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VALIDATION OF INDICATORS LIPE, CHROMIC OXIDE AND TITANIUM DIOXIDE COMPARED WITH THE REFERENCE METHOD (TOTAL COLLECTION), FOR DETERMINATION OF DIGESTIBILITY, FECAL PRODUCTION AND CONSUMPTION FOR GROWING PIGS1

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Abstract: A digestibility experiment was conducted in the Animal Nutrition Laboratory of the Veterinary School of Federal University of Minas Gerais (UFMG), in order to validate the indicators LIPE, Cr_2O_3 and TiO_2 as replacements to the reference method, total collection, for the determination of fecal output, digestibility and intake in growing pigs. The experiment lasted 12 d (7 d for adaptation to the cage and diet and 5 d of total collection of feces). Fifteen Agroceres barrows of the DanBred lineage with average weight of ± 25 kg and 65 days old were used. A completely randomized design with split plots was adopted, where the plots were the diets (Reference, Reference + inclusion of 30% micronized soybean) and the subplots were the indicators and the Total collection, with five repetitions (animals) per diet. The results obtained with the indicators were compared to the reference method of total collection. There was no statistical difference (P>0.05) for the LIPE and titanium dioxide indicators when compared with the reference method. However, the indicator chromic oxide was shown to be different from the reference method for (P> 0.05) for all variables tested not being recommended to estimate fecal output, digestibility and intake for this animal category in these trial conditions. The marker overestimated the variables at some points, and underestimated them in others, demonstrating to be an unstable method. The indicators LIPE and TiO_2 may be recommended and validated for fecal output determination and intake in these animals and these trial conditions.

Keywords: marker, fecal output, digestibility, intake

INTRODUCTION

Research in the area of nutrition, has long been looking for alternatives to evaluate the nutritional value of feeds. The process of total collection in trials for evaluation of consumption, fecal output and nutrient digestibility is very painstaking and sometimes difficult to measure, therefore, substances called indicators, have enabled the measurement of these variables safely and accurately. These substances may be present as indigestible components of the diet or indigestible substances added to diets or provided separately, for example in capsule form. To know the reliability of an indicator it is necessary to make digestibility trials comparing the results obtained with the indicator with those obtained with the total collection (reference method). Although it is extensive the use of indicators for estimating fecal excretion and digestibility of diets, few studies have verified the accuracy of such estimates, which makes it necessary to compare the total collection with the estimates (Moraes, 2007). Therefore the objective of this study was to compare estimates of fecal output, digestibility and intake of nutrients through direct methods (total collection) with the indirect method, using external indicators chromic oxide, titanium dioxide and LIPE in growing pigs fed diets based on corn and soybeans, thereby validating these indirect methods.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Animal Metabolism, Department of Animal Science, School of Veterinary Medicine, Federal University of Minas Gerais.

Fifteen barrows of the commercial line DB Danbred were used in this experiment aged 65 d and with starting weight of ± 25 kg. The animals were housed individually in metabolic cages fitted with feeder, drinker, individual collector of feces and urine, during a period of 7 d for adaptation and 5 d of collection. The trial lasted 12 d. The experimental diets were produced from a basal diet (T1) described in Table 1.

The diets corresponding to the other dietary treatments (T3 and T2) are characterized by substitution in the second diet of 30% of the basal diet with micronized soybean and in the third diet, replacement of 30% of the basal diet with blood plasma. Diets were formulated to meet the requirements of animals, according to the dietary recommendations of (Rostagno et al., 2005). Diets were fed moistened twice a day, one at 6:00 in the morning and the other at 18:00 in the afternoon. Established feed intake was according to the metabolic weight of each animal. Water was provided *ad libitum*. The digestibility indicators were administered orally. LIPE was supplied in capsule form, one capsule of 0.25 g per animal per day, chromic oxide and titanium dioxide were mixed to the ration when they were prepared, with 0.8 kg of indicators (Cr_2O_3 and TiO_2) to every 100kg of diet. Each pig received an average of 0.8% of each indicator (TiO_2 and Cr_2O_3) daily with the feed during the whole experimental period. Table 2 describes the chemical composition of the diets for the ratio of 100% DM* of the experimental diets.

