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Nitrogen balance, microbial protein synthesis and ingestive behavior of lambs fed diets containing cottonseed cake in substitution of soybean meal

Balanço de nitrogênio, síntese de proteína microbiana e comportamento ingestivo de cordeiros alimentados com dietas contendo torta de algodão em substituição do farelo de soja

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Abstract

The cottonseed cake is a co-product obtained after extraction of oil cottonseed, and because of their bromatological characteristics has potential use in ruminant feed, can increase the economy efficiency of animal production systems. The objective of this study was to evaluate diets with cottonseed cake in substitution of soybean meal through nitrogen balance, microbial protein synthesis and the ingestive behavior of lambs. Forty crossbred Santa Inês × Dorper non-castrated rams with average initial weight of 20.9 ± 2.5 kg were distributed into a completely randomized design with four treatments and ten replicates, and fed diets containing cottonseed cake in substitution of 0, 33, 66 and 100% of soybean meal. The diets were composed of corn, soybean meal, cottonseed meal and hay, with forage:concentrate reason of 50:50, isonitrogenous, with 14% crude protein. The animals were confined individually in pens with slatted floor, the evaluations of ingestive behavior, which included observations of the feeding, rumination and idle times were performed in a period of 24 hours, in 5-minute intervals. At 82 days of experiment, a spot urine sample was collected from all animals to quantify the concentrations of nitrogen, creatinine, allantoin, uric acid, xanthine and hypoxanthine in the urine. The data were subjected to variance analysis and regression, adopting α= 0.05. There was no significant difference between treatments for nitrogen balance, the synthesis of nitrogen compounds, and microbial efficiency. In addition, there was no effect of replacing soybean meal by cottonseed meal on feeding behavior of animals. Cottonseed cake can replace soybean meal up to 100% in diets for lambs.
Key words: Biodiesel. Co-products. Efficiency. Feeding. Metabolism. Rumination.

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Resumo

A torta de algodão é um coproduto obtido após a extração do oléo do caroço de algodão, e devido às suas características bromatológicas apresenta potencial de uso na alimentação de ruminantes, podendo aumentar a economicidade dos sistemas de produção animal. O objetivo com este estudo foi avaliar dietas com torta de algodão em substituição ao farelo de soja sobre o balanço de nitrogênio, a síntese de proteína microbiana e o comportamento ingestivo de cordeiros. Foram utilizados quarenta cordeiros mestiços Santa Inês x Dorper não castrados, com peso médio inicial de 20,9±2,5 kg, distribuídos em um delineamento experimental inteiramente casualizado, com quatro tratamentos e dez repetições, alimentados com dietas contendo torta de algodão em substituição à 0, 33, 66 e 100% do farelo de soja. As dietas foram compostas de milho, farelo de soja, torta de algodão e feno, com razão volumoso:concentrado de 50:50, isonitrogenadas, com aproximadamente14% de proteína bruta. Os animais foram confinados em baias individuais com piso ripado, as avaliações do comportamento ingestivo, que incluíram observações dos tempos de alimentação, ruminação e ócio foram realizadas em um período de 24 horas, em intervalos de 5 minutos. No 82º dia do período experimental, foram coletadas amostras de urina de todos os animais para quantificar as concentrações de nitrogênio, creatinina, alantoína, ácido úrico, xantina e hipoxantina. Os dados foram submetidos à análise de variância e de regressão, adotando-se α = 0,05. Não houve diferença significativa entre tratamentos sobre o balanço de nitrogênio, a síntese de compostos de nitrogenados, e a eficiência de síntese microbiana. Além disso, não se observou efeito da substituição do farelo de soja pela torta de algodão sobre o comportamento ingestivo dos animais. A torta de algodão pode substituir em até 100% o farelo de soja em dietas para cordeiros.

Palavras-chave: Alimentação. Biodiesel. Co-produtos. Eficiência. Metabolismo. Ruminação.

