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External markers for the estimation of intake by dairy cows in feedlots

Marcadores externos para estimativa de consumo por vacas leiteiras em confinamento

Dayana Alves da Costa[*1], Jailton da Costa Carneiro[2], Fernando César Ferraz Lopes[2], Clebson Lucas de Souza[1], Guilherme Rocha Moreira[3], Eloísa de Oliveira Simões Saliba[4], Mércia Regina Pereira de Figueiredo[5], Lucas Silva do Amaral[3]

1 Instituto Federal do Acre (IFAC), Sena Madureira, Acre, Brazil
2 Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil
3 Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco, Brazil
4 Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Zootecnia, Belo Horizonte, Minas Gerais, Brazil
5 Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), Vitória, Espírito Santo, Brazil
*corresponding author: dayanazoo@yahoo.com.br

Abstract: The accurate determination of individual dry matter intake (DMI) is an important parameter for evaluating the nutritional value of a diet and allows for an adequate supply of food according to the nutritional requirements of each animal. When it is not possible to measure this parameter using a direct method, the DMI can be estimated based on faecal production markers and the *in vitro* digestibility of dry matter (IVDMD) of the diet. The objective of this study was to evaluate the effects of the external marker titanium dioxide (TiO2) and enriched and modified hydroxyphenylpropane (LIPE®) on DMI. Four multiparous Holstein × Gyr cows with an average of 60 days in milk and 488 kg of body weight were used. The animals were housed in a free stall barn and fed diets based on sugarcane silage supplemented with concentrate composed of ground corn, cottonseed meal and 0%, 5%, 10% and 15% whole cottonseed on a dry matter (DM) basis. A 4 x 4 Latin square design was adopted. The DMI was determined by the ratio between the faecal production estimated by the markers and the IVDMD of the diet, with faeces collected by rectal grabbing twice a day for a period of seven days. There was no difference (P>0.05) in the DMI estimated by the external markers TiO2 and LIPE® (10.3 vs. 12.1; 11.1 vs. 12.5; 10.2 vs. 12.9; and 12.7 vs. 10.9 kg/cow/day in relation to the measured DMI for diets with 0%, 5%, 10% and 15% inclusion of whole cottonseed, respectively). The external markers LIPE® and TiO2 proved to be suitable for estimating cow DMI. Regardless of the diet offered, both external markers can be used to replace the external marker chromic oxide, as the DMI estimated by them did not differ from the DMI measured in the trough.

Keywords: fecal production; LIPE®; silage; sugarcane; titanium dioxide; whole cottonseed.

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Resumo: A determinação acurada do consumo individual é um parâmetro importante para avaliação do valor nutritivo da dieta e permite o fornecimento adequado de alimentos, conforme as exigências nutricionais de cada animal. Quando há impossibilidade da sua mensuração pelo método direto, o consumo pode ser calculado a partir da produção fecal estimada por indicadores e pela digestibilidade *in vitro* da matéria seca (DIVMS) da dieta. Foi objetivo deste estudo avaliar os indicadores externos dióxido de titânio (TiO2) e hidroxifenilpropano enriquecido e modificado (LIPE®) nas estimativas de consumo de matéria seca (MS). Foram utilizadas quatro vacas multíparas Holandês × Gir com média de 60 dias em lactação e 488 kg de peso corporal. Os animais foram alojados em estábulo tipo *free stall* e alimentados com dietas à base de silagem de cana-de-açúcar suplementada com concentrado composto de milho moído, farelo de algodão e 0%, 5%, 10% e 15% de caroço de algodão (base da MS). Adotou-se delineamento quadrado latino 4 x 4. O consumo foi determinado pela razão entre a produção fecal estimada pelos indicadores e pela DIVMS da dieta, sendo as fezes coletadas diretamente na ampola retal, duas vezes por dia, por um período de sete dias. Não houve diferença (P>0,05) nos consumos de MS estimados pelos indicadores TiO2 e LIPE® (10,3 *vs.* 12,1; 11,1 *vs.* 12,5; 10,2 *vs.* 12,9; e 12,7 *vs.* 10,9 kg/vaca/dia em relação ao consumo mensurado, para as dietas com 0%, 5%, 10% e 15% de inclusão do caroço de algodão, respectivamente). Os indicadores externos LIPE® e TiO2 mostraram-se adequados para estimar o consumo de MS das vacas. Independente da dieta oferecida, ambos mostraram que podem ser utilizados em substituição ao indicador externo óxido crômico, pois, as estimativas de consumo estimadas por eles não diferiram do consumo mensurado no cocho.

