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Study of methodological variations in apparent nutrient metabolism determination in broiler chickens

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ABSTRACT - An experiment was conducted to define a protocol to determine metabolizable nutrient and energy values of diets. The metabolizability (M) was calculated of the dry matter (DM); crude protein (CP); gross energy (GE) and AME_n of a single diet. Eighty-one 21-day old (d) male birds were used. The tested methodologies were: Cr_2O_3 (0.5%) as an indicator (partial collection) or Fe_2O_3 (1%) as a marker; fasting (0, 4, 6 and 8 h) prior to excreta collection and at the end of the feeding period on the last day of collection (total collection). The excreta collection periods were also tested (3 and 5 days). Twenty 31-day old male broilers from the same group of birds were used to assess the effect of fasting on digestive organ weight. At the end of fasting the digestive organs were removed and weighed. Metabolism coefficients and energy were not different between 3 and 5 days of total collection. CPM was lower for marker utilization and 3 days excreta collection compared to the total collection. Eight hours fasting resulted in significantly lower CPM compared to the other periods or the no fasting. With the methodology of partial collection with 5 days of collection, the lowest values were observed for all the responses, compared to the 3 day collection period. The use of the total collection methodology produced the highest DMM and CPM compared to partial collection. No influence of fasting was observed on the digestive organ sizes, indicating that until 8 hours of fasting no changes were observed in either relative or absolute organ weight. However, the relative jejunum weight of birds submitted to 4 hours fasting was higher than that of birds under no fasting. Total collection, during a 3 day period, without fasting and marker use, is the best methodology for ingredient and feed evaluation of growing birds.

Key Words: chromic oxide, excreta collection, fasting, ferric oxide, metabolizable energy organ size

Estudo de variações metodológicas na determinação do metabolismo aparente de nutrientes em frangos de corte

RESUMO - Realizou-se um ensaio com frangos de corte para a definição de um protocolo de determinação da metabolizabilidade dos nutrientes e da energia da dieta. Foram estimados os coeficientes de metabolizabilidade de matéria seca (MS), proteína bruta (PB), energia bruta (EB) e a energia metabolizável aparente corrigida para nitrogênio (EMA_n) de uma mesma dieta, utilizando-se 81 aves, de 21 dias de idade. As metodologias testadas foram as seguintes: utilização de 0,5% de Cr_2O_3 como indicador (coleta parcial) ou 1% de Fe_2O_3 como marcador e aplicação ou não de jejum (0, 4, 6 e 8 horas) pré-início de coleta e após a última refeição, no último dia de coleta (coletas totais). Períodos de coleta de três ou cinco dias também foram testados. Retiradas do mesmo grupo original, 20 aves de 31 dias de idade foram usadas para avaliar o efeito do jejum sobre o tamanho dos órgãos digestivos. Após o jejum, os órgãos foram retirados e pesados. Os coeficientes de metabolizabilidade e a EMA_n determinados pelo método de coleta total não diferiram dos obtidos pelo método de três ou cinco dias de coleta. O uso do marcador e três dias de coleta resultou em menor metabolizabilidade da PB em relação à coleta total sem marcador. Jejum de 8 horas resultou em metabolizabilidade da PB significativamente menor em comparação aos demais períodos de jejum ou à ausência de jejum. Na coleta parcial de cinco dias, os coeficientes de metabolizabilidade foram menores para todas as respostas. Pelo método de coleta total, obtiveram-se maiores metabolizabilidades da MS e PB em comparação ao método de coleta parcial. Não foi observada influência do jejum sobre o peso absoluto ou relativo da maioria dos órgãos. No entanto, o peso relativo do jejuno das aves submetidas a 4 horas de jejum foi maior que o das aves sem jejum. A coleta total de excretas durante três dias, sem jejum e sem o uso de marcador, consiste no melhor método de avaliação de ingredientes e ração em frangos de corte em crescimento.