Introduction

By-products from biodiesel are sources of protein and energy feeds largely utilized in diets for ruminants (OLIVEIRA et al., 2012; ARAÚJO et al., 2014). However, because the particularities of each by-products, some care must be taken prior to supply to animals because the presence of toxic factors or anti-nutritional compounds (ABDALLA et al., 2008).

For the cottonseed cake to be used in substitution of the traditional protein foods such as soybean meal, it is essential that the nitrogen compounds present in the protein source be evaluated so as to make sure that they will meet the nitrogen requirements of the ruminal microorganisms.

There has been a growing interest in further studying the sources of true protein used in ruminant feeding as well as the amount of protein produced by the rumen microorganisms to better meet the protein requirements of animals (CABRAL et al., 2001; ÍTAVO et al., 2002).

Evaluating the nitrogen balance and microbial protein synthesis in ruminants provides information concerning the protein nutrition of these animals, which can be important to avoid productive, reproductive and environmental damages resulting from the supply of excessive amounts of protein or an inappropriate synchronism between energy and protein in the rumen (PESSOA et al., 2009).

The supply of amino acids from the microbial protein in the small intestine is essential for the protein metabolism in ruminants. The efficiency of microbial production and the microbial flow are determining factors of the amount of microbial protein that will reach the small intestine. According to the NRC (2001), the protein synthesized by the ruminal microorganisms has an excellent amino acid profile and a rather constant composition.

Feeding behaviors studies are great important, and according to Correia et al. (2015) allows adjusting feeding and management techniques to improve the production performance of the animals. Feeding conditions and feed characteristics can alter the ingestive behavior parameters because interactions among the diet nutrients can increase microbial efficiency and improve digestibility, shortening rumen retention time (BASTOS et al., 2014).

In this way, cottonseed cake is an alternative food, whose assessments of feeding behavior and nitrogen metabolism are of great importance for appropriate use recommendations. The aim of this research was to evaluate the nitrogen balance, the synthesis of microbial protein and the feeding behavior of lambs fed diets containing cottonseed cake replacing soybean meal.

Material and Methods

Experimental area

The experiment was conducted on the Experimental Farm of the College of Veterinary Medicine and Animal Science of the Federal University of Bahia, located in the municipality of São Gonçalo dos Campos, BA, Brazil.

Animals and diets

Forty crossbred Santa Inês × Dorper non-castrated males with initial body weight of 20.9±2.5 kg were distributed into a completely randomized design with ten replicates per treatment, and housed in individual pens with slatted floor provided with feeding and drinking troughs.

The diets were composed of corn, soybean meal, mineral supplement, urea and cottonseed cake substituting 0, 33, 66 and 100% of the soybean meal, corresponding to 0, 4, 8 and 12% of cottonseed cake, respectively, in the total diet. The roughage used was hay of Tifton 85 (*Cynodon* sp) grass, at a roughage-to-concentrate ratio of 50:50. Each animal had free access to water at all times, and the drinking troughs were monitored every day so as to prevent water deficit in the containers.

The experimental assay lasted 84 days, divided into three periods of 28 days each, and started 15 days after the period used for animals to acclimate to the environment, management and diet. The animals were weighed at the beginning of the experimental period, and at the end of every 28-day period. The diets were formulated based on the NRC (2007), for an average daily gain of 200 g, and were supplied as a mixture.

The amount of feed supplied to the lambs was readjusted so that the leftovers would remain between 10 and 20% of the total natural matter offered, thereby promoting maximum voluntary intake by the animals. The animals were individually weighed at the beginning and final of the experimental period to obtain the total weight gain of the lambs.

Laboratory analysis

The analysis of the gossypol content in the cottonseed cake used in the experiment was performed at Center for Chromatographic Studies and Analyses (CEACRON, *Centro de estudos e análises cromatográficas*), at The State University of Bahia (UESB), located in Itapetinga, BA, Brazil, according to the methodology of Ribani et al. (2004).