The determination of the apparent digestibility of the diets was carried out through the traditional method of total collection and using the external indicator of digestibility LIPE according to Saliba et al., 2003, Chromium according to EDIM, 1918 and Titanium according to Jagger et al. 1992 and Short et al. 1996;through calculations and laboratory analyzes. The experimental period was 12 d being 7 for adjustment to the cages, diets and for determination of intake, and 5 d for collection. The amount of ration given to animals, leftovers and feces were weighed daily over a period of 24 hours. The collected material was weighed, placed in plastic bags and stored frozen until the end of the collection period for further analysis.

The laboratory analyzes of the diets and feces of the experiment were conducted in the Laboratory of Animal Nutrition, School of Veterinary Medicine of UFMG.

Feces were placed in a forced ventilation greenhouse at 55°C for 72 hours for pre-drying. After pre-drying, the material was exposed for two hours at room temperature, then weighed, homogenized and ground to 1mm in a Willey mill type with sieves, and packaged in plastic pots properly identified. Analyzes were conducted for Dry Matter (DM) at 105 º C, according to Cunniff (1995), Ether Extract (EE) , being Goldfisch the apparatus used for extraction of the ether extract, calcium (Ca), analyzed through Atomic Absorption Spectrophotometer, phosphorus (P), by Spectrophotometer calorimetric, Ashes (MM), (crude Protein Kjeldahl method, according to AOAC, 1995, neutral detergent fiber (NDF), crude protein (CP), gross energy in the bomb calorimeter (EB), according to techniques described by (Silva, 1981). The analysis performed for LIPE was infrared spectrophotometry, chromium analysis by atomic absorption spectrophotometer, Titanium analysis by visible colorimetric spectrophotometry.

FORMULAS USED FOR CALCULATIONS

* Pre dried matter: PDM = (weight of wet feces) - (pre-dried fecal weight) x 100

* Dry matter: Moisture = 100 - MS105 C

* Total dry matter: TDM = (DM 105 º C/100) x (MPS/100)

* Chromic oxide: determined by atomic absorption spectroscopy on samples of 0.5 g of feces.

> LIPE: determined by IR spectroscopy with Fourier transform, in samples of 0.1 g of pre-dried feces at ICEX pre-UFMG.

* Fecal output (FO) estimated by LIPE was obtained by the following equation:

$$FO = I/[I_f]$$

Where:

FO (g/day) = daily fecal output;

I (g/day) = daily dose of indicator provided;

I_f = concentration of the indicator in fecal dry matter (g / g).

DATA FOR FORMULAS

* Fecal Output (FO)
* dry matter intake (DMI)
* dry matter digestibility (DMD)

*FO = DMI x (1 – DMD)

*DMI = FO /(1 - DMD)

*DMD = 1 - (FO /DMI)

* Chromic Oxide: FO = chromium administered (g/day) / chromium in feces (g / kg DM) (Pond et al., 1989).

* Titanium dioxide:

* (Digestibility of dry Matter) DMD (%) =

$$\frac{\text{Ingested DM - DM Fecal x 100}}{\text{DM Ingested}}$$

$$\text{* FO} = \frac{\text{Quantity of LIPE administered (g) / LIPE concentration in feces}}{\text{DM 105 ° C}}$$

Where FO = Fecal Output

* FO Cr. (g/day) = Cr intake (g/day) (Sample/100% Cr) / (° C/100% DM105)

Where FOCr. = Fecal output estimated by Chromic Oxide.

$$\text{* FOTit. (g DM / day)} = \frac{\text{Tit. provided (g/day)}}{\text{(Tit\%. Faeces / DM 105 ° C)}}$$

Where FOtit. = Fecal output obtained by titanium dioxide, Tit. Supplied and Tit. in feces, the amount of titanium dioxide supplied and excreted% Tit. in feces, percentage of titanium in the feces and DM, the dry matter at 105 º C.

