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Use of indigestible markers to estimate the apparent dry matter digestibility of diets containing a cocoa by-product

Indicadores na estimativa da digestibilidade aparente em dietas com inclusão de coproduto do cacau

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Abstract

Fecal production and apparent dry matter digestibility (ADMD) were evaluated using external markers (chromium oxide; titanium dioxide; isolated, purified, and enriched lignin (LIPE®); and isolated, purified, and enriched lignin nanoparticles (NANOLIPE®) and internal markers (indigestible DM (IDM), indigestible neutral detergent fiber (INDF), and indigestible acid detergent fiber (IADF) in diets based on Tifton 85 bermuda grass (*Cynodon* sp.) hay containing different concentrations of a cocoa by-product. Sixteen crossbred (Holstein × Zebu) dairy heifers with a mean live weight of 363.00 ± 27.70 kg were evaluated and distributed in a completely randomized block design with a split-plot arrangement. The plots corresponded to the diets, which differed in the substitution of bermuda grass hay with different concentrations (0, 8, 16, and 24% of DM) of the cocoa by-product, whereas the split-plots represented the indigestible markers. Chromic oxide, LIPE®, NANOLIPE®, and INDF accurately estimated ADMD and fecal production whereas titanium dioxide, IDM, and IADF did not accurately estimate these parameters.

Key words: Total collection method. Markers. Chromic oxide. Fecal production. Accuracy.

Resumo

Foram avaliados a produção fecal e a digestibilidade dos nutrientes com o uso de indicadores externos (óxido crômico, dióxido de titânio, lignina isolada, purificada e enriquecida - LIPE® e lignina isolada, purificada e enriquecida em nanopartículas -NANOLIPE®) e internos (matéria seca indigestível - MSi, fibra em detergente neutro indigestível - FDNi e fibra em detergente ácido indigestível -FDAi) em dietas a base de feno de capim tifton 85 com inclusão de coproduto de cacau. Foram utilizadas dezesseis novilhas leiteiras mestiças Holandês X Zebu, peso vivo médio (363,00 ± 27,70 kg), distribuídas em delineamento inteiramente casualizado com arranjo em parcelas subdivididas. As dietas oferecidas foram as parcelas, que se diferenciavam quanto à substituição do feno de capim Tifton 85 (*Cynodon sp*)

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pelo coproduto do cacau em diferentes níveis (0, 8, 16 e 24% da MS) e, os indicadores, as subparcelas. Os indicadores óxido crômico, LIPE®, NANOLIPE® e FDNi estimaram com acurácia e precisão a digestibilidade aparente dos nutrientes e a produção fecal. Os indicadores dióxido de titânio, MSi e a FDAi foram ineficientes para estimar esses parâmetros.
Palavras-chave: Coleta total. Marcadores. Óxido crômico. Produção fecal. Acurácia.

Introduction

The nutritional value of the forage consumed by animals needs to be determined when assessing the efficiency of animal production systems. Forage quality is determined by nutrient digestibility and consumption. Digestibility measures are used to determine the nutritional value of forage and indicate the amount of each nutrient that can be used by the animal. Mertens (1994) and Van Soest (1994) have shown that forage consumption is the main influencing factor for animal performance because it is directly related to nutrient intake and therefore is essential for assessing nutritional requirements.

Total fecal collection (TC) is used as a reference method in nutrient consumption and digestibility tests. However, the high cost, long evaluation period, need for continuous labor, need for large sample size, extended time to adapt to the diet, and strict control of feed intake and excretion make indigestible markers a feasible option for this purpose (POMBO et al., 2016).

Indigestible markers (IMs) are compounds present in a feed (internal) or added to feed (external) and are not absorbed or digested in the gastrointestinal tract of animals (OWENS; HANSON, 1992). These indicators are supplied to the animals via diet and should be fully recovered in the feces (ITURBIDE, 1967).

The external marker (EM) chromium oxide (CO) is the most used in the literature (COSTA et al., 2018; SCHAAFSTRA et al., 2019). Studies on the EMs titanium dioxide (TD) (COSTA et al., 2018; SCHAAFSTRA et al., 2019) and isolated, purified, and enriched lignin nanoparticles (NANOLIPE®) (MOSS et al., 2017) are promising; however, further studies with different animal species and diets are needed. The EM Isolated, Purified, and Enriched Lignin (LIPE®) was used in several animal species (MARCONDES et al., 2008; FERREIRA et al., 2009; LANZETTA et al., 2009).