Palavras-chave: cana-de-açúcar; caroço de algodão; dióxido de titânio; LIPE®; produção fecal; silagem.

1. Introduction

Feeding programs are based on the assumption that animals will consume a certain amount of the diet, and when they consume less than this amount, their needs will not be met as planned. On the other hand, overestimated variations in individual consumption will increase the costs of dietary supplementation and may have negative effects on food consumption and digestibility, which, according to Velásquez et al. [(1)], could cause negative impacts on animal production.

Whole cottonseed has been used as an alternative for energy supplementation. The main advantage of this feed is its high energy content, which reflects its high contents of oil and crude protein (24%). The oil is rich in unsaturated fatty acids, with linoleic acid comprising 62% and oleic acid comprising 15% of the total fatty acids. In addition to these advantages, whole cottonseed has the ability to supply fibre for rations when compared to soybeans. The use of whole cottonseed in the diet of lactating cows has increased in Brazil in recent years, mainly due to the availability of this product in some regions and the high cost of protein supplementation [(2)].

In diets formulated for ruminants, even when alternative food sources are used, it is essential to provide roughage to maintain a balanced rumen environment. According to Costa et al. [(3)], sugarcane (*Saccharum officinarum*, L.) is a suitable forage for this purpose, especially for feeding dairy herds during the dry period of the year, and the conservation of sugarcane in the form of silage has attracted the interest of researchers and producers due to the benefits in logistics and operability that this technique can present.

To estimate individual consumption, several techniques have been developed, including electronic animal identification systems and the use of individual pens. According to Peres et al. [4], when livestock farmers do not have the infrastructure to directly measure the amount of diet consumed, an alternative technique for estimating individual intake in production animals is based on the use of substances called markers, which estimate total faecal production.

Among the techniques used to estimate total faecal production, the use of markers that may (internal) or may not (external) be naturally present in the diet stands out. This technique is based on the ratio between the amount of the marker ingested by the animal and its concentration in the faeces. Subsequently, the animal's intake can be estimated using *in vitro* digestibility values of the diet or food(s), which also allows the estimation of the digestibility of a specific nutrient in the diet [1].

Despite the scientific enrichment in research for dairy cattle on the use of markers to estimate intake, the limitations inherent to the use of different markers drive the search for others that are more accurate and less expensive and, preferably, have simpler analytical requirements. Given this scenario and the impossibility of directly measuring faecal production, the present study aimed to estimate, using the external markers titanium dioxide (TiO2) and enriched and modified hydroxyphenylpropane (LIPE®), dry matter intake by Holstein × Gyr dairy cows fed diets based on sugarcane silage and increasing levels of whole cottonseed.

2. Material and methods

This study was approved by the Ethics Committee on the Use of Animals of the Federal University of Minas Gerais – CETEA/UFMG (Protocol #163/2009) and was carried out at Campo Experimental José Henrique Brusqui, belonging to Embrapa Dairy Cattle located in Coronel Pacheco, Minas Gerais, Brazil, from September 2009 to January 2010.

Four 3/4 to 15/16 Holstein × Gir dairy cows were used; these cows were rumen fistulated, multiparous (3 to 4 lactations), and in the initial third of lactation (60 days in milk), with a milk production of 14.4±3.3 kg/day and an initial average weight of 488±35 kg. The experimental design adopted was a 4 x 4 Latin square, with a total duration of 72 days. The trial was subdivided into four periods of 18 days each, with 10 days for the animals to adapt to the diet and the remaining eight days for faeces and food samples to be collected.

Cows were fed *ad libitum* with diets based on sugarcane silage (ensiled with 1% urea on an as-fed basis) supplemented with concentrate composed of ground corn, cottonseed meal and increasing levels of whole cottonseed (0, 5, 10 and 15% on a dry matter basis – DM). The cows were allocated to a free stall with individual sand beds, a collective drinking fountain and a flushing track cleaning system.

