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Review

Internal Indicators of Digestibility in Diets Containing Ammoniated Hay from Leaves of the Babassu Palm in the Maintenance of Goats

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Abstract: The efficiency of the indigestible Dry Matter (DMi), indigestible Neutral Detergent Fibre (NDFi), indigestible Acid Detergent Fibre (ADFi) and Indigestible Lignin (LIGi) internal indicators was evaluated as a replacement for the method of total faecal collection in estimating faecal dry matter excretion and Dry Matter (DM) digestibility in diets containing hay from leaves of the babassu palm ammoniated with 4% urea in the maintenance of goats. Total diets, which included 70% hay from Guinea grass or ammoniated hay from leaves of the babassu palm were used. Twenty male goats in metabolic cages, were fed the above diets, with water and a mineral mixture at will. Mixed-model statistical analysis was adopted, with treatments consisting of the diets and the methods for evaluating faecal DM excretion and apparent DM digestibility. The diets were tested by linear regression and t-test to compare the mean values for faecal DM excretion and DM digestibility by total collection and internal indicators. The source of fibre in diets with a high proportion of bulk had an influence ($P<0.05$) on the estimation of faecal DM excretion and DM digestibility. The DMi NDFi, ADFi and LIGi indicators were recovered efficiently from the faeces and were effective in estimating faecal DM excretion by goats fed 33% hay from leaves of the babassu palm ammoniated with 4% urea as a replacement for Guinea-grass hay. The DMi, NDFi and ADFi indicators are accurate in estimating faecal DM excretion and DM digestibility in goat diets containing fibrous ammoniated hay from leaves of the babassu palm.

Keywords: Digestibility Indicators, Faecal Excretion, *Orbignya phalerata*, *Panicum maximum*

Introduction

Digestibility is one of the main parameters for evaluating the nutritive value of ruminant feed; however, determining digestibility by the traditional method of total faecal collection requires controlling intake and excretion, which often makes it laborious and costly. The use of indicators is therefore seen as an alternative for estimating this parameter. Indicators are indigestible substances, usually easily determined, which may be part of or offered with the feed and are later identified and quantified in the faeces (Cavalcanti, 2013).

The indicators may be external, when added to the diet, or internal, when present in the chemical composition. Carvalho *et al.* (2007) consider a good indicator as having the properties of being inert, totally indigestible and non-absorbable, with no physiological function, remaining uniformly distributed in the digesta, not influencing and not being influenced by intestinal secretions, absorption or motility, or by the rumen microbial population and having a specific and sensitive method for determination and quantification.

Internal indicators consist of the non-degradable dry matter fractions of chemical constituents of the feed, where it is common to use indigestible Dry Matter (DMi) or specific components of the cell wall, such as indigestible Neutral Detergent Fibre (NDFi), indigestible Acid Detergent Fibre (ADFi) and Indigestible Lignin (LIGi) (Barros *et al*., 2007). These indicators have the advantage of being present in the feed and generally remaining uniformly distributed in the digesta during the process of digestion and excretion (Moreira Filho *et al*., 2017). However, the degree of faecal recovery varies widely, which may compromise the precision and accuracy of the estimates of digestibility (Kozloski *et al*., 2009).

Evaluation of the effects of internal indicators considers their different behaviour as regards faecal recovery, the potential for estimating faecal dry matter excretion and consequently dry matter digestibility in ruminants; these observations are influenced by the experimental conditions, such as the animals, type of diet, type of indicator and how they combine with the diet (Rodrigues *et al*., 2010).

Babassu (*Orbignya phalerata* Mart ex) is a palm tree (up to 20 m high), native to the Northern and Northeast states of Brazil, found between Cerrado and Amazon rainforest (Albiero *et al*., 2011). Despite the advantages in terms of cost and availability, babassu pindoba leaves earlier roughage feed with high fiber content (67.6% NDFap and 49.6% ADFap). Thus, the ammoniation process with 4% urea is an easy and low-cost alternative for improving its nutritional value (Garcez *et al.*, 2014).

The aim of this research was to evaluate the efficiency of internal indicators in estimating faecal dry matter excretion and the apparent dry matter digestibility of diets containing hay from leaves of the babassu palm ammoniated with 4% urea in the maintenance of goats.