IMs are natural constituents of animal feed, including silica, acid-insoluble ash, lignin, fecal nitrogen, indigestible dry matter (IDM), indigestible neutral detergent fiber (INDF), and indigestible acid detergent fiber (IADF) (KOZLOSKI et al., 2009; LEE; HRISTOV, 2013; ALVES et al., 2016; MAGALHÃES et al., 2018). The results using known indicators may vary because of the adoption of different analytical techniques, partial fecal recovery rates, and different responses to diets, among other reasons, leading to precision errors in digestibility estimates.

Fecal production, apparent dry matter digestibility, and the accuracy and precision of EMs and IMs were evaluated in diets based on Tifton 85 bermuda grass hay containing different concentrations of a cocoa by-product in dairy heifers.

Materials and Methods

This study was carried out in accordance with the Ethical Principles of Animal Experimentation established by the Animal Research Ethics Committee of the Federal University of Minas Gerais (Comitê de Ética em Experimentação Animal da Universidade Federal de Minas Gerais–CETEA/UFMG) under Protocol No. 225/2015.

The study was performed in the facilities of the Agricultural Education and Development Center of the Federal University of Viçosa (Universidade Federal de Viçosa–UFV), located in Florestal, Minas Gerais, Brazil. Sixteen crossbred (Holstein × Zebu) heifers with a mean weight of 363.00 ±

27.70 kg were identified, weighed, dewormed, and individually housed in cement-floor pens.

The study period was 17 days, including 12 days of adaptation to the diets, animal husbandry, and rearing facilities, and 5 days of TC. The animals were fed daily and were equally divided into two groups, such that one group was fed at 7h00 a.m. and the other group was fed at 4h00 p.m. Leftovers were collected daily in the morning, weighed, and the amount of supplemented feed was adjusted to leave 10–15% of leftovers in the troughs.

The diets were based on Tifton 85 bermuda grass hay, corn meal, and different dry matter (DM) concentrations (0, 8, 16, and 24%) of a cocoa by-product (CB). The percentage compositions and chemical analyses are presented in Table 1.

Table 1. Percentage of ingredients (g kg^{-1}) based on the dry matter and chemical composition of experimental diets containing increasing levels of a cocoa by-product.

Ingredients	Diets (%)			
	0	8	16	24
Tifton 85 bermuda grass hay	790.00	710.00	620.00	530.00
Corn meal	180.00	180.00	190.00	200.00
Cocoa by-product	0.00	80.00	160.00	240.00
Urea + ammonium sulfate	10.00	10.00	10.00	10.00
Vitamins/minerals	20.00	20.00	20.00	20.00
Total	1000.00	1000.00	1000.00	1000.00
	Chemical composition of diets			
Dry matter	870.00	820.10	770.20	722.20
Mineral matter	50.00	54.90	59.10	63.20
Crude protein	97.00	98.00	100.00	101.00
Neutral detergent fiber	642.40	641.50	633.90	626.10
Acid detergent fiber	320.10	336.80	349.80	362.80
Lignin	25.20	45.80	65.80	86.00
Ether extract	15.70	18.40	19.20	20.00
Total digestible nutrients (%)	657.20	612.40	641.30	572.30

Fecal production (FP) was estimated using the EMs CO; TD; isolated, purified, and enriched lignin (LIPE®; modified and enriched hydroxyphenylpropane [P2S2], Florestal, Minas Gerais, Brazil); and isolated, purified, and enriched lignin nanoparticles (NANOLIPE®) (P2S2; Florestal, Minas Gerais, Brazil) and the IMs IDM, INDF, and IADF.

CO and TD were mixed to the diet at the dosage of ten grams $animal^{-1}$ day^{-1} for 12 days, including seven days of dietary adaptation and five days of TC. LIPE® and NANOLIPE® were administered as capsules at the dosage of 500 mg $animal^{-1}$ day^{-1}. LIPE® was administered for seven days, including two days of adaptation and five days of TC, and NANOLIPE® was administered for two days, including one day of adaptation and one day of TC. IDM, INDF, and IADF were determined in samples of feed, leftovers, and feces. The samples were milled in a Willey type mill with a 1-mm sieve and transferred to non-woven fabric bags (size, 4 × 5 cm; density, 100 g per cm^2) at the concentration of 20 mg of DM per cm^2 of surface (NOCEK, 1988). The samples were incubated in the rumen of two male

adult bovine animals for 264h00, as suggested by Casali et al. (2008), to obtain indigestible fractions in situ.

