ISSN 1516-635X Apr - Jun 2010 / v.12 / n.2 / 129 - 132

Poultry Feed Metabolizable Energy Determination using Total or Partial Excreta Collection Methods

■ **Author(s)**

Dourado LRB[1,2]
Siqueira JC[2]
Sakomura NK[2]
Pinheiro SRF[2]
Marcato SM[3]
Fernandes JBK[4]
Silva JHV[5]

1 Universidade Federal do Piauí - Campus Profa. Cinobelina Elvas. Bom Jesus, PI.
2 Departamento de Zootecnia. Faculdade de Ciências Agrárias e Veterinárias - UNESP/ Jaboticabal, SP.
e-mail: sakomura@fcav.unesp.br;
3 Departamento de Zootecnia. Universidade Estadual de Maringá - UEM. Maringá, PR.
4 Centro de Aquicultura da UNESP/ Jaboticabal, SP.
5 Universidade Federal da Paraíba - Convênio PROCAD - UNESP/Jaboticabal-UFPB. Areia, PA.

■ **Mail Address**

LRB Dourado
Universidade Federal do Piauí
Campus Profa. Cinobelina Elvas
BR 135, Km 03, Planalto Horizonte
64.900-000. Bom Jesus, PI.

E-mail:leilane@ufpi.br

■ **Keywords**

Acid insoluble ash, AIA, broiler, digestibility, marker, partial collection method.

■ **Acknowledgements**

The authors thank DIACEL for supplying Celite™, and Nutron Alimentos Ltda. for donating mineral and vitamin supplements, as well as the coccidiostat product.

Part of this article was published in the proceedings of Reunião Anual da Sociedade Brasileira de Zootecnia, 2008.

Arrived: November/2009
Approved: March/2010

ABSTRACT

The aim of this work was to compare the efficiency of total (TC) or partial (PC) collection excreta methods to determine metabolizable energy in poultry feeds. A number of 180 12- to 21-day-old broilers were distributed into two treatments of six replicates of 10 birds each. A reference-diet was formulated to supply broiler requirements, and the test-diets consisted of 60% of reference diets and 40% of corn or soybean meal. Celite was added at 1% to the diets as a marker. Excreta and diet samples were analyzed for dry matter, energy, nitrogen, and acid-insoluble ash (AIA). AME of corn determined by partial collection (PC) was higher (3544 kcal/kg) as compared to total collection (TC) (3133 kcal/kg). However, no difference were observed for soybean meal (1797 vs. 1821 kcal/kg) between both methods. Marker recovery rates in the excreta were 101, 111, and 96% for the basal-diet, and the test-diets with corn or soybean meal, respectively. This result indicates the importance of marker recovery rate in the excreta to evaluate feed AME and digestibility.

INTRODUCTION

The method of total excreta collection is the most frequently used to determine metabolizable energy (ME) in broiler feeds, and it is based on quantifying feed intake and total fecal excretion for a determined period (Sakomura & Rostagno, 2007). However, this method has several limitations, including the contamination of excreta with feed, feathers, intestinal mucosa sloughing, which interferes with the final results. An alternative method is to perform partial excreta collection, determining ME by the ratio of indigestible substances, called markers, present in diets and excreta. According to Vasconcellos *et al*. (2007), indirect methods of ME determination are characterized by the use of markers.

According to Ramos (2003), marker is the term used to call the material used to qualitatively or quantitatively estimate nutritional phenomena, and it is considered a physical-chemical monitor of digestive processes. A good marker is a known, non-toxic substance that remains unchanged during the passage along the gastrointestinal tract. It must also not have any influence on the physiological processes of the digestive tract, associate with other substances, and must be easy to analyze and be completely recovered in the excreta (Kobt & Luckey, 1972, quoted by Sakomura & Rostagno, 2007). However, to date, there are no markers that comply with all these requirements (Marais, 2000).

Among the external markers used in studies with monogastric animals, chromium oxide is highlighted, but it has some inconveniences, such as the incomplete recovery and analytical variation when colorimetric methods are used (Sakomura & Rostagno, 2007).