Palavras-chave: coleta de excreta, energia metabolizável, jejum, óxido férrico, óxido crômico, tamanho de órgão

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Introduction

The method of total excreta collection is the direct method most commonly used to determine the metabolizability of nutrients and metabolizable energy (ME) of feeds or of their ingredients (Sakomura & Rostagno, 2007). Nevertheless, over the years researchers have introduced variations in the method, one of which is duration of collection period. Shang et al. (1982) observed that the minimum time required for the determination of metabolizable energy is 2 days, while Avila et al. (2006) suggested that 4 days are needed to collect excreta in order to obtain reliable results.

To obtain a more precise definition of the collection period, markers are added to feeds on the first and last collection day (Sakomura & Rostagno, 2007). The explanation for the adoption of markers is that they move together with the digesta along the lumen of the gastrointestinal tract (Rodriguez, 2005). Nevertheless, this may not be true in birds, due to anatomical differences and the variations in digesta retention times in specific sites of their gastrointestinal tract (Vergara et al., 1989). Another variation is the adoption of fasting to reduce errors by guaranteeing that the excreta collected is generated from the feed actually ingested. However, fasting plays a direct role in the gastrointestinal tract and triggers changes in sizes of digestive organs and in nutrient transport (Starck, 1999).

Among the indirect methodologies currently used is partial collection using indicators. Adding indicators to feeds eliminates the need to measure feed intake and to collect all the excreta produced (Sales & Janssens, 2003b). This method produces good results compared to total collection, although indicator recovery in excreta may vary (Jagger et al., 1992).

Considering the changes that have been introduced over the years in *in vivo* methodologies, it has become necessary to investigate their impact on metabolic responses of birds and to assess the actual relevance of such regimes. The present study compares these methods, aiming at defining a simple and accurate protocol.

Material and Methods

This experiment was conducted in the Laboratório de Ensino Zootécnico (LEZO), Universidade Federal do Rio Grande do Sul (UFRGS), and the of feed and excreta were analyzed in the Laboratório de Nutrição Animal, UFRGS. Chromium content analyses were carried out in the Laboratório de Minerais, Faculdade de Zootecnia e Engenharia de Alimentos (USP).

Eighty-one male Ross × Ross 308 chickens (21 days of age; mean weight 766 ± 75 g) were used in this study. All birds were housed in individual 0.10 m^2 metabolic cages equipped with individual feeders and watering troughs and kept in a controlled environment with constant lighting throughout the experiment. Water was offered *ad libitum*, and all birds were fed twice a day the same baseline meal diet prepared with corn and soy meal as recommended by Rostagno et al. (2000), according to the studied methodologies.

The methodologies to determine metabolizability included total or partial excreta collection for 3 or 5 days, with or without fasting of solid feeds (for 4, 6 or 8 hours), and with or without the use of ferric oxide (Fe_2O_3) as marker or chromium oxide (Cr_2O_3) as indicator.

The broilers were grouped following a randomized design for the 9 methodologies and 9 replications. One bird was defined as one experimental unit. An initial 4-day adaptation period was observed to adapt birds to the diet and environment, after which the collection periods were initiated for each methodology (3 or 5 days). The experimental diet was consumed throughout the experimental period, except for the methodologies that included Fe_2O_3 as marker. Chromium oxide was administered as of the first day of the adaptation period.

In the methodologies that included total collection for 3 days with 4, 6 or 8 h fasting regimens, fasting was observed on the first day of the experimental period, before the first collection was conducted. Once the fasting that preceded the first feeding period had finished, the excreta collection trays were cleaned and the accumulated material was disposed of. On the last day of the experimental period, the excreta produced during the interval between the last feed offer and the end of the fasting period was collected, for each methodology.

For the methodologies designed to include a marker, 1% Fe_2O_3 was added to the first meal given on the first and on the last collection days. The beginning and the end of the collection period were defined based on the observation of the marker in excreta. Thus, the excreta that were not marked in the first collection and those marked on the second collection were discarded. In the methodologies using indicator, 0.5% Cr_2O_3 was added to the experimental diet. For the partial excreta collection, samples were collected, at 5 different sites on the tray and pooled to obtain a representative sample.

Excreta were collected twice a day (at 8 a.m. and 4 p.m.) to prevent fermentation. Next, excreta were stored at -10°C.