The dry matter (DM; method 967.03), ash (MM; method 942.05), crude protein (CP; method 981.10) and ether extract (EE; method 920.29) contents were determined according to the AOAC (1990), whereas the neutral detergent fiber (NDF) was determined according Mertens (2002), and acid detergent fiber (ADF) according Van Soest et al. (1991). Lignin was obtained by applying the methodology of Silva and Queiroz (2002), with the residue from the ADF treated with sulfuric acid 72% (Table 1).

Using the methodology of Mertens (1997), the concentration of non-fibrous carbohydrates (NFC) of the feeds was quantified, considering the NDF corrected for residual ash and protein, and the neutral detergent insoluble protein (NDIP) contents were quantified by the methodology of Licitra et al. (1996).

Table 1. Chemical composition of the experimental diets.

Item (%DM)	Levels of cottonseed cake in substitution for soybean meal (%DM)			
	0	33	66	100
Dry matter(% of fresh matter)	87.36	87.28	87.19	87.11
Organic matter	82.57	82.59	82.60	82.62
Crude protein	13.75	13.70	13.65	13.60
Ether extract	2.49	2.70	2.92	3.13
Neutral detergent fiber	42.14	43.27	44.40	45.52
Indigestible neutral detergent fiber	22.91	23.79	24.67	25.55
Neutral detergent insoluble protein (% crude protein)	3.24	3.12	2.99	2.87
Acid detergent fiber	9.78	10.44	11.09	11.75
Cellulose	19.95	20.42	20.89	21.35
Hemicellulose	19.23	19.47	19.72	19.97
Lignin	2.96	3.37	3.73	4.20
Non-fibrous carbohydrates	39.73	38.90	38.06	37.25
Total carbohydrates	81.87	82.17	82.46	82.77
Total digestible nutrients	69.24	69.45	63.47	64.97
Gossypol content (ppm)	-	340	680	1020

The TDN concentrations were calculated according to the digestibility estimation formulas for each analytical fraction (NRC, 2001):

$$DNFC = 0.98(\%NFC)$$

$$DCP = \% CP \times [1 - (0.4 \times ADIP/CP)]$$

$$DEE = \%EE - 1$$

$$DNDF = 0.75 \times (\%NDFp - \% lignin) \times [1 - (\% lignin/ \% NDFp) 0.0667] - 7$$

where, DNFC stands for the digestible nonfibrous carbohydrates, DCP indicates digestible crude protein, ADIP is the acid detergent isoluble protein, DEE is the digestible ether extract, DNDF is the digestible neutral detergent fiber, and NDFp is the neutral detergent fiber corrected for protein.

After estimating the digestible analytical fractions, TDN was estimated according to the following equation:

$$TDN = DCP + (2.25 \times DEE) + DNDF + DNFC$$

where TDN is estimated according to NRC (2001).

Urine analysis

At the 82th experimental day, spot samples of urine were collected four hours after feeding, during spontaneous urination. Immediately after collection, the urine was filtered through gauze and mixed to the sulfuric acid 0.036 N solution at the ratio of one part urine to four parts acid, and frozen for later quantification of the concentrations of nitrogen, creatinine, allantoin, uric acid, xanthine and hypoxanthine

The concentrations of creatinine and uric acid in the urine were determined using commercial kits. The urine allantoin, xanthine and hypoxanthine contents were estimated by colorimetry, according to the specifications given by Chen and Gomes (1992). Total nitrogen content was determined by the Kjeldhal method (SILVA; QUEIROZ, 2002).

To estimate the urine volume with the spot samples, the creatinine concentration was used as a marker, considering that an animal excretes 17.05 mg of creatinine per kg of body weight (PEREIRA, 2012); and based on the creatinine concentration in the spot urine sample, the daily excreted volume was calculated as follows:

$$DUV\ (mL) = [(BW \times 17{,}05) \times 100] \div \text{creatinine content}$$

in which the DUV is the daily urine volume; BW is the body weight, in kilograms; and creatinine content, as milligrams per deciliter of urine.

Feces were collected by the total collection method, with the aid of collecting bags, at 06h00 and 18h00, for five consecutive days in each experimental period. After they were collected, the feces from each animal were weighed, and aliquots of approximately 10% of them were taken to make composite samples per animal per diet. Composite samples were then conditioned in labeled plastic bags and frozen at –20 ºC for subsequent analyses of fecal nitrogen.

