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Digestibility of diets with passion fruit by-product estimated through external and internal markers in dairy heifers

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ABSTRACT. This study examined the influence of the external markers chromium oxide (CO), titanium dioxide (TD), isolated, purified and enriched lignin (LIPE®) and isolated, purified, enriched lignin in nanoparticles (NANOLIPE®) as well as the internal markers indigestible dry matter (iDM), indigestible neutral detergent fiber (iNDF) and indigestible acid detergent fiber (iADF) in diets with inclusion of passion fruit by-product for dairy heifers on the estimation of fecal output and nutrient digestibility. Sixteen Holstein × Zebu crossbred heifers at an average live weight of 363 ± 28 kg were randomly distributed in a completely randomized design where they received diets in which Tifton 85 (*Cynodon* sp.) hay was replaced with passion fruit by-product (0, 12, 24 and 36%, as-fed basis). The CO, LIPE®, NANOLIPE® and iNDF markers did not differ from the total collection method ($p > 0.05$) in the estimation of fecal output and nutrient digestibility. The TD and iDM markers overestimated, while iADF underestimated fecal output. Under the presented conditions, we recommend using the CO, LIPE®, NANOLIPE® and iNDF markers to estimate fecal output and nutrient digestibility in diets with inclusion of passion fruit by-product for dairy heifers.

Keywords: fecal recovery; *in situ*; passion fruit by-product; total collection.

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Introduction

Intake and digestibility are variables of great importance in animal production systems, since they are directly related to nutrient uptake and, consequently, to whether the nutritional requirements of animals are being met. According to Mertens (1994), animal performance is a direct reflection of digestible dry matter intake, with 60 to 90% of its variations resulting from changes in intake and 10 to 40% from changes in digestibility. These parameters can be estimated via total fecal collection and/or with the use of markers, which have exhibited promising results and an easy evaluation methodology. Markers are substances that are naturally present in or can be added to the animal diet, which characterizes them as internal or external, respectively. By using them, one can accurately estimate the nutrient digestibility and intake of the most diverse animal species and categories.

Although none of the substances used as markers fulfills all the ideal characteristics, many of them are sufficiently adequate to provide significant data (Berchielli, Garcia, & Oliveira, 2006). For this reason, the search for ideal markers is one of the subjects of great interest in the discovery of techniques that facilitate animal nutrition studies.

The seasonality of herbage production has been responsible, among other factors, for the reduced yields of herds. Coupled with the production cost invested in animal feed, this has aroused interest in the use of alternative feedstuffs. In this respect, by-products of fruits such as passion fruit have been constantly evaluated mainly in terms of their adequate level of inclusion in ruminant diets. In grass silage, these by-products have shown good results (Alves et al., 2015; Bonfá et al., 2015; Lira Júnior et al., 2018).

In addition to contributing to reducing environmental contamination caused by improper disposal, the use of industrial by-products in animal feed serves as an alternative source of nutrients for the period of food scarcity, constituting an important fiber source. The use of a non-forage fiber source such as passion fruit by-product in ruminant diets contributes to the supply of nutrients and may lower the diet cost, representing a viable option in regions where it is available. Studies have focused on the evaluation of markers in different diets because the varied results obtained may be related to the animal diet.

Therefore, this study proposes to examine the effectiveness of the external markers chromium oxide (CO), titanium dioxide (TD), LIPE® and NANOLIPE® and the internal markers indigestible dry matter (iDM), indigestible neutral detergent fiber (iNDF) and indigestible acid detergent fiber (iADF) in the estimation of fecal output and nutrient digestibility in Tifton 85 grass hay-based diets with increasing inclusion levels of passion fruit by-product for dairy heifers.

Material and methods

This study was carried out in accordance with the Ethical Principles of Animal Experimentation adopted by the Ethics Committee on Animal Experimentation (CETEA/UFMG) (approval no. 225/2015).

The experiment was carried out in the facilities of the Center for Agrarian Education and Development of Florestal at the Federal University of Viçosa, located in Florestal - MG, Brazil, from May to August 2012. Sixteen Holstein × Zebu crossbred heifers at an average weight of 363 ± 28 kg were identified, weighed, dewormed and housed individually in cement-floored stalls.