The free stall was equipped with electronic troughs (American Calan Inc., Northwood, NH, USA), which allowed individual control of food intake. Water and mineralized salt were available *ad libitum*. The diets were divided into two daily meals provided at 7:00 am and 5:00 pm, with the amount offered adjusted daily to allow approximately 10% remaining feed. The chemical composition and percentage of ingredients in the experimental diets are shown in Table 1.

Table 1 Ingredients and chemical composition of the experimental diets, according to the inclusion levels of whole cottonseed, on a dry matter (DM) basis.

	Whole cottonseed in the diet (%DM)			
Chemical composition (%DM)	0	5	10	15
Dry matter (%)	32.37	32.92	31.44	32.31
Crude protein	8.38	8.05	8.56	8.08
Ether extract	1.82	2.50	2.71	4.84
Neutral detergent fiber	49.89	47.98	48.41	44.72
Acid detergent fiber	31.91	31.51	30.38	29.34
Gross energy (Mcal/kg of DM)	4.30	4.17	4.05	4.27
	Whole cottonseed in the diet (%DM)			
Ingredients (%DM)	0	5	10	15
Sugarcane silage[1]	59.1	59.2	59.0	59.2
Ground corn	23.0	21.7	21.0	19.7
Cottonseed meal	16.4	12.6	8.5	4.6
Whole cottonseed	0	5.0	10.0	15.0
Mineral/vitamin supplement[2]	1.5	1.5	1.5	1.5

[1]Ensiled with 1% urea (as-fed basis). [2]Warranty levels guaranteed by the manufacturer: 120 g of Ca; 87 g of P; 18 g of S; 147 g of Na; 0.59 g of Cu; 0.04 g of Co; 0.02 g of Cr; 1.8 g of Fe; 0.08 g of I; 1.3 g of Mn; 15 mg of Se; 3.8 g of Zn; maximum F of 0.87 g; solubility of P in 2% citric acid (min.); vitamin A, 4 x 109 IU/kg; vitamin D3, 1 x 109 IU/kg; vitamin E, 3 x 104 IU/kg.

After the animals had adapted to the experimental diets, external markers were supplied. LIPE® was administered in a 500 mg/cow/day capsule, supplied once a day (before morning milking), while titanium dioxide (TiO2) was administered twice a day (before morning milking and afternoon) at doses of 5 g/cow/day, wrapped in paper. To simulate the conditions in which they are normally applied, both were administered orally, at the same time, after the cows were restrained to an appropriate trunk.

Titanium dioxide was supplied for 11 consecutive days (six days of adaptation and five days for faecal sampling), as proposed by Titgemeyer et al. [(5)], and LIPE® for a period of seven days (two days for adaptation and five days for faecal sampling). Faeces were collected by rectal grabbing twice a day after the morning and afternoon milkings, according to the methodology proposed by Saliba et al. [(6)].

In addition to faecal samples, daily samples of food and leftovers were also collected during each phase of the Latin square. All samples were stored at -15 °C, and at the end of the experiment, they were thawed at room temperature, predried for 72 h in a forced ventilation oven set at 55 °C, ground in a mill using a sieve with 1 mm screens and subjected to laboratory analyses. Faecal and food samples were analysed for DM content at 105 °C, in accordance with AOAC recommendations [7]. The *in vitro* DM digestibility (IVDMD) of the experimental diets was determined according to Tilley and Terry [8].

The concentration of titanium dioxide was determined by calorimetry according to Detmann et al. [9], and the concentration of LIPE® in faeces was determined at the Animal Nutrition Laboratory of the Veterinary School of the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) in a spectrophotometer (model Varian 099-2243) equipped with an FTIV infrared detector; the samples were subsequently dried, ground to 2 mm and pressed into tablets KBr, according to Saliba et al. [6].

The estimate of faecal DM production (kg/cow/day) was based on the ratio between the amount of the marker ingested by the animal and its concentration in the faeces (faecal DM production = grams of the marker ingested/concentration of the marker in the faeces). Individual DM intake (DMI) was calculated by the ratio between the faecal DM production estimated by the markers and the inverse of the IVDMD of the diets, according to the following equation: DMI (kg/day of DM) = faecal production (kg/day of DM)/(1 - IVDMD) reported by Prigge et al. [10].

The faecal DM production and DMI data were subjected to analysis of variance, depending on the inclusion of whole cottonseed in the diet (0, 5, 10 and 15%) and the method of estimating the measured DMI (real, LIPE® and TiO2). These data were analysed using ANOVA at the 5% probability level.