The total excretion of purine derivatives was estimated by summing the quantities of allantoin, uric acid, xanthine and hypoxanthine excreted in the urine. The number of absorbed microbial purines (AP), as mmol/day, was estimated based on the total amount of purine derivatives excreted (TP), as mmol/day, using the equation proposed by Chen e Gomes (1992) for sheep:`

$$TP = 0.84AP + (0.150\ BW\ 0.75e - 0.25AP)$$

in which TP is total purine derivatives (mmol day^{-1}), BW is the body weight; AP is theabsorbed purines (mmol day^{-1}).

The intestinal flow of microbial nitrogen compounds (MN; g N day^{-1}) is calculated based on the absorbed microbial purines (AP; mmol/day) using the following equation:

$$MN = (70 \times AP) / (0{,}83 \times 0{,}116 \times 1000)$$

where 70 represents the amount of N in the purines (mg N/mmol), 0.83 is the digestibility of the microbial purines, and 0.116 is the purine N:total N ratio in ruminal microorganisms (CHEN; GOMES, 1992).

Ingestive behavior

In the 14th day of each experimental period, they were realized evaluations of the ingestive behavior including the feeding, rumination and idle times, which were determined by visual observation of the animals during 24 hours, in five-minute intervals. During the night observations, the animals had artificial illumination.

On the same day, each animal was observed three times, in three periods of the day (morning, afternoon and night). In these periods, the number of chews per cud and the time spent on the rumination of each cud were recorded.

The data collection to determine the time spent on each activity was performed with the aid of digital stopwatches, handled by four observers arranged so as not to interfere with the animal activities.

To estimate the feeding and rumination behavioral variables (min kg DM^{-1} and NDF), feed efficiency (gDM and NDF $hour^{-1}$) and the average DM and NDF intakes per feeding period, the values of voluntary intake of DM and NDF collected in the same evaluation period as the ingestive behavior were used. The behavior variables data were obtained according to the methodology described by Bürger et al. (2000).

The number of daily ruminated cuds was calculated by dividing the total rumination time (min) by the average time spent on the rumination of a cud. For the concentration of DM and NDF in each ruminated cud (g), the quantity of DM and NDF consumed (g day^{-1}) in 24 hours was divided by the number of ruminated cuds in one day.

The feed and rumination efficiencies were obtained as follows:

$$DMFE = DMI / FEED$$

$$NDFFE = NDFI / FEED$$

in which DMFE is the feed efficiency as g DM consumed $hour^{-1}$, NDFFE is thefeed efficiency as g NDF consumed $hour^{-1}$, DMI and NDFI are the daily intakes of dry matter and neutral detergent fiber, respectively, and FEED is the time spent feeding per day.

$$DMRE = DMI / RUM$$

$$NDFRE = NDFI / RUM$$

in which DMRE is rumination efficiency as g DM ruminated hour^{-1}, NDFRE is the rumination efficiency as g NDF ruminated hour^{-1}, DMI and NDFI are the daily intakes of dry matter and neutral detergent fiber, respectively, and RUM is the time spent ruminating per day.

$$TCT = FEED + RUM$$

in which TCT is the total chewing time, as min day^{-1}.

The number of feeding, rumination and idle periods were counted by observing the sequential number of activities on the data spreadsheet. The mean daily time spent on these periods was calculated by dividing the total duration of each activity (feeding, rumination and idle) by its respective number of periods.

After the evaluations of ingestive behavior, samples of roughage, concentrate and leftovers of each animal were conditioned in labeled plastic bags and stored in a freezer at –20 ºC for later analysis of DM and NDF.

Statistical analysis

The obtained data were statistically interpreted by the parametric technique of variance analysis (ANOVA) and regression at 5% probability.