The diets were based on Tifton 85 grass hay, corn meal and passion fruit by-product. The chemical composition of the feedstuffs and the average nutrient levels determined in the diets are shown in Tables 1 and 2.

Table 1. Chemical composition (%) and gross energy levels (kcal g^{-1}) of the ingredients of the diets supplied in the experiment (DM basis).

Nutrient	Tifton 85 hay	Corn meal	Passion fruit by-product
Dry matter (% as fed)	91.91	87.00	31.00
Mineral matter	6.01	1.60	9.71
Crude protein	5.50	9.10	13.55
Neutral detergent fiber	79.42	8.30	60.36
Acid detergent fiber	39.76	3.51	49.27
Total carbohydrates	85.98	85.90	64.34
Non-fibrous carbohydrates	14.84	86.35	17.78
Lignin	5.08	1.34	21.89
NDIN*/Total N	38.64	5.95	14.30
ADIN*/Total N	15.91	10.99	10.61
Ether extract	1.41	3.75	12.40
Gross energy	4.01	4.73	4.68
IVDMD	56.70	89.14	53.48
TDN*	57.74	83.55	53.32

*NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; TDN = total digestible nutrients, estimated according to Cappelle, Valadares Filho, Silva, & Cecon (2001).

The experimental period was 17 days, which consisted of 12 days of adaptation to diets, management and facilities plus five days of experimental collections. Feed was supplied daily, in two meals: 50% at 07h00 and 50% at 16h00. Orts were collected daily in the morning and weighed and the amount of feed provided was adjusted to allow 10% orts.

The diets and orts were dried for 72 h at 55ºC in an air oven and crushed to 1 mm through a knife mill. Chemical analytical methods (Association Official Analytical Chemists International [AOAC], 2012) were used to determine the dry matter (DM; method 934.01), mineral matter (MM; Method 942.05), crude protein (CP; method 984.13) and ether extract (EE; method 920.39) contents. For neutral detergent fiber (NDF; method INCT-CA F-001/1), acid detergent fiber (ADF; method INCT-CA F-003/1), total and non-fibrous carbohydrates and lignin (method INCT-CA F-007/1), the analysis procedures proposed by Detmann et al. (2012) were adopted. Gross energy was determined by combustion in an adiabatic calorimeter (PARR 6300). *In vitro* dry matter digestibility (IVDMD) was determined by the method proposed by Tilley and Terry (1963).

Fecal output was estimated using the external markers chromium oxide (CO), titanium dioxide (TD), isolated, purified and enriched lignin (LIPE®) and isolated, purified and enriched lignin in nanoparticles

(NANOLIPE®) and the internal markers indigestible dry matter (iDM), indigestible neutral detergent fiber (NDF) and indigestible acid detergent fiber (iADF). The CO and TD markers were mixed with the diet at the dose of 10 g animal^{-1} day^{-1}, for an adaptation period of seven days followed by five days of feces collection. The LIPE® and NANOLIPE® markers were administered orally, in the form of capsules, at the dose of 500 mg animal^{-1}, for an adaptation period of two days (Lima, Graça, Borges, Saliba, & Simão, 2008) followed by five days of total fecal collection for LIPE® and one day of adaptation followed by one day of total fecal collection for NANOLIPE® (Figueiredo et al., 2019).

Table 2. Percentage (%) and chemical compositions and mean gross energy (kcal g^{-1}) of diets containing increasing levels of passion fruit by-product (DM basis).

Ingredient	Experimental diet			
	0	12	24	36
	Percentage composition			
Tifton 85 grass hay	79.00	70.00	56.00	44.00
Corn meal	18.00	15.00	17.00	17.00
Passion fruit by-product	0.00	12.00	24.00	36.00
Urea + ammonium sulfate	1.00	1.00	1.00	1.00
Vitamin/mineral supplement	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
	Chemical composition			
Dry matter	88.27	81.10	73.70	66.30
Mineral matter	4.11	4.79	5.31	5.90
Crude protein	9.10	10.00	11.10	12.10
Neutral detergent fiber	67.04	66.51	62.64	60.10
Acid detergent fiber	33.48	35.55	35.70	36.62
Non-fibrous carbohydrates	25.84	24.27	25.97	26.36
Lignin	4.25	6.38	8.33	10.34
NDIN*	0.29	0.28	0.28	0.27
NDIN/Total N	31.76	29.81	27.55	25.28
ADIN*	0.15	0.16	0.17	0.18
ADIN/Total N	15.29	14.86	14.43	14.00
Ether extract	1.57	2.84	4.23	5.58
Gross energy	3.81	3.86	3.91	3.96
TDN*	51.12	54.30	54.09	63.18

*NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; TDN = total digestible nutrients.