3. Results and discussion

With the impossibility of collecting total faeces, estimates of faecal DM production (kg/cow/day) were made with the external markers LIPE® and TiO2, where no significant effect of the diet x marker interaction was observed for this variable. Table 2 shows the average estimates of faecal DM production (kg/cow/day) obtained from cows fed diets based on sugarcane silage and increasing levels of whole cottonseed, according to external markers.

Table 2 Average estimates of faecal dry matter production (kg/cow/day) of Holstein × Gyr cows fed diets based on sugarcane silage supplemented with increasing levels of whole cottonseed obtained from the external markers titanium dioxide (TiO2) and LIPE®.

	Whole cottonseed in the diet (%DM)			
External marker	0	5	10	15
LIPE®	4.43±0.41	4.71±1.16	4.77±0.47	3.82±0.54
TiO_2	4.94±0.16	4.38±1.62	4.76±0.67	5.75±0.83

Diet (P = 0.91), Marker (P = 0.50), Diet × Marker (P = 0.12)

The LIPE® marker estimated an average faecal DM production of 4.43 kg/cow/day, regardless of the diet offered, while TiO2 estimated an average value for faecal DM production that was numerically greater, at 4.96 kg/cow/day. The variability in average estimates of faecal

DM production may be related to the variation in the nutritional quality of the daily portion of sugarcane silage offered since, each day, it may have been removed from different locations in the silo, mainly having different moisture levels depending on the location of removal, which is more humid in the lower parts.

Lima et al. [11] observed elephant grass silage (*Pennisetum purpureum*, Schum.) reported that the estimates of faecal production obtained from the external markers chromic oxide (Cr2O3) and LIPE® did not differ from those obtained from total faeces collection. However, the faecal production estimated by TiO_2 overestimated the total faecal production.

Faecal production was evaluated by Figueiredo et al. [12] in Holstein × Zebu heifers, with an average live weight of 363.0±27.7 kg, receiving diets based on Tifton 85 hay with the inclusion of cocoa coproduct at different levels (0, 8, 16 and 24% of MS). These authors concluded that the chromic oxide and LIPE® markers accurately estimated faecal production, while the TiO2 marker was inefficient in estimating this variable. Faecal TiO2 recovery varied depending on the characteristics of the diet.

Estimates of the apparent digestibility of diets based on elephant grass and cassava root scrapings were obtained by Magalhães et al. [13] from the internal markers indigestible neutral detergent fibre (NDFi) and indigestible acid detergent fibre (ADFi) and the external marker LIPE® in sheep. These authors observed that the faecal production estimated by LIPE® differed (P<0.05) from that obtained by the total faeces collection method, concluding that LIPE® was not suitable for the study of digestibility in sheep.

The diets presented IVDMDs of 63.4%, 62.3%, 62.4% and 65.1% for 0, 5, 10 and 15% whole cottonseed, respectively. The estimates of DMI provided by the markers did not differ (P>0.05) from the DMI measured in the trough in any of the treatments evaluated (Table 3).

Table 3 Dry matter intake (DMI, kg/cow/day) measured in the trough or estimated by the external markers LIPE® and titanium dioxide (TiO2) for Holstein × Gyr cows fed diets based on sugarcane silage supplemented with increasing levels of whole cottonseed.

	Whole cottonseed in the diet (%DM)			
Treatment	0	5	10	15
Measured DMI	12.35±1.18	12.23 ±3.11	13.06±1.18	10.92±1.35
LIPE®	12.10±1.13	12.49±3.09	12.87±1.26	10.95±1.54
TiO2	10.29±0.43	11.13±2.76	10.23±1.80	12.67±2.38

Diet (P = 0.80), Treatment (P = 0.12), Diet x Treatment (P = 0.09)

DMI estimates obtained from LIPE® and TiO_2 showed that both can be alternative markers for the use of chromic oxide in digestion studies, and according to Myers et al. [14], TiO2 can be legally added to food in amounts of up to 1.0% of the final product, while chromic oxide is not approved by the Food and Drug Administration (FDA) as a food additive.