Results

The results for the balance of nitrogen compounds did not differ (P=0.9300) among the diets (Table 2). Positive nitrogen balance (retained N, g day^{-1}) was verified for all diets, although this variable was not affected by the levels of substitution of soybean meal for cottonseed cake.

The microbial syntheses of nitrogen compounds and protein were also not affected (P=0.1971) by the levels of cottonseed cake used in replacement of soybean meal in the diets (Table 3). The microbial production behaved similarly, with no alterations (P=0.1971) caused by the diets. The microbial efficiency results observed in this study also were not affected (P=0.6175) by substitution of soybean meal for cottonseed cake, and were between 129.50 and 148.59 g CP kg TDN^{-1} (Table 3).

Table 2. Nitrogen balance values and total weight gain in lambs fed diets containing cottonseed cake in substitution of soybean meal.

Item	Levels of cottonseed cake in substitution for soybean meal (%DM)				P-value		SEM
	0	33	66	100	L	Q	
N intake (g day^{-1})	36.11	36.94	36.60	37.89	0.3101	0.7828	0.329
N feces (g day^{-1})	7.58	7.67	8.28	8.43	0.3118	0.9999	0.216
N urinary (g day^{-1})	13.83	15.04	14.38	15.75	0.4185	0.9011	0.404
N retained (g day^{-1})	14.70	14.23	13.93	13.71	0.6346	0.9300	0.451
N retained (g kg BW$^{0.75}$ day^{-1})	0.99	0.57	0.94	0.87	0.6704	0.8425	0.046
N retained N intake^{-1}	39.35	38.52	38.33	35.47	0.4060	0.9650	1.235

L = Significance for linear effect; Q = Significance for quadratic effect.

Table 3. Absorbed microbial purines and microbial synthesis and efficiency in lambs fed diets containing cottonseed cake in substitution of soybean meal.

Item	Levels of cottonseed cake in substitution for soybean meal (% DM)				P-value		SEM
	Urinary excretions (µmol day^{-1})				L	Q	
Allantoin	7.95	9.58	10.85	9.15	0.6512	0.1790	0.348
Uric acid	1.80	1.46	1.68	1.70	0.9507	0.8238	0.158
Xanthine and Hypoxanthine	0.44	0.38	0.60	0.54	0.4139	0.7773	0.035
Purine derivatives	10.19	11.42	13.14	11.40	0.6066	0.2380	0.382
Microbial purines absorbed (µmol day^{-1})	11.15	12.99	15.14	12.98	0.5312	0.1971	0.456
	Microbial production (g/day^{-1})						
Microbial N	8.11	9.44	11.00	9.43	0.5312	0.1971	0.332
Microbial protein	50.67	59.01	68.80	58.97	0.5312	0.1971	2.072
	Microbial efficiency						
g CP kg TDN^{-1}	148.59	150.58	150.39	129.50	0.4843	0.6175	6.601

L = Significance for linear effect; Q = Significance for quadratic effect.

The feeding, rumination and idle activities also were not affected (P=0.3652, P=0.4858, and P=0.7542, respectively) by the levels of cottonseed cake in substitution of soybean meal in the diet (Table 4). The number of ruminated cuds also did not differ (P=0.3290) among the diets, averaging 664.5 cuds per day. The feed and rumination efficiencies of DM and NDF did not differ (P=0.4058 and P=0.4155, respectively) among the cottonseed cake levels evaluated (Table 5).

With substitution of the soybean meal for cottonseed cake, the NDF increased (Table 1). The changes in the neutral detergent fiber levels in the diets were small, but sufficient to linearly increase (P=0.5905) the intake of this nutrient (Table 6).

Table 4. Behavioral activities of lambs fed diets containing cottonseed cake in substitution of soybean meal.

