

## Sources of acid insoluble ash as marker of corn digestibility in broilers

### Fontes de cinza insolúvel em ácido como indicador de digestibilidade do milho em frangos de corte

Edna Teles dos Santos<sup>1</sup>; Flávio Ferreira da Silva<sup>2</sup>; Fabrício Coelho Barbosa<sup>3</sup>; Genilson Bezerra de Carvalho<sup>4</sup>; Leonardo Atta Farias<sup>5</sup>; Daniel Biagiotti<sup>6</sup>; Roseane Madeira Bezerra<sup>7\*</sup>; Leilane Rocha Barros Dourado<sup>8</sup>

#### Highlights

AME and DMDC are similar in the two methods of excreta collection using kaolin.

CPDC is similar in the two methods of excreta collection using kaolin and celite.

Sand used with partial excreta collection method provided low AME and CPDC values.

#### Abstract

The objective was to evaluate three sources of acid insoluble ash (AIA) (celite, kaolin, and sand), as indicators, to determine the apparent metabolizable energy of corn on a natural matter (AME) basis, dry matter digestibility coefficient (DMDC), and crude protein digestibility coefficient (CPDC) using total and partial excreta collection methods. Two hundred and ten Ross broiler chicks of 18 to 27 days of age were used. Broilers were adapted to experimental the diet for four days, and excreta were collected for 5 days. A reference diet (RD) based on corn and soybean meal was prepared to meet the nutritional requirements of the birds. A test diet was prepared with 40% replacement of RD with corn. The formulated diets were: D1, 99% Reference diet + 1% celite; D2, 59.4% RD + 39.6 % corn + 1% celite; D3, 99% Reference diet + 1% kaolin; D4, 59.4% RD + 39.6 % corn + 1% kaolin; D5, 99% Reference diet + 1% sand; D6, 59.4% RD + 39.6 % corn + 1% sand. The treatments were distributed in a completely randomized design, with six diets and five replicates of seven birds each in a 2x3 factorial arrangement. There were significant interactions for

<sup>1</sup> Dra in Animal Science, Universidade Federal do Piauí, UFPI, Campus Profa Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: edna1906@hotmail.com

<sup>2</sup> Veterinarian, UFPI, Campus Profa Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: flavioferreira\_26@hotmail.com

<sup>3</sup> Master's Degree in Animal Science, UFPI, Campus Profa. Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: fabricio.fcb.barbosa@gmail.com

<sup>4</sup> Dr. in Animal Science, UFPI, Campus Profa. Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: ge.nilson.bezerra@hotmail.com

<sup>5</sup> Prof. Dr., UFPI, Campus Profa Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: leonardoatta@yahoo.com.br

<sup>6</sup> Prof. Dr., Colégio Técnico, UFPI, Campus Profa Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: biagiotti@ufpi.edu.br

<sup>7</sup> Dra in Animal Nutrition, UFPI, Campus Profa Cinobelina Elvas, Bom Jesus, PI, Brazil. E-mail: roseanemadeira@hotmail.com

<sup>8</sup> Profa Dra, UFPI, Campus Petrônio Portela, Teresina, PI, Brazil. E-mail: leilane@ufpi.edu.br

\* Author for correspondence

all variables investigated. It was observed that the AME and DMDC values of corn were similar in the two methods of collection, and CPDC values using kaolin and celite makers were similar. However, the AME and CPDC of corn using sand with partial collection method were underestimated by 17.70 and 15.53%, respectively compared to those with the total collection method. The AME values of corn with the collection methods using celite and, the DMDC using celite and sand were significantly different. It was observed that the DMDC using celite and sand provided significantly lower values (4.67 and 5.15%), respectively, and the AME using celite was 2.86% lower than that obtained by total collection. To determine the EMA, DMDC, and CPDC in broilers, it is more efficient to use celite<sup>TM</sup> and kaolin as markers with partial collection of excreta. Sand should not be used with partial excreta collection method, because it provides lower values of AME and CPDC compared to with total excreta collection method.