The internal markers iDM, iNDF and iADF were obtained from samples of feed, orts and feces, which were packed in non-woven fabric bags ("TNT"; 100 g cm^{-2}) measuring 4 × 5 cm and incubated in the rumen of two adult cattle for 264h, as suggested by Casali et al. (2008), to obtain the indigestible fractions *in situ*.

After 264 h of *in situ* incubation, the bags were removed from the rumen and washed in running water, dried in a forced-air oven and weighed and the residue was used to determine iDM. The iNDF and iADF markers were determined after washing the bags with neutral and acid detergent solutions, respectively (in a non-sequential manner), oven-drying and weighing, and the residue was used to determine the indigestible fractions.

Fecal output (FO) as estimated by the markers was determined by the following formula:

$$FO = \frac{\text{Marker intake (g)}}{\text{Marker content in feces (\%) (DM 105ºC)}}$$

The LIPE® and NANOLIPE® markers were analyzed by Near Infrared Spectroscopy using a FTIV - 800 (Varian) instrument, following Saliba et al. (2015) and Moss et al. (2017), respectively. Chromium oxide was determined as suggested by Detmann et al. (2012) (INCT method - CA M-006/1). The TD content was determined according to Myers, Ludden, Nayigihugu, and Hess (2004).

The apparent digestibility coefficients of nutrients as obtained by the external and internal marker methods were calculated as proposed by Silva and Leão (1979):

$$\text{Digestibility (\%)} = 100 - 100 \times \frac{\text{\% of marker in feed}}{\text{\% of marker in feces}}$$

The equation proposed by Lanzetta et al. (2009) was applied to calculate the fecal recovery rate of each marker:

$$\text{Fecal recovery rate} = \frac{\text{Fecal output by marker}}{\text{Fecal output by total fecal collection}} \text{ x } 100$$

The experiment was laid out in a completely randomized split-plot design where the plots were the treatments (diets) and the markers represented the subplots. Data analysis was performed using SISVAR (Statistical Analysis and Experimental Design Program) software (Ferreira, 2011). Fecal output as well as the apparent digestibility and fecal recovery coefficients were subjected to analysis of variance and means were compared by the SNK test ($p < 0.05$).

Results and discussion

The external markers CO, LIPE®, NANOLIPE® and the internal marker iNDF did not differ from the total collection method ($p > 0.05$) in the estimation of fecal output. Divergent results were obtained for the other evaluated markers. The external marker TD and the internal marker iDM overestimated fecal output by 27.12 and 34.51%, respectively, whereas the internal marker iADF underestimated it by almost 41% (Table 3). The fecal recovery rates of the iNDF and CO markers were 0.54% and 5.75% higher than observed. With LIPE® and NANOLIPE®, however, fecal recovery rate was 7.4% and 6% lower, respectively.

Table 3. Mean fecal output values (kg DM day^{-1}), standard error of the mean (SEM) and fecal recovery rate (FRR) as obtained by total fecal collection and estimated using external and internal markers.

Treatment	Fecal output	SEM	FRR
Total fecal collection	3.65b*	0.20	100.00b
Chromium oxide	3.86b	0.15	105.75b
Titanium dioxide	4.64a	0.34	127.12ab
LIPE®	3.38b	0.21	92.60bc
NANOLIPE®	3.43b	0.14	94.00bc
Indigestible dry matter	4.91a	0.19	134.51a
Indigestible neutral detergent fiber	3.67b	0.10	100.54b
Indigestible acid detergent fiber	2.16c	0.16	59.20c
CV (%)	18.84		19.44

*Means followed by different letters in the column differ statistically according to the SNK test ($p < 0.05$). LIPE® - isolated, purified and enriched lignin; NANOLIPE® - isolated, purified and enriched lignin in nanoparticles; CV - coefficient of variation.

The results obtained with the external markers CO, LIPE® and NANOLIPE® and the internal marker iNDF for the digestibility of DM, CP and NDF did not differ ($p > 0.05$) from the actual values found (Table 4), at any of the passion fruit by-product inclusion levels.