Item	Levels of cottonseed cake in substitution for soybean meal (% DM)				P-value		SEM
	0	33	66	100	L	Q	
Feeding (min)	281	272	312	282	0.3918	0.3652	3.493
Rumination (min)	508	517	477	511	0.7393	0.4858	6.529
Idle (min)	644	647	626	617	0.2265	0.7542	7.955
Cuds day^{-1}	614	688	677	679	0.1757	0.2143	13.14
Chewing number $cuds^{-1}$	58	57	56	59	0.7834	0.3290	0.873
Chewing $cuds^{-1}$ (sec)	49	46	46	45	0.1641	0.5458	0.658
Total chewing time (h day^{-1})	13.3	13.1	14.0	13.7	0.0870	0.9340	0.116

L = Significance for linear effect; Q = Significance for quadratic effect; min = minutes; sec = seconds; h = hours.

Table 5. Ingestive behavior of lambs fed diets containing cottonseed cake in substitution of soybean meal.

Item	Levels of cottonseed cake in substitution for soybean meal (% DM)				P-value		SEM
	0	33	66	100	L	Q	
	Feed efficiency (g hour^{-1})						
DM	277.06	270.15	230.85	228.27	0.0568	0.8940	9.754
NDF	129.67	129.28	113.33	114.38	0.1812	0.9250	4.694
	Rumination efficiency (g hour^{-1})						
DM	134.27	127.44	131.95	136.15	0.6999	0.4058	3.008
NDF	62.84	60.99	64.77	68.22	0.1575	0.4155	1.462
	Periods (nº day^{-1})						
Feeding	17.66	17.46	17.03	15.73	0.2201	0.6316	0.523
Rumination	25.44	28.66	24.76	26.26	0.6504	0.3573	0.396
Idle	37.48	40.06	35.86	36.86	0.1573	0.4585	0.790
	Min periods^{-1}						
Feeding	14.45	14.60	18.17	21.30	0.0004	0.3083	0.644
Rumination	19.73	18.25	21.00	19.94	0.3930	0.8569	0.420
Idle	18.49	16.77	17.16	16.62	0.1573	0.4585	0.363

L = Significance for linear effect; Q = Significance for quadratic effect.

Table 6. Intake of dry matter (DM), neutral detergent fiber (NDF) and weight gain in lambs fed diets containing cottonseed cake in substitution of soybean meal.

Item	Levels of cottonseed cake in substitution for soybean meal (% DM)				Effect		SEM
	0	33	66	100	L	Q	
	Intake (g day^{-1})						
DM	1101	1097	1124	1172	0.2602	0.5905	22.010
NDF	515	525	552	587	0.0246	0.5905	10.735
	Weight gain (kg)						
Total weight gain	15.97	15.35	15.96	15.50	0.8569	0.9387	0.422
	Intake (min kg^{-1})						
DM	231	235	275	272	0.0586	0.8279	8.672
NDF	493	492	560	544	0.2138	0.8261	17.943
	Intake (g feed periods^{-1})						
DM	65.0	64.0	69.3	77.0	0.0589	0.7784	2.236
NDF	30.4	30.6	34.0	38.6	0.0108	0.3645	1.086
	Intake (g cuds^{-1})						
DM	1.83	1.61	1.69	1.74	0.7660	0.2162	0.048
NDF	0.85	0.77	0.83	0.87	0.5717	0.2290	0.023

L = Significance for linear effect; Q = Significance for quadratic effect.

Discussion

The results for the balance of nitrogen compounds indicates that the animals did not need to change their rates of excretion of nitrogen compounds in the urine and feces due to replacement of soybean meal by cottonseed cake in the diets. The protein supplement in quantity and quality, observing its relations with other dietary ingredients, it is very important because protein is the second limiting nutrient in diets for ruminants (PINA et al., 2006)

The positive and statistically similar nitrogen balance indicates balance between the dietary protein and energy. Therefore, absence of effect on the retained N (Table 2) indicates that even with addition of urea to the diets, substituting soybean meal for cottonseed cake caused no changes in the balance of non-protein nitrogen and true nitrogen.

Similarly, Alves et al. (2010a) evaluated the replacement of soybean meal by high energy cottonseed meal in diets for dairy cows, and observed that the inclusion of cottonseed meal by up to 34.8% DM, did not result in differences in the balance of nitrogen and the efficiency of nitrogen utilization.