**Key words:** Kaolin. Metabolizable energy. Sand. Total collection.

## Resumo

Objetivou-se avaliar três fontes de cinza insolúvel em ácido (CIA) (celite, caulim, e areia), como indicadores para determinação da energia metabolizável aparente do milho na matéria natural (EMA), coeficiente de digestibilidade da matéria seca (CDMS), e coeficiente de digestibilidade da proteína bruta (CDPB) por meio dos métodos de coleta total e parcial de excretas. Foram utilizados duzentos e dez frangos da linhagem comercial Ross no período de 18 a 27 dias de idade. Os frangos foram adaptados à dieta experimental por quatro dias, e as excretas foram coletadas por 5 dias. Foi formulada uma dieta referência (DR) a base de milho e farelo de soja para atender as exigências nutricionais das aves. A dieta teste com substituição de 40% de milho à DR. As dietas formuladas foram: D1, 99% dieta referência + 1% celite; D2, 59.4% DR + 39.6 % milho + 1% celite; D3, 99% dieta referência + 1% caulim; D4, 59.4% RD + 39.6 % milho + 1% caulim; D5, 99% dieta referência + 1% areia; D6, 59.4% DR + 39.6 % milho + 1% areia. Os tratamentos foram distribuídos em delineamento inteiramente casualizado, com seis dietas e cinco repetições de 7 aves cada em esquema fatorial 2x3. Houve interações significativas para todas as variáveis investigadas. Observou-se que os valores de EMA e CDMS do milho são semelhantes pelos dois métodos de coleta utilizando caulim, e CDPB utilizando caulim e celite. Contudo, a EMA e o CDPB do milho usando areia na coleta parcial foram subestimados em 17,70 e 15,53%, respectivamente, em relação à coleta total. Os valores EMA do milho para os métodos de coleta usando celite e, o CDMS usando celite e areia foram significativamente diferentes. Observou-se que o CDMS com celite e areia proporcionou valores significativamente menores (4,67 e 5,15%), respectivamente, e a EMA com celite foi 2,86% menor que o obtido pela coleta total. Para determinar a EMA, CDMS e CDPB em frangos de corte, é mais eficiente usar celite<sup>TM</sup> e caulim como indicadores na coleta parcial de excretas. A areia não deve ser usada com o método de coleta parcial de excretas, pois fornece valores menores de EMA e CDPB em comparação com o método de coleta total de excretas.

**Palavras-chave:** Areia. Caulim. Coleta total. Energia metabolizável.

Current broilers have high weight gain, owing to high feed intake. Feed represents the largest fraction of production costs in commercial poultry. Energy content is the most expensive component of feed and one of the main factors that limits the performance of poultry animals. An accurate estimate of the energy value of foods is reflected in increases in weight gain and better feed conversion rates (Melo et al., 2014). Thus, it is necessary to accurately analyze the digestibility of nutrients and energy in feed.

The digestibility of nutrients and energy in diets indicates the portion of food consumed by the animal that can be digested and absorbed in the gastrointestinal tract (Moss et al., 2017). With this information, it is possible to formulate more accurate diets to meet the requirements for maintenance and productive performance of broilers.

Total excreta collection method has been used as a reference for estimating the digestibility coefficients of nutrients in diets, and consists of strict control of the amount of food ingested and that eliminated via excreta by the animal (A. G. M. Silva et al., 2018). However, high cost, long evaluation period, need for continuous labor, need for large sample size, extended time to adapt to experimental diet, and strict control of feed intake and excretion make indigestible marker method a feasible option for this purpose (Pombo, Valle, Bradi, & Bueno, 2016). Digestibility indicators more reliably determine the initial and final period

of excreta collection, obtaining more reliable results (Moss et al., 2017).

Therefore, the objective of this study was to evaluate three sources of acid insoluble ash (AIA) (celite, kaolin, and sand) as external markers to determine the apparent metabolizable energy of corn on a natural matter (AME) basis, dry matter digestibility coefficient (DMDC), and crude protein digestibility coefficient (CPDC) using the methods of total and partial excreta collection.

The experiment was carried out in the Poultry Section of the Campus Professora Cinobelina Elvas, at the Federal University of Piau  (UFPI), municipality of Bom Jesus, State of Piau . A total of 210 male Ross broilers of 18 to 27 days of age, weighing an average of 510+26g were used. The birds were initially housed in a conventional shed until 17 days of age, where they were given feed formulated to meet the nutritional requirements according to Rostagno et al. (2005).