Dry matter (DM), nitrogen (N) and gross energy (GE) were analyzed in diets and excreta in accordance with the recommendations by AOAC (1995). In the DM analysis, excreta were acidified with HCl to prevent nitrogen loss during the drying process (Ribeiro et al., 2001). The chromium content was determined following the methodology described by Graner (1972) and samples were analyzed by atomic absorption spectrometry (AOAC, 1995). Metabolizability coefficients of DM, of crude protein (CP) and of GE were calculated. Apparent metabolizable energy corrected for N content (AMEn) was calculated using equations described by Matterson et al. (1965). In the methodology using Cr_2O_3, the of AMEn and DM metabolizability was calculated using the chromium indigestibility factor (Rodriguez et al., 2005).

In the assessment of the influence of fasting on organ size, 5 birds used in the total excreta collection for 3 days, with or without fasting for 4, 6 and 8 h were used. After the last collection birds were weighed, killed by cervical dislocation and eviscerated to obtain the organs of the digestive tract. Crop, gizzard + proventriculus, duodenum + pancreas, jejunum, ileum and caeca were removed and individually weighed including content. Subsequently, these organs were emptied and weighed again. The time the digesta moved through the gastrointestinal tract was measured using the birds that received Fe_2O_3 as marker. A chronometer was used to measure the time between the moment the feed was offered and the appearance of the first marked excreta. The rate of passage was individually measured for each replication.

The analyses of variance were conducted according to the GLM procedure (General Linear Models) in SAS (2001). The methodologies were compared as follows: 3 days with total collection without fasting and 5 days without fasting; 3 days with total collection without fasting and 3 days using the marker Fe_2O_3; 5 days total collection without fasting and 5 days total collection with the Fe_2O_3 marker; 3 days with partial collection using the Cr_2O_3 indicator and 5 days partial collection with the Cr_2O_3 indicator; 3 days total collection without fasting and 3 days partial collection using the Cr_2O_3 indicator; 3 or 5 days with collection without fasting and 3 days with fasting for 4, 6 and 8 h. ANOVA was also used to analyze the 3-day collections after 0, 4, 6 and 8h fasting and their respective means were analyzed using the Tukey test.

Results and Discussion

Considering the metabolizabilities of DM, CE, CP and AMEn (Table 1) determined using the different collection methodologies, it is possible to state that the method used promoted significant variations across the results obtained and that the lowest values were observed mostly in the partial excreta collection regimen for 5 days, followed by the method that used partial collection for 3 days.

The metabolizability values determined were similar to those observed in other assays with broiler chickens and were between 70 and 77% for DM (Oliveira et al., 2007; Vasconcellos et al., 2007; Rodrigues et al., 2005; Corrêa et al., 2002), 74 and 77% for CE (Vasconcellos et al., 2007; Rodrigues et al.; 2005) and 63 and 67% for CP (López et al., 2007; Oliveira et al., 2007). Apart from this, the values of AMEn obtained in this experiment were very near the formulated values of 3.055 kcal ME/kg (Table 2).

When contrasts were analyzed (Table 2), no statistically significant difference (P>0,05) was observed between the collection periods of three and five days, for any of the results obtained. Likewise, Sales & Janssens (2003b) did

Table 1 - Metabolizability of dry matter, crude energy, crude protein and apparent metabolizable energy corrected for nitrogen balance (AMEn) and determined using different *in vivo* methodologies in 21-day-old broiler chickens

