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Ovine feed intake, digestibility, and nitrogen balance in feeds containing different amounts of cupuaçu meal

Consumo, digestibilidade e balanço de nitrogênio de rações contendo diferentes níveis de torta de cupuaçu em ovinos

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Abstract

Twenty neutered male sheep were subjected to 26 days of a metabolic assay at Embrapa Eastern Amazon in Belém, PA, Brazil (01°26'02"S and 48°26'21"W; altitude 8 m). The trial followed a completely randomized design, with five treatments and four repetitions. Treatment A (control) consisted of a diet containing 100% grass. The diet in treatment B contained 10% CM and 90% grass; that in treatment C contained 20% CM and 80% grass; that in treatment D contained 40% CM and 60% grass; and that in treatment E contained 60% CM and 40% grass. Intake of experimental diet and apparent digestibility coefficient of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ethereal extract (EE), cellulose (CEL), hemicellulose (HEM), and nitrogen balance (NB) were assessed. Results showed that the level of CM replacement significantly affected intake of mineral matter (IMM), crude protein (ICP), ethereal extract (IEE), and cellulose (ICEL), with the highest ($P < 0.05$) values of IMM, ICP, and IEE being observed for feed in which 60% of grass was replaced by CM, indicating that the acceptance of the experimental diet was good. ICEL decreased as levels of CM replacement increased. At 40% replacement, the apparent DM digestibility coefficient was the lowest (41.54%) and did not statistically differ from diets with 60% (45.74%) and 20% (54.19%) CM replacement. The lowest values for the apparent digestibility coefficients of OM, CP, NDF, and ADF were observed when grass was replaced with 40% and 60% CM. For diets with up to 20% replacement of Mombaça grass by CM, the apparent digestibility coefficients of DM, OM, NDF, and ADF were the highest and did not significantly differ from those of the control treatment. Nitrogen excretion in feces differed significantly; the lowest values were found with 0%, 10%, and 20% CM replacement. Use of CM represents an alternative method of dietary supplementation for ruminants and is a good source of protein and energy, since replacing Mombaça grass with increasing levels of CM led to an increase in the voluntary feed intake by ovines.

Key words: Agro-industrial byproduct, Amazon, dietary supplementation, ruminants

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Resumo

Realizou-se ensaio metabólico, com 20 ovinos machos, castrados, na Embrapa Amazônia Oriental, Belém, Pará (1°28' S 48°27' W), durante 26 dias. O delineamento foi inteiramente casualizado, em cinco tratamentos e quatro repetições. Tratamento A (Controle): 100% de gramínea; Tratamento B: 10% de TC e 90% de gramínea; Tratamento C: 20% de TC e 80% de gramínea; Tratamento D: 40% de TC e 60% de gramínea; e Tratamento E: 60% de TC e 40% de gramínea. Foram avaliados consumo e coeficientes de digestibilidade aparente da matéria seca (MS), matéria orgânica (MO), proteína bruta (PB), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), extrato etéreo (EE), celulose (CEL), hemicelulose (HEM) e balanço de nitrogênio (BN) das dietas experimentais. Os níveis de substituição de gramínea por TC promoveram diferenças significativas nos consumos de matéria mineral (CMM), proteína bruta (CPB), extrato etéreo (CEE) e celulose (CCEL), sendo que, com 60% de substituição da gramínea por TC, os valores de CMM, CPB e CEE foram mais elevados ($P < 0,05$), indicando que a dieta experimental teve boa aceitabilidade. Houve decréscimo nos CCEL em função do aumento dos níveis de substituição de TC. No nível de 40% de substituição da gramínea Mombaça por TC, houve menor coeficiente de digestibilidade aparente da MS (41,54%), que não diferenciou de 60% (45,74) e de 20% (54,19). Foi observado em 40% e 60% de substituição da gramínea por TC, os menores valores de coeficiente de digestibilidade aparente da MO, PB, FDN e FDA. Até 20% de substituição da gramínea Mombaça por TC, o coeficiente de digestibilidade aparente da MS, MO, FDN e FDA foram mais elevados, não se diferenciando significativamente do tratamento controle (0%). Ocorreu efeito significativo para a variável excreção de N das fezes, com menor excreção de N, nos níveis de 0, 10 e 20% de substituição de gramínea. A TC constitui alternativa para suplementação alimentar de ruminantes, pois os níveis crescentes do subproduto do cupuaçu, em substituição da gramínea Mombaça, proporcionam aumento no consumo voluntário por ovinos.