Materials and Methods

This experiment was carried out at the Animal Science Department of the Centre for Agricultural Sciences of the Federal University of Piauí (DZOO/CCA/UFPI), in Teresina in the State of Piauí, Brazil. Leaves of the babassu palm were harvested from plants with a height of up to two metres, in an area of the DZOO/CCA/UFPI. These were ground into particles of up to 2.0 cm in a forage maker and left to dry in the sun for two days. The hay was ammoniated with 4% urea in the ratio of the dry matter.

The urea was dissolved in water in sufficient quantity to raise the moisture content of the hay to 30% (Gobbi *et al*., 2005) and the solution was evenly sprinkled onto the hay. Ammoniation took place with the hay on wooden pallets wrapped in plastic canvas, in an open, ventilated area. After 35 days ammoniation, the plastic was removed and the ammoniated hay aired for 48 h to remove the excess ammonia.

Four diets were evaluated, formulated with 0, 33, 67 and 100% hay from leaves of the babassu palm ammoniated with 4% urea (% of DM) replacing the Guinea-grass hay (*Pannicum maximum* 'colonial'), harvested after 30 days regrowth and crushed in a forage maker into particles of up to 2.0 cm. The hays comprised the bulk of the total is fibrous diets for maintaining the goats, with a bulk to concentrate ratio of 70:30, formulated as per the NRC (2007).

The chemical composition of the diet ingredients consumed by goats is shown in Table 1, while the chemical composition and percentage composition of the diets are presented in Table 2.

Twenty uncastrated, male Anglo-Nubian goats, with body weight 34.3±10.2 kg and approximately twelve months, in good health and nutritional status, were kept in metabolic cages, with access to the diets offered at 0800 and 1600 h and water and mineral supplement available at will. The goats were weighed after fasting at the start of the experimental period and distributed into the blocks and treatments (diets).

The experimental period lasted five days to collect samples of the feed, leftovers and faeces, when the feed supplied was equivalent to 80% of voluntary consumption in order to avoid excess; this was preceded by seven days for the animals to adapt to the facilities, management and diets intake registration, allowing 20% excess, resulting a total of 12 days, according to Rymer (2000). Leftovers were collected before each meal, removing aliquots of 20%, which were packed into plastic bags and stored at -5 to -10°C.

The faeces were collected before offering the diets; aliquots of 20% of the total amount excreted were removed, which were packed into plastic bags and stored at -5 to -10°C. At the end of the experiment, they were pre-dried in a forced air circulation oven at 60±5°C for 72 h and, as with the ingredients, feed and leftovers, were ground in a Willey mill into 1-mm particles for analysis of the fibrous fractions and 2-mm particles for the remaining chemical analyses (Detmann *et al*., 2012).

The ingredients of the diets were analysed for DM content and based on the DM, the Crude Protein (CP), Ether Extract (EE) and ash were analysed, as per methodologies of the AOAC (2012). The Neutral Detergent Fibre (NDFap) and Acid Detergent Fibre

(ADFap) corrected for ash and protein and the lignin content were analysed as per the method of Van Soest *et al*. (1991). The Neutral Detergent Insoluble Nitrogen (NDIN) and Acid Detergent Insoluble Nitrogen (ADIN) were evaluated based on the total nitrogen, as described by Licitra *et al*. (1996).

Table 1: Chemical composition of the diet ingredients

Item	CGH	$BPLH_{U4\%}$	Corn meal	Soybean meal	Urea	Flower of sulphur
Dry matter						
(% of NM)	87.00	85.39	90.50	91.21	100.00	100.00
% of DM						
Crude protein	9.21	17.35	8.72	49.58	281.25	
Ether extract	1.09	2.20	4.14	0.91		
Ash	7.87	4.49	1.24	6.96		
NDFap	63.96	66.89	10.98	14.35		
ADFap	42.18	44.08	4.62	9.77		
Hemicellulose	21.78	22.81	6.36	4.58		
Cellulose	37.27	31.78	3.59	8.31		
Lignin	4.91	12.30	1.03	1.46		
Non-fibrous carbohydrates	17.87	11.91	74.92	28.20		
% of total N						
NDIN	35.77	30.99	3.75	5.70		
ADIN	21.52	15.77	1.57	2.71		