Total collection	Dry matter (%)	Crude energy (%)	Crude protein (%)	AMEn (kcal/kg NM)
5 days without fasting	76.0 ± 1.0a	79.5 ± 1.1a	69.2 ± 1.6a	3084 ± 40abc
3 days without fasting	74.0 ± 0.46ab	78.5 ± 0.38a	67.6 ± 0.82ab	3044 ± 15abc
3 days with 4h fasting	74.8 ± 0.79ab	77.0 ± 0.88ab	66.5 ± 1.0abc	2987 ± 34bcd
3 days with 6h fasting	75.0 ± 0.42ab	77.5 ± 0.45ab	66.1 ± 0.75abc	3005 ± 17abcd
3 days with 8h fasting	72.9 ± 0.84 ab	76.0 ± 0.88ab	62.4 ± 1.3cd	2949 ± 34cd
3 days with marker Fe_2O_3	73.5 ± 0.63ab	78.9 ± 0.47a	63.9 ± 0.94bc	3121 ± 19ab
5 days with marker Fe_2O_3	75.3 ± 0.95ab	79.2 ± 0.95a	67.2 ± 1.1abc	3135 ± 38a
Partial collection				
3 days with indicator Cr_2O_3	71.6 ± 0.91bc	77.4 ± 0.84ab	62.9 ± 0.99bcd	3029 ± 33abcd
5 days with indicator Cr_2O_3	68.9 ± 1.0c	74.1 ± 1.1b	57.9 ± 1.3d	2898 ± 43d
Coefficient of variation (%)	3.3	3.2	5.2	3.2
P value	0.0001	0.0002	<0.0001	<0.0001

Means in one same column followed by different letters (P<0.05) in the Tukey test.
NM = natural matter.

Table 2 - Comparisons by orthogonal contrast between metabolizabilities and AMEn determined using different *in vivo* methodologies in 21-day-old broiler chickens

Comparison	Dry matter (%)	P	Crude Protein (%)	P	Crude energy (%)	P	AMEn (kcal/kg NM)	P
Total collection (3 days) vsTotal collection (5 days)	74.0 ± 0.46 *vs* 76.0 ± 1.0	0.08	67.6 ± 0.82 *vs* 69.2 ± 1.6	0.31	78.5 ± 0.38 *vs* 79.5 ± 1.1	0.37	3044 ± 15 *vs* 3084 ± 40	0.37
Total collection (3 days) *vs* Total collection (3 days) with Fe_2O_3	74.0 ± 0.46 *vs* 73.5 ± 0.63	0.64	67.6 ± 0.82 *vs* 63.9 ± 0.94	0.02	78.5 ± 0.38 *vs* 78.9 ± 0.47	0.72	3044 ± 15 *vs* 3121 ± 19	0.08
Total collection (5 days) *vs* Total collection (5 days) with Fe_2O_3	76.0 ± 1.0 *vs* 75.3 ± 0.95	0.52	69.2 ± 1.6 *vs* 67.2 ± 1.1	0.20	79.5 ± 1.1 *vs* 79.2 ± 0.95	0.80	3084 ± 40 *vs* 3135 ± 38	0.26
Partial collection (3 days) with Cr_2O_3 *vs* Partial collection (5 days) with Cr_2O_3	71.6 ± 0.91 *vs* 68.9 ± 1.0	0.02	62.9 ± 0.99 *vs* 57.9 ± 1.3	0.01	77.4 ± 0.84 *vs* 74.1 ± 1.1	0.01	3029 ± 33 *vs* 2898 ± 43	0.005
Total collection (3 days) *vs* Partial collection (3 days) with Cr_2O_3	74.0 ± 0.46 *vs* 71.6 ± 0.91	0.04	67.6 ± 0.82 *vs* 62.9 ± 0.99	0.01	78.5 ± 0.38 *vs* 77.4 ± 0.84	0.35	3044 ± 15 *vs* 3029 ± 33	0.73
Partial collection (3 and 5 days) without fasting *vs* total collection (3 days) with fasting	75.0 ± 0.59 *vs* 74.2 ± 0.44	0.29	68.4 ± 0.88 *vs* 65.0 ± 0.69	0.01	79.0 ± 0.56 *vs* 76.8 ± 0.44	0.01	3064 ± 21 *vs* 2980 ± 17	0.01

not observe differences in DM metabolizabilities in collections that lasted 1, 3, 6, 10 or 14 days. Similarly, Avila et al. (2006) and Rodrigues et al. (2005) did not observe differences in metabolism results within 2, 3, 4 and 5 days of collection. Nevertheless, in the present paper the lowest coefficient of variation obtained for all the results evaluated was observed in the 3-day collection period without fasting in comparison with the 5-day collection period without fasting. The coefficients were: 1.75 *vs* 3.74 for DM metabolizability, 3.58 *vs* 6.74 for CP, 1.38 *vs* 3.74 for CE and 1.45 *vs* 3.93 for AMEn, respectively, revealing that this method is the most appropriate for this type of assay.