Palavras-chave: Amazônia, ruminantes, subproduto agroindustrial, suplementação alimentar

Introduction

Brazil is the 15th largest producer of sheep worldwide (FAO, 2007), with a herd of approximately 17 million animals (IBGE, 2014). In Amazonia, this number is growing; however, the main obstacle to the further development of this activity is the investment expenditure borne by producers to feed the animals. Considering this, the use of agroindustry by-products as alternative feeding products addresses this need because it reduces the costs of feeds, which make up a large portion of the total production costs. Therefore, it is important to understand the nutritional characteristics of alternative feeds so that their use as substitutes for conventional feeds is optimized.

The cupuaçu (*Theobroma grandiflorum* Schum) is a native fruit of the Amazon region and contains pulp that is highly appreciated by the local population. It belongs to the same family as cocoa (*Theobroma cacao* L.), and its roasted seeds

have been used since 1930 for the production of “cupulate,” a regional alternative to chocolate. Cupuaçu mass is generated from the extraction of oil from the dry seeds, which are free of pulp, by mechanical pressing. Through this process, 80% of the seeds’ oil is removed, which generates a by-product that contains approximately 11% ethereal extract (EE), 19% crude protein (CP), and 89% dry matter (DM) (CARVALHO et al., 2004). A large amount of seeds is processed in the state of Pará throughout the year; therefore, this by-product is easily obtained and may pose an environmental problem if not adequately disposed of (MURTA FILHO et al., 2011).

The possibility of using this agroindustry by-product in the diet of ruminants requires research to determine its nutritional value and that of feeds containing different amounts of this ingredient. Thus, the present study aimed to assess the inclusion of cupuaçu (*Theobroma grandiflorum* Schum) mass in the diet of ovinos, as a substitute

for Mombaça grass (*Panicum maximum* Jacq) via the determination of feed intake, digestibility, and nitrogen balance.

Materials and Methods

The present experiment was conducted in the Unit of Animal Research “Senador Álvaro Adolpho” (01°26’02”S and 48°26’21”W; altitude 8 m), located in Embrapa Eastern Amazonia, Belém, Pará, during the period between June and July 2010. The climate is classified as Af (Köppen); the mean annual rainfall is 2,876.9 mm, with the highest rainfall being obtained between January and June and the lowest rainfall being obtained between July and December. The mean annual temperature is 26.8°C, the mean air relative humidity is approximately 83%, and the amount of annual sunshine is 2,279.6 h (PACHÊCO et al., 2009). The soil is classified as Yellow Latosol, stony phase I, and is of clayey texture.

The gramineae *Panicum maximum* Jacq Mombaça was used, which received 36kg of NPK fertilizer (10:28:20) during cultivation, across an area of 1,800 m² divided into 30 plots of 60 m² (10 m x 6 m) each. The selected area was cut level to the height of the grass. After a period of 30 days, daily cutting was performance (25 cm from the ground) to allow the bulk of the crop to reach the same physiological stage, with 30 days of rest.

Twenty male, neutered, and de-wormed sheep of undefined breed, with a live weight of 25 kg, were used to estimate voluntary intake and apparent digestibility of the experimental feed. Animals were kept in individual metabolic cages for 26 days (21 days of adaptation and 5 days for the experimental collection of supplied feed, leftovers, feces, and urine).

The animals’ diet consisted of Mombaça grass (*Panicum maximum* Mombaça) and different percentages of cupuaçu mass (CM) as replacement feed, based on the dry matter (DM), and was

provided in the trough. The animals received two daily meals (at 08.00 and 15.00), the quantities of which were adjusted every day such that 10% of the meal would be left over, and water and mineral salts were provided *ad libitum*. The grass was cut early in the morning, shredded, and mixed with the experimental feed manually. The leftovers were weighed every morning, before the first meal of the day, to determine feed intake by the animal and to adjust the intake.