The bags were removed from the rumen, washed with running water, dried in forced ventilation oven, and used for IDM quantification. INDF and IADF were determined by washing the bags with a neutral and acid detergent solution, respectively. After that, the bags were dried in an oven at 55 °C, weighed, and used for quantifying indigestible fractions.

The general equation for calculating FP (kg day^{-1} of DM) using external and IMs was based on the ratio between the amount of marker ingested by each animal and its concentration in the feces:

$$FP = \frac{Consumption\ of\ the\ marker\ (g)}{Concentration\ of\ the\ marker\ in\ the\ feces\ (\%)\ (DM\ at\ 105°C)}$$

The CO concentration was analyzed in fecal samples by atomic absorption spectrophotometry according to the methodology described by Silva and Queiroz (2002). TD content was determined according to Myers et al. (2004) using colorimetry-based molecular electron spectroscopy. LIPE® was evaluated by near-infrared spectroscopy in a FTIR equipment. NANOLIPE® was analyzed by infrared spectroscopy with Fourier transform according to Saliba et al. (2015).

The ADMD coefficients of nutrients using external and IMs were calculated according to Silva and Leão (1979), as follows:

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

The fecal recovery rate of each marker was measured according to Lanzetta et al. (2009):

$$Fecal\ recovery\ rate = \frac{Fecal\ production\ using\ a\ marker}{Fecal\ production\ using\ the\ total\ collection\ method} \times 100$$

The accuracy, precision, and robustness of these substances to estimate the ADMD were evaluated according to Kohn et al. (1998). Accuracy was evaluated by the mean bias, which is the difference between the value predicted by the indicator and the value measured by TC. The mean bias was calculated as follows:

$$Mean\ bias = \sum \frac{(predicted - measured)}{Number\ of\ observations}$$

Precision is a measure of the dispersion between predicted and measured values, i.e., the mean variability in the difference between predicted and measured values. Precision can be measured by the root mean square error of prediction (RMSEP):

$$RMSEP = \frac{\sqrt{(predicted - measured)2}}{Number\ of\ observations}$$

The residual error is also referred to as the prediction error, excluding the mean bias, and is obtained by the equation:

$$Residual\ error = \sqrt{[RMSEP^2 - (Mean\ bias)]2}$$

The Shapiro-Wilk test was used to assess the normality of residuals, and the Hartley test was used to evaluate the homogeneity of variances. The Kruskal-Wallis non-parametric test with Dunn's post-hoc test was used to evaluate the residual error of the markers.

The study used a completely randomized block design with a split-plot arrangement. The plots consisted of the treatments (diets), and the split plots corresponded to the indicators. Data were analyzed using the Sisvar program (FERREIRA, 2011). The data were subjected to analysis of variance and regression, and the means were compared using the Student-Newman-Keuls test ($p<0.05$).

Results and Discussion

The FP values estimated using CO, LIPE®, NANOLIPE®, and INDF were similar ($p>0.05$) to those obtained using TC according to the percentage of CB in the diets (Table 2). These substances were efficient in estimating FP at all evaluated CB concentrations, i.e., the estimated amounts were similar to the actual amounts obtained using TC.

Table 2. Mean fecal production (kg dry matter day^{-1}) estimated using the total collection (TC) method and external and internal markers in diets containing different concentrations of a cocoa by-product in dairy heifers.

Markers	Percentage of cocoa by-product				Regression equation	SEM
	0%	8%	16%	24%		
TC	3.07a*	3.86b	3.43a	3.94b	$Y=^{NS}$	0.18
CO	2.94a	3.65b	3.68a	3.35b	$Y=^{NS}$	0.19
TD	2.19b	2.97b	3.45a	2.75c	$Y=2.14+0.16X–0.006X^2$ $r^2=95.50$	0.24
LIPE®	3.16a	3.27b	3.28a	3.63b	$Y=^{NS}$	0.10
NANOLIPE®	3.21a	3.22b	3.28a	3.74b	$Y=^{NS}$	0.10
IDM	3.21a	4.31a	4.18a	5.25a	$Y=3.34+0.074X$ $r^2=86.06$	0.23
INDF	3.07a	3.87b	3.32a	3.77b	$Y=^{NS}$	0.13
IADF	1.39c	1.60c	1.73b	1.85d	$Y=1.41+0.018X–r^2=98.05$	0.10

*The means followed by different letters in each column were not significantly different from each other using the SNK test ($p<0.05$).
CO, chromic oxide; TD, titanium dioxide; LIPE®, isolated, purified, and enriched lignin; NANOLIPE®, isolated, purified, and enriched lignin nanoparticles; IDM, indigestible dry matter; INDF, indigestible neutral detergent fiber; IADF, indigestible acid detergent fiber; NS, not significant; SEM, standard error of the mean.