The average individual DMIs measured in the trough (control diet) and with 15% whole cottonseed were 12.35 and 10.92 kg/cow/day, respectively. Regardless of the method used for estimating faecal DM production, the average DMIs were included in the respective confidence intervals, suggesting that both LIPE® and TiO2 are suitable for estimating individual DMIs.

The markers used to estimate the individual DMI of cows fed sugarcane silage and increasing levels of whole cottonseed in the diet allowed the DMI to be efficiently estimated, regardless of the feeding group. The values estimated by the markers were very close to those measured. The greatest variation observed with the use of TiO2 occurred in the treatment with 15% whole cottonseed, in which the DMI was overestimated by approximately 51%. For the LIPE® marker, the greatest variation in estimated DMI occurred in animals fed the control diet, where the DMI was underestimated by only 2%.

Dairy cows are social animals that form hierarchical dominance classes. Dominance and competition occur in relation to access to environmental resources, such as food. According to HÖTZEL et al. [(15)], competition can develop when cattle are placed in groups, and there is insufficient space for all cattle to feed at the same time. In this study, the food supply and trough space were not limiting and did not influence the cows' consumption, as food was provided twice a day and the percentage of remaining feed was approximately 10%, allowing for an increase in feeding activities, especially among submissive animals.

The ingestive behaviour of goats on *Panicum maximum* cv. Tanzania pasture supplemented with palm fruits and the use of the external marker TiO2 to estimate DMI were evaluated by Garcez et al. [(16)]. Unlike what was observed in the present study, these authors observed that the supplement influenced the pasture DMI, with a reduction of 8.61%, and the TiO2 marker was efficient in detecting this change in DMI.

Saliba et al. [(17)] evaluated the DMI of crossbred heifers fed diets based on sugarcane silage and different levels of concentrate supplementation. Faecal production estimates were obtained from LIPE® and TiO2 markers and from the total faeces collection method. The authors concluded that both markers were effective and did not differ statistically in relation to the DMI estimates, corroborating the results obtained in the present study.

Moreira Filho et al. [(18)] evaluated the correlations of faecal production estimates obtained from TiO2 and LIPE® markers *versus* values obtained by the total faeces collection method for sheep fed diets composed of untreated or hydrolysed sugarcane top hay with 3% and 6% urea or 1.5% and 3% calcium oxide (CaO). The authors reported that there was a positive correlation greater than 90% between the faecal production estimated by TiO2 and LIPE® markers and that measured, indicating that both markers were effective in estimating faecal production and the digestibility of DM and nutrients.

In diets based on Tifton 85 hay and the inclusion of passion fruit byproduct for Holstein × Zebu dairy heifers with an average live weight of 363±28 kg, Figueiredo et al. [(19)] evaluated the accuracy of TiO2 and LIPE® markers in estimating faecal production and digestibility. The authors observed that the results obtained from LIPE® did not differ from those obtained from the total collection method (P>0.05); however, for the TiO_2 indicator, the values obtained for faecal production were overestimated. These authors concluded that the use of LIPE® was adequate for estimating the consumption and digestibility of nutrients in diets.

Silva et al. [20] reported that the consumption estimates provided by LIPE® for Holstein × Zebu heifers kept in confinement and fed diets based on elephant grass silage, chopped sugarcane, urea and concentrate did not differ from the consumption measured in the trough in any of the diets studied, corroborating the data obtained in the present study, in which the consumption estimated by LIPE® was similar to that measured in the trough.

Faecal production estimated by the marker TiO2 in confined sheep fed diets based on *Digitaria decumbens* hay and concentrate was evaluated by Silva et al. [21]. The authors concluded that TiO2 promoted accurate faecal production estimates, and the estimated faecal excretion was similar to the measured total faecal excretion. In digestibility studies using LIPE® in diets based on cassava root scrapings and elephant grass by rumen fistulated sheep, Magalhães et al. [13] reported that the DMI was overestimated and concluded that LIPE® does not accurately predict faecal production under the experimental conditions evaluated.

Santos et al. [22] evaluated the individual consumption of F1 Holstein × Gyr cows fed sorghum silage, corn silage, fresh sugarcane and sugarcane silage treated with 1% CaO, as estimated with the markers LIPE® and TiO2. The authors concluded that both markers were efficient in estimating consumption and that the sugarcane silage-based diet resulted in a low DMI. Divergent results were observed in the present study, when lactating cows fed diets based on sugarcane silage and concentrate containing whole cottonseed were used, where there was no effect of whole cottonseed levels in the diet on the estimates of faecal production of the external markers.