GGH = Guinea Grass Hay; $BPLH_{U4\%}$ = hay from leaves of the babassu palm ammoniated with 4% urea, as % of DM; NM = Natural Matter; NDFap = Neutral Detergent Fibre corrected for ash and protein; ADFap = Acid Detergent Fibre corrected for ash and protein; NDIN = Neutral Detergent Insoluble Nitrogen; ADIN = Acid Detergent Insoluble Nitrogen

Table 2: Percentage and chemical composition of the diets

	Proportion of $BPLH_{U4\%}$			
Ingredient/nutrient	0	33	67	100
Centesimal composition				
GGH	70.21	46.55	23.33	0.00
$BPLH_{U4\%}$	0.00	23.28	46.67	70.00
Ground cornmeal	23.91	25.44	25.51	26.78
Soybean meal	5.07	4.45	4.46	3.19
Urea	0.77	0.25	0.00	0.00
Flower of sulphur	0.04	0.03	0.03	0.03
Chemical composition				
Dry matter (% of NM)	88.16	87.74	87.33	86.95
% of DM				
Crude protein	13.23	13.45	14.68	16.06
Ether extract	1.80	2.11	2.38	2.68
Ash	6.17	5.33	4.56	3.70
NDFap	48.26	48.78	49.58	50.22
ADFap	31.21	31.51	32.03	32.40
Hemicellulose	17.04	17.27	17.55	17.82
Cellulose	27.45	26.03	24.81	23.47
Lignin	3.77	5.48	7.21	8.93
Total carbohydrates	80.15	80.18	79.68	79.52
Non-fibrous carbohydrates	31.89	31.41	30.10	29.30
% of total N				
NDIN	26.30	25.07	24.02	22.88
ADIN	15.62	14.21	12.90	11.55

GGH = Guinea Grass Hay; $BPLH_{U4\%}$ = hay from leaves of the babassu palm ammoniated with 4% urea, as% of DM; NM = Natural Matter; NDFap = Neutral Detergent Fibre corrected for ash and protein; ADFap = Acid Detergent Fibre corrected for ash and protein; NDIN = Neutral Detergent Insoluble Nitrogen; ADIN = Acid Detergent Insoluble Nitrogen

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The Cellulose (CEL) and Hemicellulose (HEM) content of the feed ingredients were calculated with the formulae:

$$CEL = ADFap - LIG \text{ and } HEM = NDFap - ADFap.$$

The Non-Fibrous Carbohydrates (NFC) and Total Carbohydrates (TCH) were estimated with the formulae proposed by Detmann and Valadares Filho (2010):

$$NFC = 100 = \left[\left(\%CP - \%CP_{urea} + \%urea\right) + \%NDFap + \%EE + \%ash\right],$$

where % CP = crude protein in the diet; % CP_{urea} = crude protein from the urea in the feed, % urea = % of urea in the feed and $TCH = NFC + NDFcp$.

Faecal dry matter (DM) excretion and apparent DM digestibility were quantified by the method of total faecal collection, while estimates were calculated using internal indicators, represented by the indigestible constituents DMi NDFi, ADFi and LIGi.

Apparent DM digestibility by the method of total faecal collection was calculated with the formula:

$$Dig(\%) = \left[\left(DM_{in} - DM_f\right) \div DM_{in}\right] \times 100,$$

where: DM_{in} = DM ingested and DM_f = DM in the faeces.

To obtain the internal indicators, samples of the feed, leftovers and faeces were incubated in the rumen of a fistulated adult bovine for 264 h (Casali *et al*., 2008), using approximately 4 g of the sample ground in a 2.0 mm sieve in 12.0 × 8.0 cm nylon bags with a porosity of 50 μm. After the incubation period, the bags were washed in running water and placed in a forced air circulation oven at 60 ± 5°C for 72 h. The samples of feed, leftovers and faeces, before and after incubation, were analysed for DM content, as per AOAC (2012) and for NDF, ADF and LIG content by the method of Van Soest *et al*. (1991).