Increasing levels of CB affected the recovery of TD, IDM, and IADF. TD presented a quadratic function, with a maximum value at 13.33% of CB. Similarly, increasing concentrations of CB influenced the recovery of IDM and IADF. The mean FP using these two indicators was 4.24 kg fecal DM day^{-1} and 1.64 kg fecal DM day^{-1}, respectively.

CO is the most used marker in nutrient consumption and digestibility tests in animal diets because it has been thoroughly researched. However, the results are variable because of several factors, including incomplete mixing with ruminal digesta, faster passage through the rumen than fibrous material, possible accumulation in the digestive tract, variations in fecal excretion throughout the day, and analytical difficulties. However, as in the present study, other authors found that CO produced satisfactory results and presented several advantages, including low cost, ease of administration, and ease of analysis (CABRAL et al., 2008; FERREIRA et al., 2009; SAMPAIO et al., 2011; POZZA et al., 2013; OLIVEIRA et al., 2016).

LIPE® was used in some studies and adequately estimated FP compared to TC, with a recovery

rate close to 100% (FERREIRA et al., 2009; LANZETTA et al., 2009; VASCONCELLOS et al., 2011; SALIBA et al., 2015). The positive results with LIPE in tests of nutrient consumption and ADMD appear to be an advantage, and its use is feasible because of the shorter period of adaptation of the animals to the diet and ease of administration, which helps reduce animal stress and behavioral changes. However, different results were obtained by Magalhães et al. (2018), who recommended using INDF and IADF because they were more accurate than LIPE®.

The results of FP using NANOLIPE® evidenced its higher potential than other markers by acting as a nanoparticle and thoroughly mixing with the ruminal digesta, reducing the adaptation and TC period, and increasing the rate of recovery of this substance (FIGUEIREDO, 2011; MOSS et al., 2017).

In the present study, the recovery of INDF was 100%, which agrees with the results of Sampaio et al. (2011), Carvalho et al. (2013), and Lee and Hristov (2013), who recommended its use to estimate the ADMD of dietary nutrients.

The FP values using CO, LIPE®, NANOLIPE®, and INDF were similar ($p>0.05$) to those using TC (Table 3), which is used to validate the use of these indicators (OLIVEIRA et al., 1997). However, TD and IADF underestimated FP ($p<0.05$) whereas IDM overestimated FP relative to TC.

Table 3. Mean fecal recovery (%) using external and internal markers compared to the total collection (TC) method.

Markers	TC	CO	TD	LIPE®	NANOLIPE®	IDM	INDF	IADF
Fecal recovery	100.00b*	95.24b	79.44c	93.30b	94.06b	118.53a	98.11b	45.94d

*The means followed by different letters in each line were significantly different from each other using the SNK test ($p<0.05$)
CO, chromic oxide; TD, titanium dioxide; LIPE®, isolated, purified, and enriched lignin; NANOLIPE®, isolated, purified, and enriched lignin nanoparticles; IDM, indigestible dry matter; INDF, indigestible neutral detergent fiber; IADF, indigestible acid detergent fiber; coefficient of variation (CV)=16.94%.

The recovery of TD and IADF was less than 100% (Table 3). The mean FP using TD was 2.84 kg DM day^{-1}, which is 20.56% lower than that using TC. In the present study, sample collection was performed once daily in the morning, which may have contributed to the underestimation of FP using TD. Other contributing factors include variations in excretion between animals and the analytical methodology. The FP values were lower than those obtained by Figueiredo (2011), who used TD and reported difficulties inherent to the administration and quantification of this marker. Glindemann et al. (2009) found that FP and the recovery of TD were similar to those obtained by TC; moreover, TD recovery was higher in diets containing hay alone compared to diets containing hay and concentrate, and fecal excretion was improved when the indicator was administered once daily and FC was performed twice daily. Similarly, Souza et al. (2015) evaluated using TD as an alternative to CO for estimating FP and ADMD and observed that CO overestimated FP and the results for these two parameters were more accurate using TD. Moreira Filho et al. (2017) indicated that CO, TD, and IDM were effective in estimating FP and ADMD, with correlation coefficients higher than 90% relative to TC.

