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Effect of cottonseed processing and chitosan supplementation on lamb performance, digestibility and nitrogen digestion

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Abstract

The current study was carried out to examine the effect of cottonseed processing and chitosan supplementation on lamb performance, digestibility and nitrogen digestion. Eighty uncastrated Santa Inês lambs (23 ± 2.2 kg average weight, 4 months old) were distributed in a completely randomized design in a 2 × 2 factorial arrangement that consisted of two cottonseed processing forms (whole or ground) and two chitosan levels (0 or 136 mg/kg live weight). Higher dry matter and organic matter apparent digestibility coefficient (ADC) was achieved with the diets containing the whole cottonseed. Ether extract ADC was higher in the animals fed the chitosan-containing diet. There was an interaction effect on the ADC of neutral detergent fibre corrected for ash and protein, which increased with chitosan inclusion associated with the whole cottonseed. The lambs that received the treatment containing the whole cottonseed showed higher microbial protein synthesis. Chitosan addition increased nitrogen retention. The animals fed chitosan-containing diets showed higher microbial protein synthesis. There was an interaction effect on microbial protein synthesis. Whole cottonseed associated with chitosan in lamb diets increases ether extract ADC and microbial protein synthesis.

Introduction

The intensification of livestock systems to produce animal protein for the human population has created a demand for information about the use of agro-industrial by-products associated with additives that allow for adequate lamb performance. Some of the main additives used in ruminant feeding are ionophores, organic acids, plant extracts (Calsamiglia *et al*., 2007) and, more recently, chitosan (Henry *et al*., 2015; Dias *et al*., 2017).

Chitosan is a biopolymer derived from chitin. It is found mainly as a component of the exoskeleton of crustaceans and insects as well as in the cell walls of some fungi and bacteria (Senel and McClure, 2004). Among its biological characteristics, chitosan is known to have antibacterial, fungicide and anticholesterolaemic properties (Dutta *et al*., 2004), which has aroused great interest in its use as a modulator of rumen fermentation in ruminants (Fadel El-Seed *et al*., 2003).

Researchers examining the effect of chitosan on ruminal fermentation and digestibility of ruminants *in vivo* (Araújo *et al*., 2015; Henry *et al*., 2015; Dias *et al*., 2017) have reported increases in the ruminal propionate content and in the digestibility of dry matter, neutral detergent fibre and crude protein. Belanche *et al*. (2016) observed a reduction in methane production and increased propionic acid production when chitosan was used in an experiment with *in vitro* cultures. Chitosan was also effective in inhibiting rumen biohydrogenation and increasing the proportions of 18:1 t11 fatty acid and conjugated linoleic acid, in addition to lowering the proportion of saturated fatty acids in *in vitro* conditions (Goiri *et al*., 2010).

So far only a few experiments have been conducted on the effects of chitosan with animals *in vivo*. The impact of this additive on the performance of feedlot lambs, for instance, is not known. The use of chitosan associated with traditional ingredients such as cottonseed, a source of protein and energy, may lead to improvements in lamb performance.

Cottonseed is used in its whole form or ground, in ruminant diets. Thus, it is hypothesized that the use of ground cottonseed in association with chitosan can improve the performance and microbial protein synthesis in lambs without altering the dietary protein-to-energy ratio.

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On this basis, the goal of the current study was to investigate the effect of cottonseed processing and chitosan supplementation on lamb performance, digestibility and nitrogen digestion.

Materials and methods

Animals and housing

Eighty uncastrated Santa Inês lambs with an average body weight (BW) of 23 ± 2.20 kg, at 4 months of age, which had been previously dewormed, vaccinated (rabies and clostridial infections) and supplemented (ADE vitamin complex), were tagged and randomly assigned to treatments in a completely randomized design. Lambs were housed in individual, covered stalls with suspended slatted floors (1 m^2 per stall), equipped with drinkers and feeding troughs.

Experimental diet and management

The animals were kept in confinement for 90 days, which were preceded by 15 days of acclimation to the facilities, diets and daily management. During this phase, they received Tifton-85 (*Cynodon spp.*) hay as roughage (*ad libitum*) and increasing amounts of the experimental diets. After this period, the experimental phase began, consisting of three consecutive 30-day periods for the collection of samples and data for the evaluation of the intake, nutrient digestibility, productive performance and microbial protein synthesis.

Diets were formulated as recommended by the NRC (2007) to meet the nutritional requirements of lambs with an estimated weight gain of 200 g day, containing a roughage-to-concentrate ratio of 50:50. The feed was supplied twice daily, at 09.00 and 16.00 h.