- For the calculations of the rate of indicator fecal recovery, the formula used was according to Vasconcellos et al. (2004) described below:

* Recovery rate =

$$\text{Recovery rate} = \frac{\text{Production by fecal indicator x 100}}{\text{Production by fecal total collection}}$$

For statistical analysis of fecal output, intake and digestibility, the statistical design was completely randomized with split plots. The plots were the diets and the subplots were the indicators and the total collection, with 5 repetitions (animals) as the framework of analysis of variance described in Annex B.

The data obtained was submitted to analysis of variance (ANOVA) using the statistical package SAEG version 9.0 (Sistema. .., 2000) and means were compared by Tukey test at 5% error probability.

RESULTS AND DISCUSSION

Estimation of fecal output in g /day on DM basis by the reference/direct method (total collection) and by the indirect method (indicators) are described in Table 3.

It can be observed in Table 3, regarding the estimation of fecal output, that there was no interaction between the indicators and diets, so the averages of each diet and the means of each indicator / method were analyzed.

The indicator LIPE and the indicator titanium dioxide have behaved in a manner similar to the reference method (total collection), with no statistical difference between them. However, indicator chromic oxide was statistically different from the reference method and other indicators used, overestimating the average fecal output for the analyzed diets. According to the data described, it can be seen that chromic oxide is not an indicator with a good performance for these diets and this animal category, under the conditions tested. This result can also come from the fact that laboratorial analysis of chromium requires a reductive flame for the atomic absorption spectrophotometer, which is difficult to achieve, therefore making this analysis time consuming and laborious with large variations. This issue is presented with agreement of other studies in the literature, and was also observed in other animal categories.

Curran et al., (1967), in a note on the use of chromic oxide as an indicator for the estimation of fecal output, said that one reason for the low recovery of fecal Cr_2O_3, can be the analytical method of dosage. The technique of atomic absorption (AAS) described by Williams et al., (1962) is still subject to doubt, since their determination usually vary. However, in some studies where this indicator and the indicator LIPE were used they did not obtain statistical difference between them and the total collection method.

Glindemann et al. (2009), when evaluating the fecal output and rate of fecal recovery of titanium dioxide in grazing sheep, found satisfactory results for these parameters and recommended the application of this indicator twice daily. Marcondes et al. (2007) to evaluate the titanium dioxide and other external and internal indicators to estimate fecal output in crossbred beef heifers fed sugar cane and concentrate, found that this indicator was effective in estimating the fecal output in dry matter basis, as well as the intake of concentrate.

Kozloski et al. (2006) evaluated the effect of sampling time on estimates of fecal excretion using chromic oxide as an external marker in three experiments with grazing cattle. In all experiments 10g of chromium were used, one daily dose for 10 d, supplied orally. In their conclusion, the fecal excretion of grazing animals can be estimated by providing chromic oxide only once a day, together with two fecal samplings between the eighth and tenth days of supply of the indicator. In studies with poultry, Rodrigues et al. (2005) evaluated the method of total excreta collection and chromic oxide method as an indicator. They determined the apparent metabolizable energy corrected for nitrogen retention (AMEn) and coefficients of apparent digestibility of dry matter (CADDM) of a diet based on corn and soybean meal, containing 19% CP and 3,100 kcal / kg. A total of 60 Leghorn roosters, weighing 2350 ± 105 g. Each rooster was evaluated by two methods simultaneously in consecutive periods. The use of three collection days was enough in the methodology of total excreta collection to determine the values of CADDM and AMEn of diets based on corn and soybean meal. Adopting 3 d of collection and 0.665% chromic oxide in the diet, it is possible to determine the values of AMEn and ADDMC similar to those obtained by the traditional method of total excreta collection.