In a study carried out on pasture evaluating the estimate of faecal production with the marker TiO2 in beef steers with an average weight of 230.5±14.4 kg equipped with faecal collection bags, Silva et al. [21] observed that TiO2 had greater precision in estimating faecal production and good faecal recovery rates, thus reinforcing the effectiveness of using the marker TiO2 in nonfistulated animals kept in grazing systems.

4. Conclusion

The external markers LIPE® and TiO2 can be used to estimate the individual dry matter intake of lactating confined cows fed diets based on sugarcane silage supplemented with concentrates containing increasing levels of whole cottonseed. Moreover, the choice of external marker to be used depends on the price, availability and laboratory analysis conditions.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

Conceptualization: D. A. Costa, J. C. Carneiro e F. C. F. Lopes. *Data curation*: G. R. Moreira, D. A. Costa, J. C. Carneiro e F. C. F. Lopes. *Formal analysis*: C. L. Souza, G. R. Moreira e D. A. Costa. *Investigation*: D. A. Costa. *Methodology*: C. L. Souza, J. C. Carneiro e D. A. Costa. *Funding acquisition*: J. C. Carneiro e F. C. F. Lopes. *Project* *administration*: D. A. Costa. *Validation and visualization*: D. A. Costa. *Supervision*: D. A. Costa. *Writing (original draft)*: D. A. Costa. *Writing (review & editing)*: E. O. S. Saliba, M. R. P. Figueiredo, L. S. Amaral, F. C. F. Lopes e D. A. Costa.

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References

1. Velásquez AV, Oliveira CA, Martins CMMR, Balieiro JCC, Silva LFP, Fukushima RS, Sousa DO. Diet, marker and fecal sampling method interactions with internal and external marker pairs when estimating dry matter intake in beef cattle. Livestock Science. 2021;253:e104730. DOI: https://doi.org/10.1016/j.livsci.2021.104730

2. Maciel FA, Dias KDG, Gasparotto PHG, Dantas Filho JV, Cavali J. Produção de leite de vacas da raça girolando alimentadas com dietas com e sem caroço de algodão (*Gossypium hirsutum* L). Tekhne e Logos. 2020; 11(3):63-74.

3. Costa DR, Ribeiro KG, Cruz GFL, Silva TC, Cardoso LL, Pereira OG. Silagens mistas de cana-de-açúcar e amendoim forrageiro tratadas com *Lactobacillus buchneri*. Ciência Animal Brasileira. 2022;23:e72352P. DOI: https://doi.org/10.1590/1809-6891v23e72352P

4. Peres, MTP, Batista R, Twardowski TS, Ribeiro Filho HMN, Monteiro ALG. Titanium dioxide (TiO2) as a marker to estimate fecal output in sheep. Ciência Rural. 2019;49(12):e20190190. DOI: https://doi.org/doi:10.1590/0103-8478cr20190190

5. Titgemeyer EC, Armendariz CK, Bindel DJ, Greenwood RH, Löest CA. Evaluation of titanium dioxide as a digestibility marker for cattle. Journal of Animal Science. 2001;79(4):1059-1063. DOI: https://doi.org/10.2527/2001.7941059x

6. Saliba EOS, Faria EP, Rodriguez NM, Moreira GR, Sampaio IBM, Saliba JS, Gonçalves LC, Borges I, Borges ALCC. Use of infrared spectroscopy to estimate fecal output with marker Lipe®. International Journal of Food Science, Nutrition and Dietetics. 2015;S4(001):1-10. DOI: https://doi.org/10.19070/2326-3350-si04001

7. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTIS - AOAC. Official methods of analysis. 19th ed. Gaithersburg: AOAC International; 2012.

8. Tilley JMA, Terry RA. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science. 1963;18(2):104–111. DOI: https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

9. Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG. Métodos para análise de alimentos. Visconde do Rio Branco, MG: Suprema; 2012. 214p.