On the 18th day, the birds were weighed and distributed into metabolic cages measuring 1 x 1 x 0.5m in length, width, height, respectively, equipped with excreta collection trays, lined with plastic. The birds received ration and water ad libitum.

A reference diet (RD) based on corn and soybean meal was formulated to meet the nutritional requirements of the birds. A test diet with a 40% replacement of RD with corn was also prepared (Table 1).

**Table 1**  
**Water already added with magnesium chloride (MgCl<sub>2</sub>)**

Ingredients	Inclusion level, %
Corn	60.84
Soybean meal	33.91
Soybean oil	1.453
Salt	0.434
DL-Methionine	0.241
L-Lysine HCl	0.190
Limestone	0.823
Dicalcium phosphate	1.777
Vitamin Supplement <sup>a</sup>	0.100
Mineral Supplement <sup>b</sup>	0.100
Choline chloride	0.070
Antimicrobial <sup>c</sup>	0.005
Coccidiostatic <sup>d</sup>	0.050
Total	100.00
Nutritional Composition	
Potassium, %	0.806
Chlorine, %	0.293
Linoleic acid, %	2.191
Apparent metabolizable energy, kcal/kg	3.000
Crude Protein, %	20.79
Calcium, %	0.884
Available phosphorus, %	0.442
Digestible Methionine, %	0.533
Digestible Methionine + Digestible cystine, %	0.814
Digestible Lysine, %	1.146
Sodium, %	0.214

<sup>a</sup> Vitamin supplement (composition per kg product): folic acid, 2,000 mg; pantothenic acid, 15 g; niacin, 60 g; biotin, 150 mg; vit. A, 13,000.00 IU; vit. B<sub>1</sub>, 4,000 mg; vit. B<sub>12</sub>, 20,000 mcg; vit. B<sub>2</sub>, 9,000 mg; vit. B<sub>6</sub>, 4,000 mg; vit. D<sub>3</sub>, 5,000.000 UI; vit. E, 80.000 UI; vit. K<sub>3</sub>, 4.000 mg;

<sup>b</sup> Mineral Supplement (composition per kg product): selenium, 300 mg; Mn, 100 g; Zn, 100 g; Fe, 40 g; Cu, 15 g; I, 1.000 mg;

<sup>c</sup> Zinc Bacitracin;

<sup>d</sup> Coxistac;

Treatments: celite, kaolin, and sand using total and partial collection;

Diets: D1- 99% Reference diet + 1% celite; D2- 59.4% RD + 39.6 % corn + 1% celite; D3- 99% Reference diet + 1% kaolin; D4- 59.4% RD + 39.6 % corn + 1% kaolin; D5- 99% Reference diet + 1% sand; D6- 59.4% RD + 39.6 % corn + 1% sand.

The treatments were distributed in a completely randomized design in a 2x3 factorial arrangement, to evaluate three sources of acid insoluble ash (celite, kaolin, and sand) as indicators and determine the apparent metabolizable energy of corn on a natural matter (AME) basis, dry matter digestibility coefficient (DMDC), and crude protein digestibility coefficient (CPDC) using two methods of excreta collection (total and partial). The treatments comprised six experimental diets (celite, kaolin, and sand using total and partial excreta collection), with five replications of seven birds each:

D1: 99% Reference diet + 1% celite;

D2: 59.4% RD + 39.6 % corn + 1% celite;

D3: 99% Reference diet + 1% kaolin;

D4: 59.4% RD + 39.6 % corn + 1% kaolin;

D5: 99% Reference diet + 1% sand;

D6: 59.4% RD + 39.6 % corn + 1% sand.

Birds of 18 to 27 days of age were adapted to the experimental diet for four days, and excreta were collected for five days. The diets were weighed and 1% ferric oxide was added on the first and last day of collection.

The excreta were collected twice a day, packed in plastic bags, identified, weighed, and stored in a freezer at -5°C. At the end of the experimental period, the amount of feed consumed and excreta produced were determined. Feces were thawed and homogenized, and approximately 10% was taken (partial collection) and dried in a forced ventilation oven at 55°C for 72 hours. Thereafter, excreta and feed samples were ground and sent to the laboratory for determination of dry matter, crude energy, and nitrogen contents according to D. J. Silva

and Queiroz (2002), and insoluble acid ash using a methodology adapted from Carvalho et al. (2013). The results obtained were used to calculate the apparent metabolizable energy (AME) values, according to the formulas described by Sakomura and Rostagno (2016).