Marcondes et al., (2006) used chromic oxide and LIPE as indicators and observed no statistically significant differences in estimates of fecal output in heifers for 3 or 5 d of collection for both chromic oxide and LIPE. It was possible to check, from the data of fecal output by total collection and use of indicators, that these authors obtained full recovery of LIPE and chromium, for 3 or 5 d of collection.

Pereira et al., (2004) used the indicator LIPE to estimate fecal output and overall apparent digestibility of dry matter in growing rabbits, evaluating the growing inclusion of citrus pulp (0, 8, 16, 24 and 32%)in their diets. An interaction was observed between the treatments and method used (LIPE). Increased inclusion levels of citrus pulp was associated to an increase in the difference between the values of fecal output and digestibility of dry matter in relation to the values obtained by total collection. These authors concluded that the LIPE was effective as external indicator, however, when there were increased levels of inclusion (16, 24 and 32%), fecal output was overestimated and dry matter digestibility was underestimated, recommending against its use under these conditions. Also, with increased inclusion of citrus pulp, animals presented clinical symptoms of diarrhea. Saliba et al., (2003) compared the fecal outputs estimated by LIPE, and obtained by total collection for sheep, rabbits and swine. These authors obtained similar results between methods, and recovery rates of 94.6% and 102.6% for pigs and 97.9 and 99.3% for rabbits, both subject to two different diets, and 95.9% for sheep fed Tifton 85 hay.

Studies in which indicators tested in this experiment did not achieve satisfactory results for the estimation of fecal output:

Sampaio et al. (2011), in order to estimate the total recovery and time biasing of estimates of fecal excretion obtained with external indicators chromic oxide and titanium dioxide and with internal indicators indigestible dry matter (iDM), indigestible neutral detergent fiber (iNDF) and indigestible acid detergent fiber (iADF) in a digestion trial with cattle fed different diets, conducted a study where 14 steers F1 Nellore × Red Angus were used, aged an average of 12 months and with average weight 287 kg. The animals were fed elephant grass silage, corn silage or hay Brachiaria grass, supplemented or not with 20% concentrated mixture. The experiment consisted of two periods of 13 d each, according to Latin square design 2 × 2, with grouping of seven squares. The animals received daily 10 g of chromic oxide and 10 g of titanium dioxide by oesophageal gavage. No effects were observed of forage, concentrate level or their interaction on estimates of fecal recovery, on both indicators, internal and external. Estimates of average fecal recovery of chromic oxide and titanium dioxide were 99,50% and 101,95%, respectively. For internal indicators, observed average fecal recovery of 99,02; 98,87 and 102.07% for iDM, and iNDF iADF, respectively. In all cases, the recoveries were equal to 100%. All indicators evaluated can be considered free of bias over long periods of time. However, greater accuracy is verified for fecal excretion estimates obtained with internal markers.

Morata et al (2006) evaluated the apparent metabolizable energy and corrected apparent metabolizable energy, and the coefficients of gross energy metabolism and digestibility of DM and OM of some feeds for emus of seven months of age, using the techniques of total collection of the excreta and using chromic oxide as an indicator. The values of ADDMC, CADOM, AME, AMEn, MCEC and CMEBn of feeds, determined by the technique of total collection were generally higher than those determined by Chromic Oxide. The technique of chromic oxide has proved inadequate to determine ADDMC, CADOM, AME, AMEn, MCEC and CMEBn food for emus compared to the total collection of excreta. It is recommended therefore more tests similar to this to overrule or not the use of this technique for this species.