Acid-insoluble ash (AIA) is an indigestible that contains mainly silica treated with hydrochloric acid, and it is considered an internal marker. However, as grains contains low AIA levels, external sources (Celite™, sand, and silica) may be added to diets to increase the accuracy of the estimates. These sources may be considered external markers (Sales & Jansen, 2003). Studies carried out at UNESP Jaboticabal using AIA (Barbosa *et al*., 2008; Santos *et al*., 2008) yielded good metabolizable energy and digestibility estimates of nutrients in broiler feeds. However, no reports were found in literature on the use of this marker to determine AME in feed ingredients.

The aim of this study was to compare the use of total or partial excreta collection using acid-insoluble ash as marker to determine apparent metabolizable of corn and soybean meal for broilers.

MATERIAL AND METHODS

The study was carried out at the Poultry Sector of the Animal Science Department of the School of Agrarian and Veterinary Sciences, UNESP, Jaboticabal, SP, Brazil, in January, 2007. A number of 180 Cobb broilers, from 12 to 21 days of age (experimental period), was used. From one to eleven days of age, birds were fed a diet formulated to supply their nutritional requirements, according to Rostagno *et al*. (2005). A reference diet (RD) was then formulated to supply the birds' nutritional requirements. Feedstuffs (corn and soybean meal) replaced RD at 40%. One percent Celite™ was used to determine AIA. Diet composition is shown in Table 1. The experimental treatments consisted of the methods used to determine corn and soybean metabolizable energy: T1 - total excreta collection method (TC) and T2 - partial excreta collection method (PC). A completely randomized statistical design, with two treatments with six replicates of 10 birds each was applied.

On day 12, birds were individually weighed and randomly distributed in battery cages (0.72m^2) to have similar average initial weight among experimental units. Birds were submitted to a period of adaptation of five days to the experimental diets.

The period of total excreta collection started on day 17. Trays lined with a plastic sheet were fitted under the cages to prevent the loss of excreta. Experimental feeds were weighed, and 1% ferric oxide was added on the first and on the last day of the collection period (day 21) to identify the excreta derived from the experimental diet. Therefore, in the first collection, non-marked excreta were discarded, as well as in the last collection. After removing feathers, feed residues, and other contamination sources, excreta from each experimental unit were collected early in the morning and in the evening.

Table 1 - Ingredient and nutritional composition of the experimental diets fed to broilers from 12 to 21 days of age.

Ingredients (%)	Diets		
	Reference (RD)	RD+Soybean meal	RD+Corn
Reference diet	-	59.400	59.400
Corn	58.105	-	39.600
Soybean meal	36.058	39.600	-
Soybean oil	1.233	-	-
Salt	0.489	-	-
DL-Methionine	0.202	-	-
L-Lyisine HCl	0.091	-	-
Calcitic limestone	0.941	-	-
Dicalcium phosphate	1.560	-	-
Vitamin supplement[1]	0.100	-	-
Mineral supplement[2]	0.100	-	-
Choline chloride	0.070	-	-
Coccidiostat[3]	0.050	-	-
Celite™[4]	-	1.000	1.000
Total (kg)	99 + 1[(4)]	100	100
Nutritional composition			
AME (kcal/kg)	2900	2627	3062
CP (%)	21.34	31.68	15.95
Calcium (%)	0.847	0.634	0.515
Available phosphorus (%)	0.400	0. 325	0.269
Sodium (%)	0.213	0.133	0.134

1 - Composition per kg product: Folic acid, 1000mg; pantothenic acid, 15000mg; antioxidant, 0.5g; niacin, 40000mg; selenium, 300mg; biotin, 60mg; vit. B1, 1800 mg; vit. B12, 12000mg; vit. B2, 6000 mg; vit. B6, 2800 mg; vit. A – 10,000,000 IU; vit. D3, 2,000,000 IU; vit. E, 15,000mg; vit. K3, 1800 mg; Addition of 1kg/t in the starter phase. 2 - Composition per kg product: Mn, 150,000mg; Zn, 100,000 mg; Fe, 100,000 mg; Cu, 16,000 mg; I, 1,500 mg. Addition of 0.5kg/t. 3 - Sodium monensin. 4 - Marker.