Regarding the use or not of the Fe_2O_3 marker, the metabolizabilities were similar for the two methodologies. However, the use of the marker in the 3-day collection led to the lowest CP metabolizability ($P<0.02$), which may be explained by the specific aspects concerning the digesta flow in birds. The crop and gizzard play an active role in digesta retention, while the liquid phase is retained longer in the caecum. Therefore, marker flow depends on the phase of the digesta to which it is associated (Vergara et al., 1989), and the 3-day collection period may not be enough to obtain the full appearance of the marker in the excreta and thus underestimate the metabolizability of some nutrients.

All the results obtained using partial excreta collection with the Cr_2O_3 indicator were lower ($P<0.05$) in the 5-day collection as compared to the 3-day collection. Rodriguez et al. (2005) observed a similar result, which points to the lower indicator recovery rate in the excreta of the birds analyzed. In this methodology, it is assumed that the Cr_2O_3 indicator moves along the gastrointestinal tract without reacting with nutrients and digestive enzymes, without solubilizing or being absorbed, and should be thoroughly recovered in feces, without even undergoing any interference from the natural chromium (Neto et al., 2003). However, commercially available forms of Cr_2O_3 may contain small amounts of soluble forms of the metal that, if absorbed, may affect results (Neto et al., 2003). According to Sales & Janssens (2003a), the lower results obtained with this methodology are explained by the inherent mistakes to it, such as the variability in the indicator recovery rate in excreta or the lack of uniformity due to the manner in which the indicator combines with larger particles.

In the comparison between 3-day total and partial collections (3-day total collection without fasting *vs* 3-day collection with Cr_2O_3), it was observed that metabolizability for DM and CP was lower in the partial collection, showing that the indicator underestimates values compared to the total collection. The Cr_2O_{3-} indicator, although moving mainly along with the digesta solid phase, could accumulate

partially in the watery phase affecting AME values (Palander, 2006), which explains the lower results obtained in the present study.

The last comparison, between methodologies with and without fasting, regardless of duration, proved that DM and CE metabolizabilities and that diet AMEn were higher in the birds managed without fasting (P<0.01), which confirmed that the withdrawal of food for 4 h or longer may underestimate metabolism results.

In the analysis of the methodology with and without different fasting periods and 3-day collection (Table 3), no difference (P>0.05) was observed between DM and CE metabolizabilities and AMEn of birds submitted or not to different fasting periods. No statistical difference was detected (P>0.05) between the CP metabolizability evaluated in broilers submitted to 0, 4 and 6h fasting periods. Nevertheless, the 8h fasting period led to lower metabolizability (P<0.01), which corroborated the hypothesis that fasting causes changes in nutrient absorption and metabolism (Ferraris & Carey, 2000). It is possible that the lower CP metabolizability was the outcome of increased endogenous and mainly of metabolic losses that occur with increased fasting times. In this case, the larger excretion of nitrogen as uric acid in urine during the last hours of fasting could explain the results.

It is worth stressing that bird age is an important variable. In young birds, up to 7 days of age, a gradual filling of the gastrointestinal tract may occur as of a former situation of emptiness and it is possible to obtain negative metabolism results. In such cases, fasting may be important to solve these kinds of problem.

The relative weight of the digestive tract organs of broiler chickens in the several fasting periods (Table 4) indicated that the fasting of up to 8 h did not produce any significant effect on organ weights or segments evaluated. The exception was the jejunum, that was lighter in birds that did not undergo fasting as compared to those that were submitted to the 4h fasting period. Considering the other fasting periods, the values were intermediary.

One of the explanations for the lowest jejunum weight in birds not submitted to fasting is the fact that ingesta accumulates more expressively in the jejunum, over the first hours of fasting, to start nutrient recycling (Clech & Mathias, 1995). This recycling compensates for the lack of feed in the gastrointestinal tract and thus causes changes in enzymatic activity, greater cell proliferation in this segment and therefore an increase in gastrintestinal weight.