Berchielli et al. (2005) conducted three experiments to evaluate the use of two internal markers, indigestible NDF and ADF, obtained by incubation *in vitro* and *in situ*, and an external marker, chromic oxide, to estimate the fecal output and duodenal digesta flow in cattle. The values of indigestible NDF and ADF proved to be variable for each voluminous feed, regardless of the methodology used (*in vitro* or *in situ*), possibly indicating that the incubation for 144 hours does not reproduce the total indigestible fraction. The estimates of fecal output and duodenal digesta flow, obtained with the markers evaluated, behaved quite differently in each bulky feed. The internal markers indigestible NDF and ADF can be used as predictors of the parameters evaluated, provided that certain precautions are taken in its determination.

In Table 4, there is the estimate of digestibility by the methods of chromic oxide, titanium dioxide and LIPE indicators, compared with the reference method (total collection) in %. There was no interaction between the indicators and diets. Therefore the averages of each diet and every indicator/method were analyzed.

In table 4, the averages of the indicators LIPE and titanium dioxide compared with the total collection were statistically similar, however the chromic oxide indicator was statistically different from the other indicators and the reference method (total collection). That indicates that the chromic oxide method underestimated the digestibility estimate of diets analyzed in this experiment. Analyzing the averages of the diets it can be seen that diet 2, with 30% micronized soybean replacement and diet 3, with replacement of 30% of blood plasma were statistically better than the reference diet which contained only corn and soybean. However, diet 3 was also similar to the reference diet so it may be nutritionally inferior to diet 2.

Lanzetta et al. (2009) evaluated the efficiency of LIPE to estimate the digestibility of nutrients in diets of horses compared to methods of total collection and chromic oxide methods. We used six foals of the breed Mangalarga Marchador, averaging 2 years of age and 345 kg of live weight, fed hay and commercial concentrate in a 50:50 ratio. The diet consisted of alfalfa hay (*Medicago sativa*) and concentrate (15% CP). The author concluded that the results obtained with LIPE were similar to those determined by total collection, confirming the efficiency of LIPE to estimate the digestibility of nutrients in horses.

Saliba et al. (2002) conducted a study with the objective of comparing lignin isolated from corn straw (LPM-RM) with the indicators used in digestibility and intake trials. LPM-RM, chromic oxide (Cr_2O_3), ytterbium chloride (YbCl3.6H2O), the

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indigestible acid detergent fiber (ADF-ind), the methoxyl content of lignin (OCH3), Klason lignin (LK) and lignin determined by infrared spectroscopy (LIG-IV) as markers and indicators. The digestibility data obtained with LIG-IV, Cr_2O_3 and YbCl3.6H2O were similar, but different from other indicators (21% higher in the case of Cr_2O_3 and 24% lower in the case of LIG and ytterbium-IV) compared with the total collection.

In a study with seabass of the species *Centropomus parallelus*, to determine the digestibility of ingredients used in fish diets, the animals were fed with soybean meal oat bran and rice bran, tested ingredients, in diets with 0,5% chromic oxide (Cr_2O_3) as external marker. In this experiment, treatment with soybean meal, using 20% soybean meal to replace fish meal resulted in weight gain superior even to the basal diet containing 65% fish meal. It can be concluded, that the chromic oxide was effective to determine the digestibility of these feeds. (Barroso, 2002).

Titgemeyer et al. (2001) used TiO_2 and Cr_2O_3 as indicators to determine digestibility in steers. In the first assay, eight steers consuming prairie hay were divided into four treatments: 1) control, no supplement, 2) 1.6 kg/d supplementation of corn (DM basis), and 3) 0.43 kg/d of baked syrup in block containing 30% crude protein, and 4) 5.0 g/d *Smartamine M*-(Rhone-Poulenc Animal Nutrition, Atlanta, GA). All animals received 20 g/day of white salt. Each calf received 10 g of TiO_2 with the white salt for 14 d. The collection of orts, feces and diet occurred within the following seven days. The analysis of TiO_2 was performed according to Short et al. (1996), with modification of using only 10 mL of H2O2 30% instead of 20 ml. The recovery of TiO_2 was 92.8% and was not affected by treatments. The digestibility calculated by TiO_2 was statistically equal to digestibility.