All analyzed variables were checked for the presence of outliers, and assumptions of normality of student errors (Cramer-von-Misses test) and homogeneity of variances (levene test) were tested. After verifying the non-violation of the assumptions, the data were subjected to analysis of variance using the GLM procedure of the statistical program SAS (1996). To compare the means, the SNK test was used with 95% significance.

There were significant interactions ( $P \leq 0.05$ ) for all variables investigated (Table 2). It was observed that the values of AME and DMDC of corn using kaolin were similar in the two methods of collection. The values of CPDC using kaolin and celite were also similar. However, the AME and CPDC of corn using sand with partial collection method were underestimated by 17.70 and 15.53%, respectively compared to those in total collection method. The AME values of corn using celite were significantly different in both collection methods. Similarly, DMDC using celite and sand were significantly different. It was observed that the DMDC using celite and sand provided significantly lower values of 4.67 and 5.15%, respectively, and the AME using celite obtained by partial collection was 2.86% lower than that obtained by total collection.

Some authors reported that acid insoluble ash (AIA) may overestimate the digestibility and energy values of feed ingredients (Coca-Sinova et al., 2011). This

may occur owing to incomplete solubilization of soluble minerals in hydrochloric acid, such as calcium and phosphorus in animal feces, overestimating the AIA content in excreta (Zanatta et al., 2013).

Researchers are yet to find a substance with a perfect indicator feature.

However, Carvalho et al. (2013) showed that partial collection with marker method is comparable to the standard procedure of total collection of excreta, indicating that acid insoluble ash (AIA) can be used as a marker to predict the AME and digestibility coefficients of ingredients, as they present similar results.

**Table 1**  
**Water already added with magnesium chloride (MgCl<sub>2</sub>)**

Variables	Marker	Collection		Mean	Collection	Marker	Cb x Mc	CV <sub>a</sub> (%)
		Total	Partial					
AME, kcal/kg	Celite	3.49Aa	3.28Ab	3.38				
	Kaolin	3.68Aa	3.75Aa	3.72				
	Sand	3.56Aa	2.93Bc	3.24	<0.0001	<0.0001	<0.0001	3.79
	Mean	3.58	3.33					
DMDC, %	Celite	81.59Aa	77.78Ab	79.68				
	Kaolin	82.36Aa	84.72Aa	83.54				
	Sand	82.95Aa	78.68Ab	80.81	0.0835	0.0192	0.0296	3.56
	Mean	82.30	80.39					
	Celite	67.34Aa	68.06Aa	67.70				
CPDC, %	Kaolin	67.95Aa	70.77Aa	69.36				
	Sand	72.20Aa	60.99Bb	66.60	0.2334	0.5703	0.0196	7.35
	Mean	67.79	65.79					

<sup>a</sup> Coefficient of variation (%);

<sup>b</sup> Collection;

<sup>c</sup> Marker;

Mean values followed by different letters, uppercase in the rows and lowercase in the columns, are significantly different based on SNK test ( $p \leq 0.05$ );

Treatments: celite, kaolin, and sand using total and partial collection.

Acid insoluble ash could be used as an internal marker to calculate the digestibility of a diet precisely, and the method could be used as an alternative to total collection method in many animals (Figueiredo et al., 2019; Papadomichelakis & Fegeros, 2020). However, Prawirodigdo, Gannon, Leury and

Dunshen (2021) demonstrated that the basal diet and choice of indigestible marker can substantially influence the determination of apparent total tract digestibility and that the use of AIA as an indigestible marker varies with species of animal used.

To determine EMA, DMDC, and CPDC in broilers, it is efficient to use celite<sup>TM</sup> and kaolin as markers with partial collection of excreta. Sand should not be used with partial collection method, because the values of AME and CPDC were lower compared with the values obtained by total excreta collection method.