The animals were subjected to five treatments: Treatment A (control) = diet consisting of 100% grass; Treatment B = diet consisting of 90% grass + 10% CM; Treatment C = diet consisting of 80% grass + 20% CM; Treatment D = diet consisting of 60% grass + 40% CM; and Treatment E = diet consisting of 40% grass + 60% CM. The supplied feed was sampled before the morning meal and the leftovers were collected and weighed. The samples were packaged in plastic bags, labeled, and stored at -20°C. At the end of the experimental period, the feed samples were thawed at room temperature and homogenized to prepare the composed samples for further analysis. The bromatological composition of the ingredients in the experimental diets and in the total diet (in DM) is shown in Table 1.

The samples of feed, leftovers, and feces were pre-dried in a forced-air ventilation oven at 55°C for approximately 72 h. Samples were then ground in a Willey-type mill with a 1-mm-mesh sieve and stored in plastic containers for further analysis (SILVA; QUEIROZ, 2006).

The analyses were performed in the Animal Nutrition Laboratory of the Rural Federal University of Amazonia - UFRA - and in the Soil Laboratory of the Federal Institute for Education, Science and Technology of Pará - IFPA/Castanhal Campus. The analyzed parameters were dry matter (DM), organic matter (OM), and mineral matter (MM) in the feed, leftovers, and feces, according to the recommendations of the Association of

Official Analytical Chemists (2005). Neutral detergent fiber (NDF), acid detergent fiber (FDA), cellulose (CEL), and lignin (LIG) were analyzed according to the sequential method, as described by Van Soest et al. (1991). The determination of crude protein (P) was performed using the Kjeldahl

method (AOAC, 2005). The ethereal extract (EE) was determined according to the recommendations made by Silva and Queiroz (2006). Table 1 shows the bromatological composition of the ingredients in the experimental diets and in the total diet (expressed as the percent of DM).

Table 1. Bromatological composition of the ingredients in the experimental diets and in the total diet (in DM).

Diet	Variable (% in DM)												
	DM	MM	OM	EE	CP	FND	FAD	CEL	HEM	LIG	TDN	ADIN	NDIN
Cupuaçu mass	82.48	9.11	90.89	17.93	17.79	53.51	38.2	22.76	15.11	15.44	-	47.97	50.70
Mombaça	24.26	7.54	92.65	1.62	10.32	69.21	55.85	45.17	13.32	10.68	-	24.28	33.27
0	24.26	7.54	92.65	1.62	10.32	69.21	55.85	45.17	13.32	10.68	38.02	-	-
10	30.08	7.69	92.47	3.25	11.06	67.64	54.08	42.93	13.53	11.63	56.35	-	-
20	35.90	7.85	92.30	4.88	11.81	66.07	52.32	40.69	13.71	11.63	54.89	-	-
40	47.55	8.17	91.95	8.14	13.31	62.85	48.79	36.20	14.06	12.58	60.94	-	-
60	59.19	8.48	91.59	11.40	14.80	59.95	45.26	31.72	14.41	13.56	56.79	-	-

Dry matter (MS), organic matter (OM), mineral matter (MM), ethereal extract (EE), crude protein (CP), fiber in neutral detergent (FND), fiber in acid detergent (FDA), cellulose (CEL), hemicellulose (HEM), lignin (LIG), and total digestible nutrients (TDN). NDT- Total digestible nutrients: %TDN = (TDN Intake/DM Intake) × 100 (SNIFFEN et al., 1992). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) (SILVA; QUEIROZ, 2006).

Intake of dry matter (IDM), organic matter (IOM), crude protein (ICP), ethereal extract (IEE), neutral detergent fiber (INDF), acid detergent fiber (IADF), cellulose (ICEL), and lignin (ILIG), and the coefficients of apparent digestibility of dry matter (CADDM), organic matter (CADOM), crude protein (CADCP), gross energy (CADEE), neutral detergent fiber (CADNDF), and acid detergent fiber (CADADF) were determined by analyzing fecal samples. The formula proposed by Silva and Leão (1979) was used to calculate the coefficients of apparent digestibility of DM, OM, CP, EE, NDF, ADF: $CADN (\%) = [(NCON - NEXC)/NCON] \times 100$, where: CADN = coefficient of apparent digestibility of the nutrient; NCON = quantity of nutrient intake (in grams), and NEXC = quantity of nutrient excreted in the feces (in grams).