Titgemeyer et al. (2001), in a second experiment, used two steers fed limited diets with maize base (3.3 kg rolled corn, 0.7 kg of chopped alfalfa, 0.50 kg of soybean meal and 0.10 kg of mineral vitamin supplement). Steers were adapted to the diet for 7 d, and 10g/d of TiO_2 and Cr_2O_3 were added to the diet, within the following 14 d, being 5g of the indicators fed in the morning and 5g in the afternoon diet. The animals were housed in metabolic cages to collect feces between day 2, after the addition of the indicator to the diet, and 21 of the experimental period. The fecal samples were subdivided into 5 d of collection (2-6, 7-11, 12-16, 17-21 d). The first collection period (2-6 d) resulted in low recoveries of indicators. The recovery for other periods was statistically equal to 100%, ranging from 91.2% to 98.3% for TiO_2 and 108.7% to 116.2% and Cr_2O_3. After adaptation to the indicators, digestibility calculated by TiO_2 was underestimated by 1.1 percentage points to the total collection, while the digestibility calculated based on Cr_2O_3 was overestimated by 2.2 percentage points.

Titgemeyer et al. (2001), made a third experiment with 8 ruminally cannulated steers fed diets based on corn laminate containing 8% alfalfa, 3% syrup and 0.5% urea (DM basis). Animals were offered 10 g of TiO_2 and Cr_2O_3 daily, divided into two equal portions. Steers were adapted to the diet for 9 d and the total collection occurred during the four subsequent days. When the indicators technique was used for the calculation of digestibility, TiO_2 led to a higher estimate of fecal output and a lower digestibility of the diet. The authors concluded that titanium dioxide can be used as an indicator for digestibility of ruminant animals. In this work, they found recoveries above 90% 95% TiO_2 but digestibility was underestimated, ranging from 1.1 to 5.5 units percentages.

Kavanagh et al. (2001) used Cr_2O_3, TiO_2 and acid insoluble ash (AIA) to determine digestibility in pigs. They used eight boars divided into two treatments. Treatment A consisted in adding to the diet based on cereals, 1 g/kg of indicators TiO_2 and Cr_2O_3, and Treatment B did not have indicators. The pigs were fed twice a day with 3.3% to 3.7% of their body weight in ration. The period of adaptation to diets indicators was 7 d. The total collection occurred in 5 d, with the aid of ferric oxide (20 g/kg). Analyses of titanium were made according to Short et al. (1996). The recovery of indicators in the diets were 0.93 g / kg of Cr_2O_3, 1.02 g / kg of TiO_2 and 1.336 (without fractions of TiO_2 and Cr_2O_3) and 2.906 (with fractions of indicators) for AIA. The recovery in the feces was 96.0%, 92.3% and 99.9% respectively for Cr_2O_3, TiO_2 and AIA. The digestibility estimated with TiO_2 in this study was underestimated in relation to the total collection. The authors explain it was due to the low recovery of the TiO_2 in feces.

Jagger et al. (1992) evaluated the indicators TiO_2, Cr_2O_3 and lignin in the study of apparent ileal digestibility of amino acids in pigs with type "T" reentrant cannulas. 1g/kg and 5 g/kg of TiO_2 and Cr_2O_3 were added to the diets. There was a decrease in initial intake when the amounts of the indicators were 5 g, but this was transient and no longer observed after a period of 7 to 10 d. The recovery rate was 74.6% and 79.7% for quantities of 1 to 5 g / kg of Cr_2O_3, 98.3% and 96.9% for quantities of 1 to 5 g / kg of TiO_2 and 98.1% for lignin. There was a significant difference between indicators determining the digestibility, except for nitrogen and histidine. The amino acid digestibility values were underestimated when Cr_2O_3 was used. The authors concluded that the best indicator in this study was 1 g / kg of TiO_2 to determine the apparent ileal digestibility of amino acids.