The pattern of results obtained in this experiment is the same obtained by Hinton et al. (2000b), in a study that revealed that the adoption of fasting for 0, 6, 12 18 and 24 h did not diminish caecum weight. Also, Buhr et al. 1998, 2003) verified that most of this weight loss was explained by the emptying of the gastrintestinal tract, not by organ weight loss *per se*, during the first 6 hours of fasting. Cabrera & Saadoun (2006) and Karasov & McWilliams (2005) observed that feed restriction led to losses of small intestine weight only after fasting periods in excess of 21 h.

The age of the birds used in this experiment may also offer an explanation for the lack of effects of fasting on the size of the gastrointestinal organs. In these animals, the gastrointestinal tract was fully developed and the organism placed greater emphasis on enzymatic responses, absorptive mechanisms and even behavioral mechanisms, in order to save energy. Therefore, no organ or segment of any organ suffered the influence of fasting or of different fasting periods on absolute weight. Yet, in 1-day-old birds the effects of fasting may be quite remarkable (Geyra et al., 2001).

Contents of the crop, proventriculus + gizzard, small intestine , jejunum, and ileum were respectively larger in birds that did not undergo fasting compared to the fasting ones (P<0.05), but these contents did not vary across the different fasting periods (Table 5). The contents of duodenum and caecum + colon, however, did not differ for the different methodologies tested.

The content of the crop of the birds submitted to 4, 6 and 8h fasting periods was essentially water. Similarly,

Table 3 - Metabolizability of dry matter, crude energy crude protein and AMEn determined using several methodologies in 21-day-old broiler chickens

	Metabolizability			
Methodology	Dry matter (%)	Crude energy (%)	Crude protein (%)	AME_N (kcal/kg NM)
Total collection (3 days) without fasting	74.0 ± 0.46	78.5 ± 0.38	67.6 ± 0.82a	3044 ± 15
Total collection (3 days) with 4-h fasting	74.8 ± 0.79	77.0 ± 0.88	66.5 ± 1.0a	2987 ± 34
Total collection (3 days) with 6-h fasting	75.0 ± 0.42	77.5 ± 0.45	66.1 ± 0.75a	3005 ± 17
Total collection (3 days) with 8-h fasting	72.9 ± 0.84	76.0 ± 0.88	62.4 ± 1.3b	2949 ± 34
Coefficient of variation (%)	2.64	2.67	4.62	2.73
P	0.10	0.10	0.01	0.08

Means in one same column followed by different letters differ (P<0.05) in the Tukey test.
NM = natural matter.

Table 4 - Relative weight (%) of emptied digestive organs of broiler chickens submitted to different fasting periods

Fasting (h)	Relative organ weight[1] (%)							
	BW (g)	Crop	Gizzard and proventriculus	Small intestine	Duodenum+ Pancreas	Jejunum	Ileum	Caecum + Colon
0	1464 ± 66	0.5 ± 0.02	2.5 ± 0.12	2.9 ± 0.11	0.9 ± 0.07	0.9 ± 0.04b	1.0 ± 0.06	0.6 ± 0.04
4	1460 ± 46	0.5 ± 0.02	2.4 ± 0.12	3.2 ± 0.06	0.9 ± 0.02	1.2 ± 0.04a	1.1 ± 0.06	0.6 ± 0.02
6	1428 ± 50	0.6 ± 0.02	2.5 ± 0.05	2.9 ± 0.09	0.8 ± 0.04	1.1 ± 0.06ab	1.0 ± 0.02	0.6 ± 0.02
8	1426 ± 29	0.6 ± 0.05	2.6 ± 0.09	3.1 ± 0.17	0.9 ± 0.03	1.1 ± 0.08ab	1.1 ± 0.09	0.5 ± 0.01
CV (%)	7.6	12.8	8.8	8.5	11.3	11.5	13.0	9.5
P	0.91	0.50	0.68	0.22	0.26	0.03	0.35	0.84

[1]Percentages correspond to the weight of the organ relative to the animal weight.
Means in one same column followed by different letters differ in the Tukey test (P<0.05).
CV = coefficient of variation.