Dietary inclusion of passion fruit by-product induced a reduction in NDF and an increase in ADF contents when compared with the diet without the ingredient. Lignin levels also increased, likely due to the presence of seeds in the passion fruit by-product. The average DM digestibility of the diets was 52% and varied according to the estimate provided by each evaluated marker.

Results obtained with CO are abundant and varied in the literature because it is the most commonly used external marker in trials to estimate intake and digestibility in several categories of animals and diets. According to Owens and Hanson (1992), variations in fecal CO excretion throughout the day are a relatively known limitation of this marker. Unlike the present results, Silva et al. (2010) found that CO underestimated the intake of feedlot heifers fed diets based on of elephant grass silage or sugarcane, regardless of the diet, with results differing from those measured at the trough and obtained with the iNDF, iADF, Klason lignin and LIPE® markers. Ribeiro Filho, Zimermann, and Kozloski (2008) reported that the different CO recovery rates described in the literature can be attributed to different protocols used and to differences in the type and quality of the diet.

The results reported here using the LIPE® marker corroborate other authors who have shown positive outcomes with the use of this marker in the estimation of intake, fecal output and nutrient digestibility in several animal species (Ferreira et al., 2009; Lanzetta et al., 2009; Andrade et al., 2013; Silva et al., 2014; Saliba et al., 2015). LIPE® has the advantage of being provided in only two days, which reduces animal stress when the marker is given orally. NANOLIPE®, in turn, offers the advantage of coming in the form of nanoparticles, which favors its incorporation into the digesta, with a better fecal recovery rate. The results obtained with this marker in the present study are promising, and the intake and digestibility trials that have used it are recent. A noteworthy advantage of NANOLIPE® is the shorter time of adaptation of the

animals and shorter period of fecal collection compared with those of the other evaluated markers, which makes it possible to minimize the work, labor and animal stress, demonstrating its use potential, as shown in the studies of Moss et al. (2017) and Figueiredo et al. (2019).

Table 4. Mean values (%) of apparent digestibility of dry matter (DMd), crude protein (CPd) and neutral detergent fiber (NDFd), standard error of the mean (SEM) and coefficient of variation (CV, %) of the diets as estimated by the external and internal markers and compared with total collection.

Treatment	Digestibility					
	0	12	24	36	Mean	SEM
			DMd			
Total collection	48.70bc	54.55bc	48.75bc	55.65bc	51.91	1.34
Chromium oxide	39.60c	48.56c	47.99c	54.08c	47.56	2.35
Titanium dioxide	34.93d	38.40d	40.68d	37.69d	37.93	3.71
LIPE®	57.08b	59.45b	49.11b	63.92b	57.39	2.25
NANOLIPE®	53.96b	64.45b	49.46b	62.04b	57.48	1.99
iDM	38.96d	33.52d	25.89d	36.35d	33.68	2.07
iNDF	50.13bc	46.35c	49.28bc	55.26bc	50.26	1.48
iADF	67.94a	70.91a	66.01a	77.52a	70.60	2.34
			CPd			
Total collection	64.09bc	66.62bc	63.23bc	71.05bc	66.25	1.43
Chromium oxide	57.84c	64.76c	63.01c	70.78c	64.10	1.58
Titanium dioxide	54.90d	57.41d	57.72d	60.10d	57.53	2.46
LIPE®	70.01b	72.44b	63.47b	75.19b	70.28	1.77
NANOLIPE®	67.87b	75.55b	63.73b	75.19b	70.59	1.69
iDM	57.36d	54.34d	46.86d	58.19d	54.19	2.02
iNDF	65.12bc	63.13bc	63.83bc	70.92bc	65.75	1.28
iADF	77.85a	79.96a	75.82a	85.20a	79.71	1.62
			NDFd			
Total collection	41.20bc	44.84bc	39.69bc	48.00bc	43.43	2.13
Chromium oxide	30.90c	42.41c	38.87c	44.98c	39.29	2.83
Titanium dioxide	25.92d	30.72d	30.05d	37.88d	31.14	3.58
LIPE®	50.86ab	54.39ab	40.06ab	57.29ab	50.65	2.73
NANOLIPE®	42.27ab	60.20ab	40.43ab	55.03ab	49.48	2.48
iDM	30.08d	25.32d	17.73d	24.48d	24.40	2.48
iNDF	42.88bc	39.66c	40.46bc	46.74bc	42.44	1.80
iADF	54.00a	57.44a	48.55a	63.78a	55.94	3.29

*Means followed by different letters in the column differ statistically according to the SNK test ($p < 0.05$). *DMd – CV = 16.76%; CPd – CV = 9.12%; NDFd – CV = 25.47%.