FP estimated by IADF was 54.06% lower than that by TC. This result may be because IADF was influenced by the method of measurement, which was sequential and performed after measuring IDM and INDF, resulting in the accumulation of methodological errors. Detmann et al. (2004) reported that the lower concentrations of IADF in feed, leftover, and fecal samples relative to INDF

provided a lower residual mass to be quantified in vitro, and systematic errors due to failure or lack of standardization of analytical methods were more representative. However, Alves et al. (2016) found that the variability in FP was relatively lower using INDF. These authors also observed that the variability in the recovery of INDF and IDM was relatively greater and the recovery of CO was similar between the experimental diets, assuming that CO was not affected by different feeding conditions. Kozloski et al. (2009) indicated that a lower recovery of markers might be related to partial food digestion or absorption, physical-chemical changes in the digestive tract, or analytical limitations.

IDM overestimated the mean FP by 18.53%, indicating that the concentration of this indicator was relatively higher in the feces. The mean excreted amount of IDM was 660 grams higher than that using TC. In this respect, Huhtanen et al. (1994) have shown that IDM may contain contaminants because detergents are not used to purify the residues after incubation, which may compromise the results (VAN SOEST, 1994). For this reason, the fecal recovery of IDM may be increased, and nutrient digestibility may be underestimated. This result is corroborated by Kozloski et al. (2009), whereby IDM recovery ranged from 64.80% to 108.50% and was higher than the recovery of INDF. In contrast, Cabral et al. (2017) found that IDM accurately estimated FP and ADMD in sheep whereas INDF and IADF did not accurately estimate these parameters.

The ADMD values estimated using CO, LIPE®, NANOLIPE®, and INDF were similar ($p>0.05$) to those estimated by TC at all CB concentrations (Table 4). The apparent crude protein digestibility (ACPD) values estimated using these markers were different from those using TC only at 24% of CB. There was no significant difference ($p>0.05$) in ADMD values using TD at all concentrations of CB. The results of ACPD and apparent NDF digestibility (ANDFD) using TD were different from TC only at 24% of CB. The estimated values of ADMD, ACPD, and ANDFD increased as the concentration of IDM increased. The values using this marker were different from those using TC only at 24% of CB. However, the estimated values of ADMD, ACPD, and ANDFD using IADF were significantly different ($p>0.05$) at all evaluated CB concentrations. Similarly, the ADMD values overestimated by TD and IADF followed the same pattern of the underestimated FP values (Table 2).

Table 4. Mean values of apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD), apparent neutral detergent fiber digestibility (ANDFD), regression equations (RE), and coefficient of determination (r^2) of diets estimated using external and internal markers compared with the total collection (TC) method.

Markers	Percentage of cocoa by-product					
	0%	8%	16%	24%	RE	r^2
			ADMD			
TC	58.53bc*	56.09b	61.73b	47.10bc	$Y=^{NS}$	
CO	60.01bc	56.33bc	54.16bc	56.82bc	$Y=^{NS}$	
TD	70.55b	64.62b	58.30bc	63.73b	$Y=^{NS}$	
LIPE®	56.07c	60.86b	59.52bc	55.86bc	$Y=^{NS}$	
NANOLIPE®	55.50c	61.55b	59.34bc	55.28bc	$Y=^{NS}$	
IDM	56.30c	48.47c	48.60c	30.25d	Y=57.61–0.98X	82.94
INDF	58.23c	53.89bc	58.97bc	49.77c	$Y=^{NS}$	
IADF	80.57a	81.05a	78.74a	75.45a	$Y=^{NS}$	

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			ACPD			
TC	66.58b	60.05bc	65.09b	50.74c	$Y=^{NS}$	
CO	67.88b	62.15bc	62.78b	59.29bcd	$Y=^{NS}$	
TD	76.32ab	69.22b	65.78b	65.97b	$Y=^{NS}$	
LIPE®	66.06b	66.11bc	64.69b	64.38b	$Y=^{NS}$	
NANOLIPE®	63.93b	61.36bc	64.82b	62.16bd	$Y=^{NS}$	
IDM	64.79b	55.09c	58.19b	34.46e	Y=66.31–1.09X	78.84
INDF	66.37b	59.86bc	66.49b	52.80cd	$Y=^{NS}$	
IADF	84.26a	83.44a	82.59a	76.93a	Y = 85.23–0.28X	81.77
			ANDFD			
TC	55.25b	49.50bc	49.03b	37.75c	$Y=^{NS}$	
CO	54.71b	49.35bc	45.23b	48.46bc	$Y=^{NS}$	
TD	66.65ab	59.07bc	50.09b	56.83b	$Y=^{NS}$	
LIPE®	51.85b	54.53bc	48.02b	55.06bc	$Y=^{NS}$	
NANOLIPE®	48.95b	62.05b	48.27b	52.35bc	$Y=^{NS}$	
IDM	50.59b	40.56c	36.68b	17.14d	Y=51.87–1.30X	92.13
INDF	52.75b	46.64bc	51.08b	40.34bc	$Y=^{NS}$	
IADF	78.08a	78.15a	74.66a	70.84a	$Y=^{NS}$	