Using the indicators, calculation of Faecal Recovery (FR_I) and estimation of Faecal DM Excretion (FE_I) were carried out as per the formulae described by Zeoula *et al*. (2002):

$$FR_I(\%) = \left(FE_I \div FE_{CT}\right) \times 100,$$

where FE_I = total faecal excretion estimated by the indicator (gDM/day) and FE_{CT} = faecal excretion estimated by total faecal collection (gDM/day); and, $FE(g/dia) = I_{in} \div I_f$, where I_{in} = ingested indicator (g/day) and I_f = faecal indicator (g/gDM).

Apparent digestibility by the internal indicators (Dig_I) was estimated from the data for faecal DM excretion, adopting the formula described by Zeoula *et al*. (2002):

$$Dig_I(\%) = \left[\left(DM_{in} - DM_f\right) \div DM_{in}\right] \times 100.$$

The data were analysed in a completely randomised block design (animals) adopting mixed-models, with the treatments, diets (0, 33, 67 and 100% ammoniated hay from leaves of the babassu palm) and methods for evaluating faecal DM excretion and apparent digestibility (by total collection and by the DMi NDFi, ADFi and LIGi) internal indicators, considered a fixed effect [3 degrees of freedom (DF) for the diets and 4 DF for the methods] with the group (goats, 4 DF) and the residual considered a random effect, adopting the MIXED procedure of the SAS Software (2002). When the mean values for the diets were significant, the significance of the beta values of linear, quadratic or cubic effect was evaluated and the respective regression equation was obtained by the PROC MIXED procedure of the SAS (2002) software. For the methods of obtaining faecal DM excretion (total collection and DMi NDFi, ADFi and LIGi indicators), the significant mean values were compared by t-test, using the LSMEANS procedure of the SAS (2002) software.

Results

The results for faecal dry matter Excretion (FE) and Faecal Recovery of the indicator (FR_I) using the method of total collection and with the use of internal indicators in goats fed diets containing ammoniated hay from leaves of the Babassu Palm ($BPLH_{U4\%}$) replacing Guinea-Grass Hay (GGH) are presented in the Table 3.

The total recovery of faecal DM by the method of total faecal collection was equal (P>0.05) to that obtained with the DMi, NDFi and ADFi internal indicators when the Guinea grass in the diet was completely replaced by the hay from leaves of the babassu palm, except for LIGi, where faecal recovery exceeded 100% (118.0 ± 11.9%), resulting in an overestimation of faecal DM excretion (Table 3).

The results for dry matter digestibility estimated by the method of Total Collection (TC) and with the use of internal indicators in goats fed diets containing ammoniated hay from leaves of the Babassu Palm ($BPLH_{U4\%}$) replacing the Guinea-Grass Hay (GGH) are presented in the Table 4.

Estimated DM digestibility using the LIGi indicator reduced (P<0.05) by 0.0947 for every 1% of ammoniated hay from leaves of the babassu palm in the maintenance diet of the goats (Table 4).

Table 3: Faecal dry matter excretion (FE) and faecal recovery of the indicator (FR_I) using the method of total collection and with the use of internal indicators in goats fed diets containing ammoniated hay from leaves of the babassu palm ($BPLH_{U4\%}$) replacing Guinea-grass hay (GGH)

	$BPLH_{U4\%}$ content (% of GGH)				Diet		
Indicator	0	33	67	100	SEM	P	RE
	Total Collection (TC)						
FE_{TC}	280.9[d*]	234.1[a]	206.0[c]	196.4[b]	17.47	0.0336	1
FR	100.0	100.0	100.0	100.0			
	Indigestible Dry Matter (DMi)						
FE_{DMi}	345.0[c]	258.0[a]	223.2[b]	199.0[b]	22.45	0.0018	2
FR_I	121.5±9.9	113.7±18.5	108.8±9.0	95.4±12.4			
	Indigestible Neutral Detergent Fibre (NDFi)						
FE_{FDNi}	375.9[b]	271.4[a]	238.3[a]	205.9[b]	24.97	0.0006	3
FR_I	132.8±8.7	119.4±21.1	115.4±10.8	96.1±12.8			
	Indigestible Acid Detergent Fibre (ADFi)						
FE_{ADFi}	406.2[a]	266.1[a]	239.4[a]	198.1[b]	26.33	<0.0001	4
FR_I	144.7±3.8	116.8±16.7	116.6±7.7	94.0±13.0			
	Indigestible Lignin (LIGi)						
FE_{LIGi}	350.2[c]	266.0[a]	244.2[a]	237.9[a]	21.91	0.0190	5
FR_I	123.4±8.4	117.3±18.8	118.8±6.8	118.0±11.9			
SEM indicator	18.1	14.3	12.6	21.2			
P	<0.0001	0.0608	0.0002	0.0051			

