDOM.

determination of digestibility which is time-consuming and costly. Therefore, there has been a need for quick and reliable *in vitro* methods for determining nutrient digestibility in single feedstuffs for use in feed formulations and for control of complete diets (Boisen and Fernandez, 1997).

Estimation of ME values can be done by directly collecting the excreta of the animals but this method is relatively complex, expensive and needs several weeks for obtaining the results and requires separating urine from faeces which can be difficult (Noblet and van Milgen, 2007). There are several reports that metabolizable energy values vary according to the type of poultry used in their determination. Slinger et al. (1964) reported that chicks obtained more ME from high energy diets and less ME from low energy diets than did turkeys.
Several different equations to predict ME have been derived based on physical characteristics such as bulk density of grains, dry matter or organic matter digestibility, and chemical characteristics of a foodstuff or diet using proximate or other analyses (Farrel, 1999; Campbell et al., 1986). But in some cases, prediction equations may be less effective, particularly for processed ingredients. Nehring and Haenlein (1973) produced regression equations to predict ME based on the digestibility of each of the chemical components as determined in the Weende system of analysis. Furthermore, Farrel (1999) reported that general prediction equations, no matter how precisely they are derived, do not usually have universal application, particularly when used to predict ME of some individual ingredients as opposed to formulated diets.

Knowledge of the metabolisable energy of feedstuffs and complete diets for poultry is important in making decisions in poultry production, as feed cost has a major impact on economic parameters of poultry production (Tica et al., 2009)

Conclusion

Based on the results of this study, it can be concluded that the ME of feeds for poultry can be successfully predicted using the enzymatic procedure for determining the organic matter digestibility. Results of the statistical analysis showed that using only EDOM as a predictor is not as accurate as when the other variables from proximate analysis are included.

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