*The means followed by different letters in each column were significantly different from each other using the SNK test ($p<0.05$). CO, chromic oxide; TD, titanium dioxide; LIPE®, isolated, purified, and enriched lignin; NANOLIPE®, isolated, purified, and enriched lignin nanoparticles; IDM, indigestible dry matter; INDF, indigestible neutral detergent fiber; IADF, indigestible acid detergent fiber. NS, not significant.

There was a significant interaction between the indicators and the percentage of CB, which is a fiber-rich food with a high lignin content, and this characteristic might affect the concentration of the markers probably because of the degree of ruminal digestion.

Berchielli et al. (2005) reported that the most common causes of errors were the loss of markers during digestion or routine laboratory tests, which compromised the accuracy of in situ food assessment. This loss may overestimate digestibility values because the lost fraction is usually associated with the DM fraction that is rapidly degraded and readily soluble in the rumen.

Different results for IMs may be because their characteristics in the analyzed forage are different (BERCHIELLI et al., 2005). Therefore, there may be differences in the rate and extent of degradation depending on the characteristics of the fibrous portion of the silage. Watanabe et al. (2010) observed that diet composition was one of the main factors for the success and choice of an IM for estimating ADMD and concluded that non-fibrous carbohydrates might be the major interfering factors.

The studies that estimated FP, nutrient consumption, and ADMD used different incubation bags (F57, nylon, and non-woven fabric), incubation times (96h00, 144h00, 188h00, and 264h00), and particle sizes (1, 2, and 3 mm); evidencing the need to standardize these parameters (CASALI et al., 2009; DETMANN et al., 2012).

The accuracy and precision of ADMD measurements using IDM and IADF were significantly different ($p<0.05$) from those using other markers and, consequently, less reliable (Table 5).

Table 5. Accuracy and precision of apparent dry matter digestibility (ADMD) values estimated using external and internal markers.

Markers	Observed ADMD	Predicted ADMD	Accuracy*	Precision*	Residual error#
CO	63.08	56.83	-0.39 b	1.56 b	1.51 bc
TD	63.08	64.30	0.08 b	1.56 b	1.51 c
LIPE®	63.08	58.08	-0.31 b	1.94 b	1.88 bc
NANOLIPE®	63.08	57.92	-0.32 b	2.05 b	1.99 bc
IDM	63.08	45.91	-1.07 c	4.29 a	4.16 ab
INDF	63.08	55.21	-0.49 b	2.14 b	5.59 ab
IADF	63.08	78.94	0.99 a	4.18 a	18.84 a
CV			2.56	5.24	-

*The means followed by different letters in each column were significantly different from each other using the Tukey's test ($p<0.05$).
#The means followed by different letters in each column were significantly different from each other using the Kruskal-Wallis test ($p<0.05$) and Dunn's post-hoc test ($p<0.05$)
CO, chromic oxide; TD, titanium dioxide; LIPE®, isolated, purified, and enriched lignin; NANOLIPE®, isolated, purified, and enriched lignin nanoparticles; IDM, indigestible dry matter; INDF, indigestible neutral detergent fiber; IADF, indigestible acid detergent fiber.

The accuracy of the measurements was consistent with the fecal recovery of the markers (Table 3). Rodrigues et al. (2010) found that a lower recovery indicated that the concentration of markers in the feces was lower, leading to an overestimation of the excretion of the dietary fractions of interest and, consequently, underestimating digestibility values. Similarly, errors of prediction of ADMD were larger using IDM and IADF, indicating that these estimates were more distant from the actual TC values. Nonetheless, there were no significant differences in prediction errors using CO, LIPE®, NANOLIPE®, and TD ($p>0.05$). ADMD values were comparatively higher using IADF, suggesting the lower precision of this IM.

The different estimates using TD, IDM, and IADF reinforce the need for further studies to identify the factors responsible for the variability in responses and allow methodological standardization.

Conclusion

The EMs CO, LIPE®, and NANOLIPE®, and the IM INDF accurately estimated ADMD and FP.

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