*Mean values followed by different lowercase letters in a column differ by t-test (P<0.05).
RE = regression equation; SEM = standard error of the mean; P = statistical probability.
[1]$\hat{Y}_{CT} = 274.16 - 0.9711X$. $R^2 = 0.2264$. P = 0.0117
[2]$\hat{Y}_{FDNi} = 331.27 - 1.6172X$. $R^2 = 0.3803$. P = 0.0010
[3]$\hat{Y}_{FDAi} = 359.35 - 1.8746X$. $R^2 = 0.4132$. P = 0.0004
[4]$\hat{Y}_{LIGi} = 379.48 - 2.1739X$. $R^2 = 0.4996$. P = <0.0001
[5]$\hat{Y} = 332.39 - 1.2713X$. R2 = 0.2468. P = 0.0154

Table 4: Dry matter digestibility estimated by the method of Total Collection (TC) and with the use of internal indicators, in goats fed diets containing ammoniated hay from leaves of the Babassu Palm ($BPLH_{U4\%}$) replacing the Guinea-Grass Hay (GGH)

	$BPLH_{U4\%}$ contente (% of GGH)				Diet		
Indicator	0	33	67	100	SEM	P	RE
	Dry matter digestibility						
Total collection	70.5[a*]	67.4[a]	64.3[a]	61.2[a]	1.25	0.0681	
DMi	64.1[b]	63.6[b]	61.3[b]	63.3[a]	0.85	0.7496	
NDFi	60.9[c]	61.8[b]	58.9[c]	63.1[a]	0.94	0.5615	
ADFi	57.4[d]	62.5[b]	58.5[c]	63.9[a]	1.00	0.0508	
LIGi	63.5[b]	63.5[b]	57.6[c]	54.6[b]	1.24	0.0234	1
SEM (indicador)	1.1	0.6	0.7	1.4			
P (indicator)	<0.0001	0.0181	0.0001	0.0014			

*Mean values followed by different lowercase letters in a column differ by t-test (P<0.05).
DMi = indigestible Dry Matter; NDFi = Indigestible Neutral Detergent Fibre; ADFi = indigestible Acid Detergent Fibre; LIGi = indigestible Lignin; RE = Regression Equation; SEM = Standard Error of The mean; P = statistical Probability.
[1]$\hat{Y} = 64.26 - 0.0947X$, $R^2 = 0.4661$, P = 0.0047

Discussion

The equivalent values for DMi and for the NDFi and ADFi indicators to that of total faecal collection is explained by the high proportion of lignin in the cell wall of the ammoniated hay from leaves of the babassu palm, in addition to the hydrolytic effect of ammoniation, from which a cell wall structure remains that is more resistant to rumen degradation.

When evaluating total diets containing Guineas-grass hay or 33% Guineas-grass hay + 67% ammoniated hay from leaves of the babassu palm as bulk, there was an overestimation of faecal DM excretion of the order of 36.6 and 14.9% respectively when replacing the method of total faecal collection by an estimation using the DMi NDFi, ADFi and LIGi internal indicators.

The inclusion of 33% ammoniated hay from leaves of the babassu palm did not influence (P>0.05) the estimation of faecal DM excretion between the indicators and the total collection of faeces. However, when 100% ammoniated hay from leaves of the babassu palm was included in the total diet as bulk, the LIGi indicator resulted in an overestimation (P<0.05) of faecal DM excretion, while its estimation by the DMi NDFi and ADFi indicators did not differ (P>0.05) from that obtained by the total collection of faeces (Table 3).

The inadequate recovery of LIGi in the sample may be related to filtering problems during chemical analysis, since determination of the lignin follows the ADFi analysis, which in addition to the filtering process is also subjected to the acid detergent solution; this hampers the precision of the lignin analysis (Zeoula *et al*., 2002; Detmann *et al*., 2007) and explains any inconsistencies in the estimates of faecal DM excretion and apparent DM digestibility using the LIGi.