To determine the nitrogen balance, 24 h-urine

samples were collected on the last 3 days of the experimental period by using collecting funnels adapted to the animals. Rubber tubes were coupled to the funnels to collect the urine in plastic containers with 20 mL of HCl 1:1 to avoid the proliferation of bacteria and potential losses due to volatilization. Of the total volume of excreted urine, a 20-% aliquot per animal was removed, and nitrogen analysis was performed (SILVA; QUEIROZ, 2006).

The experimental design was completely randomized, with five treatments (percentage CM replacement) and four repetitions (animals). Data were subjected to analysis of variance by using the method of least squares. The significance of the effects of the replacement levels was confirmed by the F test, and the means of the treatments were compared using Tukey's test, at a significance level of 0.05 (SAS, 2003).

Results and Discussion

Table 2 shows the values relating to experimental diet intake, according to the percentage of *Panicum maximum* Mombaça grass replacement by cupuaçu (*Theobroma grandiflorum* Schum) mass (CM). Different percentages of replacement promoted

significant differences in IMM, ICP, IEE, and ICEL. When 60% of grass was replaced by CM, the values of IMM, ICP, and IEE were higher ($P < 0.05$), which indicated that the experimental diet was well accepted by the animals. Therefore, CM was shown to have good palatability, probably due to its chocolaty aroma.

Table 2. Intake of experimental diets in which *Panicum maximum* Mombaça grass was replaced by CM (*Theobroma grandiflorum* Schum) at different percentages.

Intake	Replacement level of cupuaçu mass (%)					CV (%)
	0	10	20	40	60	
IDM (g)	570.41	478.27	429.09	398.83	587.46	26.92
IDM (PV)	2.43	2.00	1.66	1.60	2.25	1.38
IOM (g)	634.6	607.3	506.0	480.4	628.6	25.95
IMM (g)	43.01 ^c	36.78 ^{abc}	33.69 ^{bc}	32.58 ^{ab}	49.82 ^a	26.99
ICP (g)	58.87 ^{ab}	52.90 ^{ab}	50.90 ^b	53.08 ^{ab}	86.94 ^a	27.25
ICP (PV)	2.54	2.22	1.96	2.13	3.33	1.60
IEE (g)	9.24 ^c	15.54 ^{bc}	20.94 ^{bc}	32.46 ^b	66.97 ^a	30.79
INDF (g)	394.78	323.50	283.50	250.98	352.18	26.88
IADF (g)	310.58	258.65	224.50	194.59	265.88	26.89
IHEM (g)	75.98	64.71	58.83	56.07	84.65	26.98
ICEL (g)	257.66 ^a	205.32 ^{ab}	174.60 ^b	144.38 ^b	186.34 ^b	26.92
ILIG (g)	60.92	55.63	49.91	50.17	79.66	27.18

Intake of dry matter (CMS), intake of organic (CMO), intake of mineral matter (CMM), intake of crude protein (CPB), intake of ethereal extract (CEE), intake of neutral detergent fiber (CFDN), intake of acid detergent fiber (CFDA), intake of cellulose (CCEL), intake of hemicellulose (CHEM), intake of lignin (CLIG). CV = Coefficient of variation.

Means followed by different letters, in the same line, differ significantly (Tukey's test at 5% probability).

The high ICP observed following use of the 60% mixture confirms that CM is a good source of protein and has good palatability and/or acceptability as a feed supplement. The composition of the experimental diet (Table 1) shows that this by-product of the cosmetic agroindustry is a good source of animal feed, with a CP content of >7%, which is the minimum amount required for the maintenance of vital functions in ruminants (NRC, 1996). This is because the protein content is higher in CM (17.79%) than in Mombaça grass (10.32%). Therefore, the replacement of grass with CM is beneficial, as CM functions as an alternative source of protein in the diet of ruminants, especially during

the dry season, when grasses of the Amazonian pastures have low nutritional value, causing nutritional deficiencies in the animals, lower animal productivity, and consequent economic losses. The reduction in ICEL was associated with the increased percentage of CM in the feed, combined with the reduced CEL content of the diet (Table 1).