The experimental diets (Table 1), which were composed of roughage and concentrate, were evaluated in a 2 × 2 factorial arrangement corresponding to the use of ground or whole cottonseed, with and without chitosan. Treatments were as follows: (1) Diet containing the whole cottonseed; (2) Diet containing whole cottonseed + 136 mg chitosan kg/BW; (3) Diet containing ground cottonseed; (4) Diet containing ground cottonseed + 136 mg chitosan kg/BW. The chitosan used in the experiment had a deacetylation degree of 0.86, an apparent density of 0.33 mg/ml and a pH of 7.9 (Polymar®, Fortaleza, Ceará, Brazil). The diets were weighed on a digital scale and were provided to allow approximately 10% refusals (dry matter basis). Throughout the entire experimental period, samples of ingredients and diets were collected and combined to form a composite sample, which was divided into four equal parts and placed in labelled plastic bags that were subsequently stored in a freezer at −20 °C for later chemical analysis.

Table 1. Proportions and chemical composition of the basal experimental diet used for feedlot-finished lambs

Ingredient (g/kg DM)	Diet
Tifton-85 hay	500
Ground corn	184
Soybean meal	145
Cottonseed	150
Urea	6.00
Mineral premix[a]	15.0
Composition chemical (g/kg DM)	
Dry matter (g/kg as fed)	865
Organic matter	951
Mineral matter	48.9
Crude protein	172
Ether extract	46.1
Neutral detergent fibre[b]	417
Acid detergent fibre	209
Hemicellulose	209
Cellulose	178
Lignin[c]	29.7
Total carbohydrates	729
Non-fibrous carbohydrates	312
Total digestible nutrients	733

[a]Assurance levels (per kg in active elements): calcium: 120 g; phosphorus: 87 g; sodium: 147 g; sulphur: 18 g; copper: 590 mg; cobalt: 40 mg; chromium: 20 mg; iron: 1800 mg; iodine: 80 mg; magnesium: 1300 mg; Se: 15 mg; zinc: 3800 mg; molybdenum: 300 mg; fluorine: 870 mg; phosphorus solubility in 2% citric acid, minimum – 95%.
[b]Using heat-stable α-amylase without the addition of sodium sulphite to the detergent.
[c]Lignin (sa)-Lignin determined by solubilization of cellulose with sulphuric acid.

Chemical composition, intake and digestibility

Production performance was evaluated in all 80 lambs (20 per treatment), whereas digestibility and the other parameters were evaluated in 40 lambs (ten per treatment). The apparent digestibility trial took place between the 30th and 37th and between the 60th and 67th days of the experimental period. Total faecal collection was performed using collection bags. The first 3 days were dedicated to the adaptation of lambs to the collection bags, followed by 5 days of total faecal collection. Faeces were collected directly from the collection bags twice daily (08.00 and 15.00 h), from the 33rd to the 37th and from the 60th to the 67th days in the individual stalls in the feedlot. Next, the total faecal production of each animal was recorded, and aliquots of approximately 10% of the total collected were separated, packed in individual, labelled plastic bags and stored in a freezer at −20 °C until further analysis. During the digestibility trial, samples of feed and refusals were collected daily. For the analysis of the supplied feed, samples of ingredients and refusals were harvested weekly. The apparent digestibility coefficient (ADC) was calculated using the following formula proposed by Wiseman (2018):

Samples of roughage, concentrate, refusals, ingredients and faeces were pre-dried in a forced-air oven at 55 °C for 72 h. Next, they were ground in Wiley knife mills with 1 mm sieves and stored in labelled plastic bottles with caps for laboratory analyses.

The dry matter (DM; method 967.03), mineral matter (MM; method 942.05), crude protein (CP; method 981.10) and ether extract (EE; method 920.29) contents of all samples of feedstuffs and refusals were determined following procedures described by the AOAC (1990). The organic matter (OM) content was obtained by the following equation: OM = DM – MM. Neutral detergent fibre (aNDFom-NDF) was analysed as suggested by Van Soest *et al.* (1991) and corrected for the residual ash in

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accordance with Mertens (2002), using heat-stable α-amylase without the addition of sodium sulphite to the detergent (Ankom Tech Corp., Fairport, NY, USA); the result was expressed free of residual ash, as proposed by Licitra *et al.* (1996). The acid detergent fibre concentration was measured by the methodology proposed by Van Soest *et al.* (1991). Lignin was determined according to method 973.18 (AOAC 2002), by solubilization of cellulose with 72% (w/v) sulphuric acid.