The high values for LIGi may also be related to the large variety of non-lignin components, as well as contaminants resulting from the treatment of the bulk, which may influence determination of the ADFi, in addition to problems related to the reduced size of the faecal particles containing the lignin that affords greater solubilisation in the sulphuric acid solution. Silva *et al*. (2010) therefore recommend using LIGi cautiously when estimating faecal DM excretion and DM digestibility.

Accuracy in estimating digestibility by internal indicators is related to their Faecal Recovery (FRI), since the closer to the FR obtained by total collection (100%) the more precise the indicator for this estimate (Rodrigues *et al*., 2010). The internal indicators evaluated in this study (DMi NDFi, ADFi and LIGi) resulted in an overestimation of faecal DM excretion as a result of the RF_I obtained for these indicators being greater than 100%, which may be associated with their higher concentration in the ammoniated hay from leaves of the babassu palm (Table 3). Berchielli *et al*. (2005a) attribute any value for RF_I of less than 100% to partial absorption of the indicator in the digestive tract or to its transformation into other compounds, which causes faecal DM excretion to be underestimated.

By both the method of total faecal collection and the use of internal indicators (DMi NDFi, ADFi and LIGi), faecal DM excretion decreased (P<0.05) with proportional increases in the ammoniated hay from leaves of the babassu palm in the bulky portion of the maintenance diet for goats (Table 3). Including ammoniated hay from leaves of the babassu palm as the only bulk in the diet, resulted in the estimation of DM digestibility using the DMi NDFi and ADFi internal indicators being equal (P>0.05) to the method of total faecal collection.

The accuracy of the indicators depends on the type of forage, which is related to the composition of the forage fibre and which can influence the rate and extent of degradation. Therefore, incubation time in the rumen should be considered for each type of fibre (Berchielli *et al*., 2005b). The DMi, NDFi and ADFi indicators were incubated in the rumen for 264 h and proved to be efficient in predicting faecal DM excretion and DM digestibility. Therefore, obtaining indicators for total diets containing hay from leaves of the babassu palm required less incubation time than the 288 h required to predict faecal DM excretion using the DMi and ADFi indicators in buffalo fed elephant grass 'Cameroon' (*Pennisetum purpureum*) (Soares *et al*., 2011).

DM digestibility calculated with the use of internal indicators was underestimated for the method of total faecal collection, on average by 12.7, 6.8 and 9.2%, for the diets formulated with Guinea grass hay, 67% Guinea grass hay + 33% ammoniated hay from leaves of the babassu palm and 33% Guinea grass hay + 67% ammoniated hay from leaves of the babassu palm respectively, demonstrating the need for attention when using these indicators in total maintenance diets with a high proportion of bulk (70%).

Conclusion

The conclusion of this study was that the determining faecal dry matter excretion and dry matter digestibility by the method of total faecal collection and the use of internal indicators is influenced by the source of fibre in the diet containing a high proportion of bulk. The DMi NDFi, ADFi and LIGi indicators are recovered efficiently from the faeces and are effective in estimating faecal dry matter excretion by goats fed 33% hay from leaves of the babassu palm ammoniated with 4% urea as a replacement for Guineas grass hay. The DMi NDFi and ADFi indicators are accurate in estimating faecal DM excretion and DM digestibility in goat diets containing fibrous ammoniated hay from leaves of the babassu palm.

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Author´s Contributions

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Miguel Arcanjo Moreira Filho: Conceptualization, methodology, investigation, review and editing, data curation, software, validation.

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Rosianne Mendes de Andrade da Silva: writing, review and editing.

Declaration of Animal Rights

International, domestic and institutional guidelines for the care and use of animals in research activities were followed, under Experimental Protocol No. 009/2016, approved by the Ethics Committee for Animal Use of the Federal University of Piauí, Teresina city, Piauí state, Brazil.

Ethics and Conflict of Interest

This article was written from data from the latest author's research and is original. Corresponding author has ensured that all authors involved in this article have read and approved this article for publication. There are no ethical issues in this study. The authors declare there to be no conflicts of interest.

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