The means of the coefficient of apparent digestibility CADDM, CADOM, CADCP, CADNDF, and CADADF exhibited significant differences ($P < 0.05$) with increasing percentage of grass Mombaça replaced by CM; however, CADEE, CADCEL, and CADHEM were not significantly different (Table 3).

When 40% of Mombaça grass was replaced by CM, there was a decrease in CADDM (41.54%), which was not different from that observed when 20% and 60% of Mombaça grass was replaced by CM (54.19% and 45.74%, respectively). This result may be explained by the difference in the

lignin content in the experimental diets (Table 1), which affects DM digestibility by interfering with the cell wall digestion of plants. It is worth noting that lignin is the main factor that negatively interferes with ruminal digestibility (PEZZONI et al., 2012).

Table 3. Means of the coefficient of apparent digestibility of diets containing *Panicum maximum* Mombaça grass and CM (*Theobroma grandiflorum* Schum).

Variable (%)	Percentage of CM replacement (%)					CV (%)
	0	10	20	40	60	
CADDM	60.01a	60.23a	54.19ab	41.54b	45.74ab	13.32
CADOM	63.03ab	64.18a	57.90abc	45.88c	48.65bc	12.23
CADCP	64.39a	57.82ab	48.46bc	30.54d	35.03cd	15.19
CADEE	57.82	57.26	58.96	71.61	73.81	19.31
CADNDF	64.05a	63.49a	57.12ab	45.47b	47.48b	12.90
CADADF	58.67a	58.16a	50.09ab	34.85b	36.43b	17.40
CADCEL	60.61	59.10	57.59	45.84	42.46	18.08
CADHEM	86.49	84.71	83.82	82.02	81.17	4.94

Coefficient of apparent digestibility of dry matter (CADDM), organic matter (CADOM), mineral matter (CDAMM), crude protein (CADCP), ethereal extract (CADEE), neutral detergent fiber (CADNDF), acid detergent fiber (CADADF), cellulose (CADCEL), and hemicellulose (CADHEM). CV = Coefficient of variation.

Means followed by different letters, in the same line, differ significantly (Tukey's test at 5% probability).

The increased lipid content in diets with increased levels of CM may be another reason for the reduction in CADDM, as was observed by Pimentel et al. (2012). A decrease in CADDM is common in diets that have high lipid content (SOUZA JÚNIOR et al., 2011).

When grass was replaced by CM at levels of 40% and 60%, lower values of CADOM, CADCP, CADNDF, and CADADF were obtained. One factor that may have contributed to the reduction in CADNDF and CADADF is the association with the high lignin content in CM (15.44%), when compared to that in traditional concentrated feeds (1.14%) and soybean (1.31%). Increased dietary lignin content contributes to reduced availability of nutrients to microorganisms in the rumen (CARVALHO et al., 2007). Most plants contain some lignin and

its content varies between 4% and 12%, but can reach 20% of DM in more fibrous forages (SILVA; QUEIROZ, 2006). Another factor that may have affected the CDNDF is the high concentration of EE in the diets, following administration of feed with higher percentages of CM, which may have slowed down the degradation of fibers in the rumen (the main site of fiber digestion) (SILVA et al., 2005). High ADF contents in the feed can hinder digestibility, and indigestible fiber and lignin represent the largest portion of ADF (CARVALHO et al., 2006).

The inhibitory effect of CM on CADCP indicates that there is a limit to the level at which by-products can be used in replacement diets because the concentration and quality of protein in the diet can influence the intake of feed by ruminants and, consequently, change both the physical and

physiological mechanisms of ingestion control (SILVA et al., 2005).

The by-products of agroindustry may be considered as valuable sources of protein, energy, and NDF in animal production. Therefore, nutritionally balanced animal feeds should consider the by-products of the agroindustry as non-forage fiber-sources (NFFS). The use of NDF from by-products as a portion of the total NDF content in the diet represents an option in ruminant feeding. However, the addition of NFFS to feeds with the aim of replacing part of the forage NDF should take into account the differences in chemical composition, physical characteristics (particle size), digestion, and food passage rates, when compared to forage fiber sources. Moreover, NFFS exhibits a higher specific gravity, which favors an increased ruminal passage rate (GRANT, 1997). The combination of these factors contributes to shorter feed retention in the rumen, a higher passage rate of potentially digestible NDF into the lower digestive tract, and consequently, lower digestibility of NDF (FINKS, 1997).