As regards the internal markers iDM, iNDF and iADF, results presented in the literature indicate that their behavior may vary depending on the analysis methodology and type of diet provided to the animals—more specifically, the fiber used. This is because the constitution of the fibrous fraction of each roughage changes the rate and extent of degradation (Berchielli, Oliveira, Carrilho, Feitosa, & Lopes, 2005). Berchielli et al. (2006) also stated that errors in the methodology of analysis of these markers lead to variations in the results and that some markers are probably more adequate than others depending on the roughage used. In addition, other factors such as the form of incubation (*in situ* or *in vitro*), incubation time and loss of particles through the pores of the nylon bags may be the main causes of the observed variations in the fecal recovery of the internal markers. The internal marker iNDF came closest to the total collection method in the estimates of fecal output and digestibility (Tables 3 and 4).

Dias et al. (2008) found that iADF accurately estimated DM digestibility and fecal recovery like total fecal collection, in a trial with cattle fed a diet based on Tifton grass hay and concentrate. In an experiment with cattle fed diets based on ground corn, soybean hulls or corn germ meal, Watanabe, Ezequiel, Galati, Biagioli, and Silva (2010) recommended using iNDF and iADF together, as they provided accurate digestibility estimates of the evaluated diets. Maeda et al. (2011) found that iDM accurately estimated omasal flow in cattle and buffalo. As for fecal flow, CO showed fecal recovery close to 100%, with iNDF overestimating and iADF underestimating fecal output. Sampaio et al. (2011) evaluated the fecal recovery of the iDM, iNDF, iADF, CO and TD markers in cattle fed elephant grass silage, corn silage or *Brachiaria* grass hay, supplemented or unsupplemented with a 20% concentrate mixture, and found satisfactory results for all evaluated markers, with fecal recovery not differing from 100%.

Pombo, Valle, Bradi, and Bueno (2016) evaluated the accuracy, precision and robustness of the internal markers iNDF, iADF, indigestible cellulose and acid detergent indigestible lignin in the prediction of apparent DM digestibility in horses and also recommended iADF. Magalhães et al. (2018) conducted a trial evaluating the digestibility of diets based on cassava chips and mature elephant grass in sheep and recommended the internal markers iNDF and iADF. Additionally, Sousa et al. (2018) reported that iDM showed satisfactory results in estimating nutrient intake and digestibility compared with iNDF and iADF, in a trial with grazing sheep.

Fecal output as estimated by TD in this study does not agree with values reported in the literature (Ferreira et al., 2009; Glindemann, Tas, Wang, Alvers, & Susenbeth, 2009). Wang, Ragland, and Adeola (2017) reported a low fecal recovery rate of the TD marker when compared with total fecal collection in pigs fed diets with corn starch, corn meal or oatmeal. Sampaio et al. (2011) investigated the excretion profile of the CO, TD, iDM, iNDF and iADF markers, as well as different fecal sampling designs, to obtain representative point samples. According to these authors, there must be at least four fecal collections, which can be distributed throughout the day, for a significant representativeness of the total excreted feces; and four days of collection, so that there the marker is accurately represented in the fecal sample. This protocol would reduce sampling errors that could interfere with the determination of fecal output and, consequently, nutrient digestibility by the marker. In the present study, the external markers were supplied once daily and the feces were also collected once, in the morning.

In view of the foregoing, it is imperative to standardize the methodologies of use and analysis of markers in digestibility and intake trials so that better responses can be obtained with the most varied diets and animal categories evaluated.

Conclusion

The chromium oxide, LIPE®, NANOLIPE® and indigestible neutral detergent fiber markers accurately estimated fecal output and nutrient digestibility.

The titanium dioxide, indigestible dry matter and indigestible acid detergent fiber markers were inefficient in estimating fecal output and nutrient digestibility.

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