In Table 5, we can observe the estimated intake of animals by the indirect method through the use of indicators, compared with the real intake method in (g/day). The table shows that there was no interaction between the indicators and diets, so the

average intake of the animals was analyzed. The average intake was also not different between diets. However in the mean consumption obtained by the methods of total collection (reference) and indicators, the averages given by chromic oxide was different for estimating intake compared to the reference method, LIPE and titanium dioxide. chromic oxide overestimated the average intake of the diets, which also occurred in studies of fecal production described in Table 3.

The indicators titanium dioxide and LIPE proved to be efficient for the determination of intake in all diets with no significant difference in the estimation of the three variables analyzed in relation to actual intake.

Ferreira et al. (2009) conducted two studies to estimate individual intake of dairy cows fed in a group. In the first, to validate the methodology, they used eight cows housed in individual pens fed corn silage and 4 kg of concentrate. Two external markers (chromic oxide and titanium dioxide) were evaluated to estimate individual consumption of concentrate. The indigestible acid detergent fiber (iADF) was used to estimate the intake of silage. The fecal dry matter production (FDMP) was determined by total collection of feces and estimated by LIPE. Both the chromic oxide and the titanium dioxide made possible to estimate efficiently the intake of concentrate regardless of the method for estimating FDMP. The iFDA satisfactorily estimated intake of corn silage. In the second experiment, 31 cows were used, divided into three feeding groups and housed in free-stall barns. They were fed corn silage and concentrate according to milk production (8.0, 5.5 and 4 kg for groups 1, 2 and 3, respectively). There was no difference between the chromic oxide and titanium dioxide on dry matter intake (DMI) of concentrate, which was very close to the average amount provided per cow / day (6.99 vs 7.12; 4.81 vs 4.96 and 3.49 vs 3.52 kg / cow / day). There was great individual variation in the consumption of concentrate, silage and total dry matter, regardless of feeding group. No relationship was observed between dry matter intake, milk production and metabolic weight of the animals. The chromic oxide and titanium dioxide can be used to estimate the individual intake of concentrate, and iFDA is suitable to estimate the intake of corn silage for lactating cows fed in group.

Lima et al. (2008) evaluated the efficiency of chromic oxide and LIPE, supplied once daily, for the estimation of DMI in beef calves, of both sexes, with an average age of 210 d and average weight of 168kg, grazing *Brachiaria decumbens*. These authors observed that the application of chromic oxide once a day underestimated values of fecal excretion and consequently DMI of animals. The average value of fecal output estimated by chromic oxide was 52.5% lower than estimated by LIPE. Therefore, in grazing, the LIPE is a more reliable option for determining the DMI indirectly since produced results that were more consistent with the requirements and performance of animals in this study.

Soares et al. (2004) evaluated the DMI of lactating crossbred Holstein-Zebu cows fistulated in the rumen. The intake was measured by the difference in weight of the food offered and orts, and estimated with aid of chromic oxide (Cr_2O_3) by fecal output (FO) and indigestibility of food. The intake was overestimated by 9.25% when the technique of chromic oxide was used, compared with measured data from the weighing offered and orts. This overestimation has been attributed to recovery of chromium in the feces. The estimates obtained with the aid of chromic oxide may be considered satisfactory when working with reviews digestibility, fecal output and DMI

CONCLUSION

The indicator LIPE and titanium dioxide were not statistically different from the method of total collection (reference) for the evaluated variables: fecal output, intake and diet digestibility, in nutritional studies for growing pigs. However the use of chromic oxide as an indicator is not recommended to estimate fecal output, digestibility and intake for these animals categories tested in the experiments, because this indicator at times overestimated the variables tested, and on other times it underestimated them, demonstrating to be a method quite variable, in relation to other tested methods. Therefore indicators LIPE and titanium dioxide can be recommended and validated for determination of fecal output, digestibility and intake under the conditions tested in this animal category.