## References

- Carvalho, G. B., Dourado, L. R. B., Lopes, J. B., Ferreira, A. H. C., Ribeiro, M. N., Silva, S. R. G.,... Silva, F. E. S. (2013). Métodos de análise da cinza insolúvel em ácido utilizada como indicador na determinação da energia metabolizável do milho para aves. *Revista Brasileira de Saúde e Produção Animal*, 14(1), 43-53. doi: 10.1590/S1519-99402013000100005
- Coca-Sinova, A., Mateos, G. G., Gonzalez-Alvarado, J. M., Centeno, C., Lazaro, R., & Jimenez-Moreno, E. (2011). Comparative study of two analytical procedures for the determination of acid insoluble ash for evaluation of nutrient retention in broilers. *Spanish Journal of Agricultural Research*, 9(3), 761-768. doi: 10.5424/sjar/20110903-439-10
- Figueiredo, M. R., Saliba, E. O. S., Barbosa, G. S. S. C., Silva, F. A., Nunes, A. N., Silva, C. R. M., & Moreira, G. R. (2019). Use of indigestible markers to estimate the apparent dry matter digestibility of diets containing a cocoa by-product, *Semina: Ciências Agrárias*, 40(6), 2771-2782. doi: 10.5433/1679-0359.2019v40n6p2771
- Melo, C. C. V., Alvarenga, R. R., Santos, L. M., Oliveira, D. M., Lago, A. A., Oliveira, E. C.,... Zangeronimo, M. G. (2014). Influência de diferentes intervalos de coleta de excretas sobre o valor energético e de nutrientes metabolizáveis de alimentos para aves. *Acta Tecnológica*, 9(1), 27-32. doi: 10.35818/acta.v9i2.293
- Moss, P. C. B., Rezende, A. S. C., Saliba, E. O. S., Lana, A. M. Q., Moura, R. S., Cassou, F.,... Alves, G. E. S. (2017). Validation of Nanoli<sup>®</sup> as method to assess the apparent digestibility of nutrients on horses. *Arquivos Brasileiro de Medicina Veterinária e Zootecnia*, 69(3), 687-694. doi: 10.1590/1678-4162-8270
- Papadomichelakis, G., & Fegeros, K. (2020). Reliability of acid-insoluble ash as internal marker for the measurement of digestibility in rabbits. *World Rabbit Science*, 28(1), 1-12. doi: 10.4995/wrs.2020.12216
- Pombo, G., Valle, T. D., Bradi, R. A., & Bueno, I. C. S. (2016). Acurácia, precisão e robustez de indicadores internos para predição da digestibilidade aparente total de matéria seca em equinos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 68(3), 769-775. doi: 10.1590/1678-4162-8441
- Prawirodigdo, S., Gannon, N. J., Leury, B. J., & Dunshea, F. R. (2021). Acid-insoluble ash is a better indigestible marker than chromic oxide to measure apparent total tract digestibility in pigs. *Animal Nutrition*, 7(1), 64-71. doi: 10.1016/j.aninu.2020.07.003
- Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R. F., Lopes, D. C.,... Barreto, S. L. T. (2005). *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais* (2a ed.). Viçosa, MG: UFV.

- Sakomura, N. K., & Rostagno, H. S. (2016). *Métodos de pesquisa em nutrição de monogástricos* (2nd ed.). Jaboticabal: FUNEP.
- Silva, A. G. M., Borges, I., Neiva, J. N. M., Rodriguez, N. M., Saliba, E. O. S., Morais, S. A.,... Valle, R. C. A. (2018). Avaliação do lipe® como indicador externo de digestibilidade em ovinos recebendo dietas com torta de babaçu. *Revista Brasileira de Nutrição Animal*, 12(1), 39-44. Recovered from <http://www.nutricaoanimal.ufc.br/seer/index.php/higieneanimal/article/view/469/2349>
- Silva, D. J., & Queiroz A. C. (2002). *Análise de alimentos: métodos químicos e biológicos* (3rd ed.). Viçosa, MG: UFV.
- SAS Institute Inc. (1996) SAS/STAT User's Guide, Version 6, Fourth Edition, Vol. 2. SAS Proprietary Software Release 6.12. SAS Institute, Inc., Cary, NC.
- Zanatta, C. P., Gabeloni, L. R., Félix, A. P., Brito, C. B. M., Oliveira, S. G., & Maiorka, A. (2013). Metodologias para determinação da digestibilidade de dietas contendo fontes proteicas vegetal ou animal em cães. *Ciência Rural*, 43(4), 696-701. doi: 10.1590/S0103-84782013005000024