A sample of approximately 20% of the excreta was randomly collected to represent partial excreta collection. For the method of total excreta collection, excreta were weighed and homogenized, placed in plastic bags identified according to collection method and experimental unit, and frozen. At the end of the experimental period, feed intake and total amount of produced excreta were determined. Samples were thawed at environmental temperature. Excreta were homogenized and dried in a forced-ventilation oven at 55°C for 72 hours.

Dried excreta samples were ground in a micro-mill and submitted to the Animal Nutrition Lab. Feed and excreta dry matter, gross energy, nitrogen, and AIA contents were determined.

Dry matter (final drying) was determined according to Silva & Queiroz (2002). Gross energy was determined by pelleting the diets, which were burnt in a calorimetric bomb (1281, PARR, Instruments, USA). Nitrogen content was analyzed by the method of Kjeldahl according to the methodology described by Silva & Queiroz (2002).

AIA was determined using an adaptation of the methodology of Van Keulen & Young (1977). Approximately 0.75g of the pre-dried samples were weighed in 15-mL borosilicate conical tubes. Samples dried in a convection oven at 80°C for 12 hours, and then burnt in a muffle at 430°C for 10 hours. After cooling, samples were treated with 5mL hydrochloric acid (4N) and placed in a digestor block at 125°C for 30 minutes. Samples were left resting until complete cooling, and were then centrifuged for 10 min at 1500 rpm. The acid was discarded, and approximately 3mL distilled-deionized water were added to the sample. Samples were agitated in a vortex apparatus, and submitted again to centrifugation at the same rotation and for the same time. Final rinsing with water was performed three consecutive times, and after all samples were washed, they were dried again in the convection oven at 80°C for 12 hours. Samples were placed in the muffle at 430°C for 10 hours, and after cooling, samples were weighed to determine AIA percentage.

Apparent metabolizable energy (AME) values were determined based on analytical results according to Sakomura & Rostagno (2007). In order to justify the results, marker recovery rates in the excreta were calculated (g excreted/ g ingested x 100) for the three diets: reference diet (RD), RD + corn, and RD + soybean meal using total excreta production and feed intake data obtained with the total excreta collection method.

Data were submitted to homoscedasticity and normality analyses, outliers were removed, and then submitted to analysis of variance using the GLM procedures of SAS software package. Means were compared by the test of Student-Newman-Keuls at 5% significance level.

RESULTS AND DISCUSSION

Apparent metabolizable energy values were determined according the two methods and are shown in Table 2. Methods were significantly different (P<00001) only for corn energy determination. There was no difference between methods for soybean meal energy determination, indicating the AIA can be used as a marker to predict soybean meal AME. On the other hand, the partial collection method overestimated corn apparent metabolizable energy when AIA as compared to total collection method. AIA has been wiely used as a marker, yielding good estimates of nutrient digestibility and feedstuff metabolizable energy (Barbosa *et al*., 2008; Li *et al*., 2002; Santos *et al*., 2008; Scott & Boldaji, 1997).

According to Sales & Janssens (2003), marker recovery rate in the excreta is the most important criteria to consider when testing markers for digestibility studies, but there are few studies that report this rate. Marker recovery rates of the reference diet (RD), RD + corn, and RD + soybean meal were approximately 101, 111, and 96%, respectively, showing that corn energy values were affected by marker recovery rate in the excreta.

Despite not being commonly performed in practice, AME values were corrected by marker recovery rate. The ratio between marker content in the excreta and its recovery rate (marker percentage in the excreta/ recovery rate) was calculated, resulting in as estimate of marker content in the excreta at 100% recovery rate. The values obtained after correction are shown in Table 3. The statistical analysis of the corrected

Table 2 - Apparent metabolizable energy (AME) of corn and soybean meal determined by total or partial excreta collection method.

Method	AME (kcal/kg FM)		AME (kcal/kg DM)	
	Corn	Soybean meal	Corn	Soybean meal
Total collection	3133±89b	1797±101	3377±96b	1931±109
Partial collection	3544±68a	1821±155	3820±88a	1957±166
CV (%)	2.26	7.21	2.26	7.21
Probability	<0.0001	0.8033	<0.0001	0.8000

Means followed by the same letters in the same column are not different by the SNK test. FM=fresh matter; DM=dry matter.