Therefore, this may explain the results obtained in the present study because CADNDF decreased as the level of CM replacement in the diet increased. Digestion and passage are competing processes; therefore, NFFS should be retained or reduced to increase ruminal digestibility of potentially digestible NDF. There is the potential to increase ruminal digestibility when NFFS is added to the diet (FINKS, 1997). França et al. (2009) emphasize that supplying larger particles of NFFS favors the biphasic stratification of ruminal content, thus retaining the particles and increasing digestion time. Therefore, the fiber included in the ruminants' diet should be of high quality and

the size of the particles should be appropriate to ensure maximum intake, mastication activity, and ruminal fermentation (GRANT, 1997).

The highest values of CADDM, CADOM, CADNDF, and CADADF were obtained when up to 20% of Mombaça grass was replaced by CM, and no significant differences were observed when compared with the control treatment (no replacement). Brito et al. (2014) assessed the digestibility of CM in the diets of confined young bulls and concluded that the inclusion of up to 20% CM in their diet did not alter digestibility. Pereira (2009) studied the use of CM as feed for sheep in Mato Grosso and showed that the inclusion of high levels of CM in the diet affected the *in vitro* digestion of ingredients with higher fiber content.

The determination of nitrogen balance, i.e., nitrogen intake minus fecal and urinary nitrogen, under controlled conditions, permits the quantification of protein metabolism and specifically indicates whether the animal is gaining or losing protein (LADEIRA et al., 2002). The nutritional value of ruminant feed depends largely on the interaction between ingested nutrients and the action of microorganisms in the digestive tract (MIOTTO et al., 2012). The quality of the protein in ruminant diets has been traditionally evaluated by determining the level of digestible protein and less frequently by evaluating the nitrogen balance. Digestible protein only takes into consideration the balance between dietary protein intake and protein excretion, whereas the nitrogen balance also reflects urinary loss (VAN SOEST, 1994). Table 4 shows the mean ingested nitrogen, fecal nitrogen, and urinary nitrogen, following consumption of the experimental diets.

Table 4. Means of ingested nitrogen (g day⁻¹), fecal nitrogen (g day⁻¹), urinary nitrogen (g day⁻¹), and retained nitrogen (% of ingested nitrogen) in diets including CM (*Theobroma grandiflorum* Schum).

Variable (g.day ⁻¹)	Percentage of CM replacement (%)					CV (%)
	0	10	20	40	60	
Ingested N	10.03	8.99	9.74	10.35	14.20	31.39
Fecal N	3.05 ^b	3.16 ^b	3.66 ^b	5.32 ^{ab}	8.10 ^a	35.44
Urinary N	3.23	1.86	1.86	4.21	2.12	48.98
N-Balance	3.75	3.97	4.21	0.82	3.98	64.39
Retained N	3.75	3.97	4.21	0.82	3.98	64.39

CV = Coefficient of variation.

Means followed by different letters, in the same line, differ significantly (Tukey's test at 5% probability).

The ingestion of nitrogen exceeded its excretion, which demonstrates that the animals receiving higher levels of CM replacement had a positive nitrogen balance. There was a significant effect on nitrogen excretion in feces, with lower nitrogen excretion being observed when 0%, 10%, and 20% of Mombaça grass was replaced by CM. Higher nitrogen losses in feces were probably caused by higher ingestion of nitrogen. There was no significant difference in the urinary excretion of nitrogen with increasing levels of CM in the diet. The nitrogen balance was positive at all levels of replacement, which indicates that there was protein retention in the animals' bodies and suggests that the cupuaçu by-product is suitable for inclusion in the ruminant diet.

Conclusion

A by-product of the cosmetic agroindustry, CM (*Theobroma grandiflorum* Schum), can be used as an alternative feed supplement in ruminant diets and is a good protein and energy source, since substituting Mombaça (*Panicum maximum* Jacq) grass with increasing levels of this by-product resulted in increased voluntary feed intake in sheep. However, a limit for the inclusion of this byproduct should be respected because nutrient digestibility decreases when CM is included at a level of 20% or more, although the nitrogen balance did not reach negative values.

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