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TABLES AND FIGURES

Ingredients	T1
Corn (grain)	69.3
Soybean meal	24.9
Meat meal	4.4
Growth Premix [1]	0.4
Calcarium	0.5
Salt	0.5
Chromic oxide inclusion	0.8
Titanium dioxide inclusion	0.8

Table 1. Percentage composition of the reference diet

[1]Vitamined mineral Premix per kg of product: vit. A, 2,000,000 UI; vit. D_3, 300,000 UI; vit. E, 5,000 mg; vit K_3, 625 mg; vit. B_1, 250 mg; vit. B_2, 1,000 mg; vit. B_6, 500 mg; vit. B_{12}, 5,000 mcg; Nicotinic acid, 625 mg;; Nelenium, 125 mg; Biotin, 12.5 mg; Antioxidant, 30,000 mg; e vehicle q.s.p., 1,000 g, Pantotenic acid 2,500mg; Folic acid 150mg; Choline 60,000mg; vit. C 12,500mg; Metionin 25,000mg; Threonine 10,000mg; Tripsin. 5,000mg; 125mg Selenium, Iodine 200mg, 125mg Cobalt, Iron 17,500mg; 100,000mg

Lysine; 5,000mg Copper, Manganese 10,000mg; 20,000mg Zinc; Tylosin 11,000mg; 500mg BHT.

Diets	Reference	30% Micronized soybean	30% Blood plasma
MS, %	87.29	89.52	87.17
EE, %	4.17	9.43	2.36
FDN, %	13.6	15.4	18.7
MM, %	0.1	0.08	0.09
CA, %	1.07	0.99	0.67
P, %	0.63	0.64	0.81
PB, %	21.14	27.34	35.85
EB, cal/g	4657.1	5008.4	4977.2

Table 2. Bromatological composition for 100% DM[1] of experimental diets

[1]DM - dry matter

Diets	Indicator,% recovery				X
	Total Collection	LIPE	Cr_2O_3	TiO_2	
Reference	119.4	122.5	137.9	118.0	124.5[a]
30% Micronized soybean	95.1	100.7	116.6	115.4	106.9[b]
30% Blood Plasma	106.0	108.6	156.1	120.4	122.8[a]
X	106.8[B]	110.6[B]	136.9[A]	117.9[B]	

Table 3. Estimation of fecal output of animals by reference method (total collection) and by indicators (g/day)

Different upper case letters on the same line and different lower case letters in a column represent a difference by the Tukey test (P<0,05). Variation Coefficient = 12.88%.

Diet	Indicator, % recovery				X
	Total Collection	LIPE	Cr_2O_3	TiO_2	
Reference	89.6	89.0	87.7	89.5	88.9[b]
30% Micronized Soybean	91.7	91.2	88.9	89.9	90.5[a]
30% Blood Plasma	91.0	90.9	86.9	90.6	89.8[ab]
X	90.8[A]	90.4[A]	87.8[B]	89.8[A]	

Table 4.: Estimation of digestibility of the diets by the reference method (total collection) and the method of indicators in %

Different upper case letters on the same column and different lower case letters in the same line represent a difference by the Tukey test (P<0,05). Variation Coefficient = 1.45%.

Diet	Indicator				X
	Total Collection	LIPE	Cr_2O_3	TiO_2	
Reference	1116	1362	1503	1122	1276
30% Micronized Soybean	1143	1074	1412	1153	1196
30% Blood plasma	1208	1249	1752	1238	1361
X	1156[B]	1228[B]	1556[a]	1171[B]	

Table 5. Estimation of diets intake by animals via the reference method (total collection) and the indicators method

Different upper case letters on the same line and different lower case letters in the same column represent a difference by the Tukey test (P<0,05). Variation Coefficient = 12.9%