According to the NRC (2001), microbial protein synthesis depends in large part on the availability of carbohydrates and nitrogen in the rumen, and the microbial growth increases with the balance of the availability of fermentable energy and degradable nitrogen. Thus, it can be inferred that in this experiment no limitation of microbial growth on any of the diets with inclusion of cottonseed cake (Table 3).

Santos et al. (2010), evaluated the nitrogen use efficiency of growing dairy heifers fed concentrate rations based on soybean or cottonseed meal, they observed that the use of concentrated with cottonseed meal did not result in differences in the amount of total purine, allantoin, uric acid, microbial efficiency and microbial nitrogen.

Additionally, Barros et al. (2011), evaluating the replacement of soybean meal by cottonseed meal in multiple supplements for beef heifers grazing, observed that the use of cottonseed meal did not cause differences from microbial nitrogen and and microbial efficiency, but the authors found that there was a linear decrease in fecal nitrogen excretion with the inclusion of cottonseed meal, and concluded that cottonseed meal in supplements to heifers grazing produces similar productive results regarding the use of soybean meal.

In relation to feeding behavior, animals fed diets with higher amounts of neutral detergent insoluble fiber and / or larger particle sizes, will spend greater time chewing activities (feeding and rumination) and shorter times in idling, which may the ability to influence food intake (CARVALHO et al., 2014). The similarity in the constitution of the fibrous fraction and in the particle size of the diets might have contributed to the normal transit of the fiber through the digestive tract of the animals, impeding the ruminoreticular fill among the different diets, which could explain the results obtained for the feeding and rumination activities.

The increase in the concentration of the cell-wall components of the diets was not sufficient to increase the total chewing time by the animals. The number of cuds depends on the rumination time and on the time spent to ruminate each cud. Absence of variation in the rumination time indicates that there was no difference in the number of ruminated cuds, i.e., the levels of cottonseed cake did not change these parameters.

According to Mertens (1997), the increase in the amount of fiber in the diets stimulates the chewing activity and reduces production of volatile fatty acids. In this study, the absence of significant effect in the number of chews per cuds, in the time spent on the chewing of each cud and in the time spent on the total chewing activity can be related to the reduced particle size of the feeds utilized in the diet, especially of the roughage (chopped Tifton 85 hay), which was the same for all diets.

Due to the effect of rumen fill, food intake by ruminants is determined by the disappearance and passage the potentially degradable neutral detergent fiber and ruminal passage of indigestible neutral detergent fiber (RUFINO JÚNIOR et al., 2015). This way, the increase in NDF intake (Table 6) was not sufficient to influence the feed and rumination efficiencies. The feed and rumination efficiencies were expected to increase when evaluated on a NDF basis, since the NDF intakes as g day^{-1} increased significantly according to the cottonseed cake levels in the diet.

In ruminants, the time spent in feed and rumination activities is influenced by food characteristic, the fiber content and the presence of anti-nutritional compounds (NICORY et al., 2015). The average time spent on rumination was 503 min day^{-1}, which is between the range of 8 to 9 hours considered normal for the rumination activity (ALVES et al., 2010b; VAN SOEST, 1994). The average time spent on the ingestion activity did not vary among the diets, and was 287 min day^{-1}.

The total chewing time was not altered with increased cottonseed cake in the diet, whose average was 13.5 hours day^{-1}(Table 4), similarly, Correia et al. (2015) also did not find significant differences for time chewing in young bulls fed diets containing different levels of substitution of soybean meal for peanut pie.

In our study, was not observed difference for feed and rumination efficiencies, and usually, the feed and rumination efficiencies are influenced by the DM and NDF intakes, and this was proved by Carvalho et al. (2004), who observed lower rumination efficiency when the animals consumed lower amounts of these nutritional components.

Conclusion

Total substitution of soybean meal for cottonseed cake in the concentrate, corresponding to 12% of the total diet, does not change the balance of nitrogen compounds or the microbial protein synthesis, nor does it compromise the ingestive behavior and feed and rumination efficiencies of feedlot lambs.

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