10. Prigge EC, Varga GA, Vicini J L, Reid RL. Comparison of ytterbium chloride and chromium sesquioxide as fecal indicators. Journal of Animal Science. 1981;53(6):1629-1633. DOI: https://doi.org/10.2527/jas1982.5361629x

11. Lima RF, Pantoja DS, Almeida BF, Silva ACT, Cunha AR. Avaliação de indicadores externos de produção fecal em bubalinos confinados. Agroecossistemas. 2020;12(1):201-211, 2020. DOI: http://dx.doi.org/10.18542/ragros.v12i1.9012

12. Figueiredo MRP, Saliba EOS, Barbosa GSSC, Silva FA, Nunes AN, Silva CRM, Moreira GR. Indicadores na estimativa da digestibilidade aparente em dietas com inclusão de coproduto do cacau. Semina: Ciências Agrárias. 2019;40(6):2771-2782. DOI: https://doi.org/10.5433/1679-0359.2019v40n6p2771

13. Magalhães ALR, Teodoro AL, Souza MFS, Nascimento Júnior JRS, Sousa DR, Santos KC, Andrade AP, Pinto CS, Barbos, IFT, Silva SMC. Dietas para ovinos contendo capim elefante maduro e raspa de mandioca: estimativa de digestibilidade aparente por meio de indicadores. Archivos de Zootecnia. 2018;67(258):244-252. DOI: https://doi.org/10.21071/az.v67i258.3660

14. Myers WD, Ludden PA, Nayigihugu V, Hess BW. Excretion patterns of titanium dioxide and chromic oxide in duodenal digesta and feces of ewes. Small Ruminant Research. 2006;63(1-2):135-141, 2006. DOI: https://doi.org/10.1016/j.smallrumres.2005.02.010

15. Hotzel MJ, Teixeira DL, Machado Filho LCP. A hierarquia social e o regime de oferta influenciam o consumo de água em bovinos leiteiros. Revista Brasileira de Agroecologia. 2013;8(1):84-91.

16. Garcez BS, Alves AA, Araújo DLC, Oliveira ME, Pompeu RCFF, Rogério MCP, Moreira AL, Lima ISS. Performance and grazing behavior of growing goats supplemented with palm tree fruit. Revista Brasileira de Zootecnia. 2019; 48:e20180159. DOI: https://doi.org/10.1590/rbz4820180159

17. Saliba EOS, Silva FA, Silva CRM, Rodriguez NM, Barbosa GSSC, Borges ALCC, Borges I, Gonçalves LC, Saliba JS. Utilization of markers Lipe and TiO2 compared to total collection of feces on determination of digestibility and fecal output in bovines. Proceedings of the Australian Society of Animal Production. 2014;30(s/n):75.

18. Moreira Filho MA, Alves AA, Garcez BS, Moreira AL, Azevêdo DMMR, Parente HN. Digestibility markers of sheep diets containing hydrolyzed sugarcane-top hay. Revista Brasileira de Saúde e Produção Animal. 2017;18(1):38-49. DOI: https://doi.org/10.1590/s1519-99402017000100005

19. Figueiredo MRP, Saliba EOS, Barbosa GSC, Colodo JCN, Almança RA, Moreira GR. Digestibility of diets with passion fruit by-product estimated through external and internal markers in dairy heifers. Acta Scientiarum. Animal Sciences, 2021;43:e50241. DOI: https://doi.org/10.4025/actascianimsci.v43i1.50241

20. Silva JJ, Saliba EOS, Borges I, Gonçalves LC, Rodriguez NM, Aroeira LJM, Silva AGM, Costa FJN. Indicadores para estimativa de consumo total por novilhas holandês x zebu mantidas em confinamento. Revista Brasileira de Saúde e Produção Animal. 2010;11(3):838-848.

21. Silva S, JC, Correa L, GA, Mayorga, M, OL, Duran, C, EN, Portilla P, D, Diaz T, FA, Granja-Salcedo YT, Valencia E, DM. Effect of marker dosage frequency and spot fecal sampling frequency in the prediction accuracy of fecal output using chromic oxide and titanium dioxide in grazing BON steers. Tropical Animal Health and Production. 2021;53(5):e448. DOI: https://doi.org/10.1007/s11250-021-02902-0

22. Santos SA, Valadares Filho SC, Detmann E, Valadares RFD, Ruas JRM, Amaral PM. Different forage sources for F1 Holstein × Gir dairy cows. Livestock Science. 2011;142(1-3):48-58. DOI: https://doi.org/10.1016/j.livsci.2011.06.017