Table 3 - Apparent metabolizable energy (AME) of corn and soybean meal as determined by total or partial excreta collection methods, and simulated by marker recovery rate.

Method	AME (kcal/kg FM)		AME (kcal/kg DM)	
	Corn	Soybean meal	Corn	Soybean meal
Total collection	3133±89	1797±101	3377±96	1931±09
Partial collection	3124±67	1760±104	3367±72	1891±112
CV (%)	2.52	5.76	2.51	5.76
Probability	0.8734	0.6303	0.8792	0.6301

FM=fresh matter; DM=dry matter.

values showed that AME values estimated by AIA were not significantly different as compared to those obtained using the method of total excreta collection, indicating that the differences observed in corn were due to the high marker recovery rate obtained with that diet.

Some factors may affect marker recovery rate, such as the amount of marker included in the diets. Cheng & Coon (1990) reported that the presence of high silica levels (higher than 2%) affects feed passage rate in the digestive tract, and therefore, digestibility. Scott & Boldaji (1997), working with wheat and oats with or with no enzyme addition and AIA levels of 0.5, 1.0, and 1.5%, found that in oats with enzyme addition, AME values varied as a function of AIA levels. Therefore, it may be assumed that diet composition may influence nutrient estimates when markers are used, as the content of some components, such as fiber, may affect feed passage rate and consequently marker recovery rate.

It must be noted that, in the present study, marker recovery rate in the reference diet (101%) was close to the optimal rate (100%), indicating the AME estimates in balanced diets are adequate when AIA is used. Based on these observations, it is evident that further studies on factors that interfere in the marker recovery rate in each individual feedstuff should be carried out in order to correctly estimate nutrient digestibility and feedstuff metabolizable energy.

CONCLUSIONS

Feedstuff metabolizable energy estimation using the partial excreta collection method with the use of acid-insoluble ash, was not similar to that obtained using total excreta collection method, demonstrating that total excreta collection is still the most adequate method.

REFERENCES

Barbosa NAA, Sakomura NK, Fernandes JBK, Dourado, LRB. Enzimas exógenas no desempenho e na digestibilidade ileal de nutrientes em frangos de corte. Pesquisa Agropecuária Brasileira 2008; 43: 755-762.

Cheng TK, Coon CN. Research note: calcium digestibility studies utilizing acid insoluble ash measurements. Poultry Science 1990; 69:2228-2230.

Li X, Dove H, Bryden WL. Comparison of digestibility markers in poultry. Asia Pacific Journal of Clinical Nutrition 2002; Suppl 11: 206.

Marais JP. Use of markers. In: D'Mello JPF, editor. Farm animal metabolism and nutrition. Wallingford : CaBI International; 2000. p. 255-277.

Ramos S. Comparação de diferentes indicadores com método de coleta total para determinação da digestibilidade aparente de diferentes dietas para eqüinos dissertação. Pirassununga (SP): Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo; 2003.

Rostagno HS. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 2a ed. Viçosa (MG): UFV; 2005. 186 p.

Santos FR, Hruby M, Pierson EEM, Remus JC, Sakomura NK. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. Journal Applied Poultry Reserach 2008; 17:191-201.

Sakomura NK, Rostagno HS. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: FUNEP; 2007. 283 p.

Sales J, Janssens GPJ. The use of markers to determine energy metabolizability and nutrient digestibility in avian species. World´s Poultry Science Journal 2003; 59:214-237.

Scott TA, Boldaji F. Comparison of inert markers [chromic oxide or insoluble ash (Celite™)] for determining apparent metabolizable energy of wheat- or barley-based broiler diets with or without enzymes. Poultry Science 1997; 76:594-598.

Silva DJ, Queiroz AC. Análise de alimentos: métodos químicos e biológicos. 3a ed. Viçosa (MG): UFV; 2002. 235p.

Van Keulen J, Young BA. Evaluation of acid-insoluble ash as natural marker in ruminant digestibility studies. Journal of Animal Science 1977; 44(2):282-287.

Vasconcellos CHF, Veloso JAF, Saliba EOS, Baião NC, Lara LJC. Uso da LIPE como indicador externo na determinação da energia metabolizável de alimentos em frangos de corte. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2007; 59(2):459-465.