Table 5 - Content of the gastrointestinal tract (g) of broiler chickens submitted to different fasting periods

Fasting (h)	Content Weight (g)						
	Crop	Gizzard + proventriculus	Small intestine	Duodenum	Jejunum	Ileum	Caecum + Colon
0	13.1 ± 2.4a	17.6 ± 1.5a	51.9 ± 5.8a	8.0 ± 0.9	28.8 ± 2.6a	19.1 ± 1.7a	7.1 ± 2.5
4	3.4 ± 0.9b	13.0 ± 1.9ab	23.3 ± 3.4b	6.4 ± 1.0	9.4 ± 1.9b	7.6 ± 1.8b	7.7 ± 1.8
6	1.5 ± 0.4b	10.2 ± 1.5b	16.0 ± 0.6b	5.2 ± 1.0	8.3 ± 0.9b	2.5 ± 0.3b	3.7 ± 0.8
8	1.0 ± 0.45b	9.8 ± 2.0b	15.8 ± 1.5b	5.5 ± 0.8	7.0 ± 0.9b	3.9 ± 1.1b	3.0 ± 0.6
CV (%)	62.4	30.8	28.8	33.0	28.9	36.8	66.7
P	0.01	0.02	0.01	0.17	0.01	0.01	0.13

Means in the same column followed by different letters differ in the Tukey test (P<0.05).
CV = coefficient of variation.

Hinton et al. (2000b) and Savage (1998) observed a decrease in crop content during the first hours of fasting periods. On the other hand, the presence of ingesta in the gizzard + proventriculus of birds that are under fasting is due to decreased pylorus activity, which obstructs the depletion of the totality of the contents, and to the lack of feed that forces the movement of the remaining gizzard contents. The decrease in peristaltic and antiperistaltic activity makes the material linger along different intestine segments, even when longer fasting times are adopted. The data obtained in the present paper are similar to those reported by Savage (1998) in a study that revealed different amounts of intestinal contents after a 10 h fasting regime, and to the data obtained by Hinton et al. (2000a), who observed digesta in the ileum, large intestine and cloaca 16 h after feed withdrawal.

The weights of the duodenum and of the caecum + colon contents did not differ with the adoption of fasting. In the case of the caecum + colon, the content in this segment was considerably larger with the 0 and 4 h fasting as compared to the 6 and 8 h fasting. Nevertheless, the high variation coefficient masked the differences across methodologies, which did not present statistical significance. A ten hour fasting period is enough to increase epithelial scurf to the point that remaining contents held up in the last third of the intestine are moved forward (Savage, 1998).

The mean time taken by excreta marked with Fe_2O_3 to appear was 228 min, in both methodologies (total collection for 3 days and total collection for 5 days), that is, 3 h 48 min (Table 6). Washburn (1991) studied 35-day-old broiler chickens and also observed that the time digesta flowed was 228 min. The variation coefficient was relatively low and no differences were observed in times at which the marker appeared in excreta, considering the 3- and 5-day collection periods. This result was predictable, since the methodology including marker use was the same in the two cases.

Table 6 - Time elapsed (min) between feeding and appearance of the Fe_2O_3 marker in the excreta of growing broiler chickens

Methodology	Time marker appeared (min)
Total collection, 3 days	223 ± 5.9
Total collection, 5 days	233 ± 9.4
Coefficient of variation (%)	10.3
P value	0.38

Conclusions

The lack of methodological standardization leads to variable metabolizatizabilty coefficients in broiler chickens. Changes in methods concerning collection period, adoption of fasting and use of an indicator or marker are factors that influence experimental results. A regimen of 3 days with total collection, without using a marker and without fasting,

is the best methodology to assess the use of nutrients and feeds by broiler chickens. Using markers produces similar results to the conventional method, but it does not increase quality in the obtained values, while indicator use underestimates metabolic results. Fasting periods of up to 8 h do not significantly influence the weights of gastrointestinal organs, but long fasting periods produce negative effects in some results, and are best avoided.

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