Total carbohydrates (TC) were estimated as proposed by Sniffen *et al.* (1992), as follows: TC = 100 – (%CP + %EE + % MM). The concentration of non-fibrous carbohydrates (NFC) in the ingredients was determined as described by Mertens (1997), considering aNDFom in the calculations.

The concentrations of NFC in the samples of diets, refusals and faeces were estimated by the following equation proposed by Hall (2003):

$$\text{NFC} = 100 - (\%\text{CP} + \%\text{EE} + \%\text{MM} + \text{aNDFom})$$

where NFC = estimated NFC content (%DM); CP = CP content (%DM); EE = EE content (%DM); MM = MM content (%DM); aNDFom = NDF content corrected for residual ash and protein (%DM).

Both TC and NFC were converted to g/kg in the current paper.

The total digestible nutrient (TDN) content was estimated by the formula proposed by Weiss (1999), as follows:

$$\text{TDN} = \text{DCP} + 2{\cdot}25 \times \text{DEE} + \text{DNFC} + \text{aDNDFom}$$

where DCP, DEE, DNFC and aDNDFom are the digestible fractions of CP, EE, NFC and aNDFom, respectively.

Additionally, the intakes of DM and aNDFom per metabolic weight were estimated by the following equation: Intake ($\text{g/kg}^{0.75}$) = amount of DM or aNDFom (kg) consumed × 100 $\text{BW}^{0.75}$, with nutrient intake calculated on a DM basis.

Urinary excretion and microbial protein synthesis

On the 18th, 20th and 22nd days of the third experimental period, urine samples were harvested approximately 4 h after the morning feed. Urine was collected during spontaneous urination, using plastic cups. At the end of each collection, samples were filtered through gauze, and a 10 ml aliquot of urine was separated. Subsequently, the samples were diluted in 40 ml of a 0.036 N sulphuric acid solution (Valadares *et al.*, 1999). These were then packed in labelled plastic bottles and stored at −20 °C for later quantification of the urinary creatinine concentration.

The daily excretion of creatinine (mg/day) was determined by multiplying the average BW of each lamb by excretion coefficient of 17.05 mg of creatinine per kilogram of BW (Pereira *et al.*, 2013), as shown below:

$$\text{DEC} = \text{BW} \times 17{\cdot}05$$

where DEC = daily excretion of creatinine (mg/day); BW = animal body weight (kg).

The urinary volume (litres) was estimated based on the daily excretion of creatinine (mg/day) and the creatinine concentration (CC) in the spot urine samples (mg/l), as follows:

$$\text{UV} = \text{DEC}/\text{CC}$$

Urine samples were used for the quantification of the urinary concentrations of urea, creatinine, total nitrogen, allantoin, uric acid, xanthine and hypoxanthine. The urinary concentrations of creatinine, uric acid and urea were determined using commercial kits (Bioclin®, Belo Horizonte, Minas Gerais, Brazil). Urinary allantoin was quantified by the colorimetric method, described by Chen and Gomes (1992). Urea values were converted to urea nitrogen by multiplying the obtained values by the factor 0.4667.

The total excretion of purine derivatives was calculated as the sum of the amounts of allantoin, uric acid, xanthine and hypoxanthine present in the urine (mmol/day). Absorbed purines (X, mmol/day) were estimated from the excretion of purine derivatives (Y, mmol/day) by the following equation proposed by Chen and Gomes (1992), for sheep:

$$Y = 0{\cdot}84X + (0.150\text{LW}^{0{\cdot}75}\text{e}^{-0.25\text{X}})$$

where 0.84 is the efficiency of absorption of exogenous purines; 0.150 $\text{LW}^{0.75}$ corresponds to the endogenous excretion of purine derivatives; and $\text{e}^{-0.25\text{X}}$ is the rate of substitution of the *de novo* synthesis for exogenous purines.

Microbial protein synthesis in the rumen (g micN/day) was calculated as a function of absorbed purines (X, mmol/day), using the equation described by Chen and Gomes (1992):

$$\text{micN} = 70X/\ (0.83 \times 0{\cdot}116 \times 1000)$$

where 70 is the purine N content (mg N/mmol); 0.83 is the digestibility coefficient of microbial purines; and 0.16 is the ratio between N in purines and total bacterial N.

The nitrogen content in the samples of consumed material, faeces and urine was determined by following the methodology described by AOAC (1990). Nitrogen retention (retained N, g/day) was calculated by the following formula:

$$\text{Retained N} = \text{N intake (g)} - \text{Faecal N (g)} - \text{Urinary N (g)}$$

Performance

Lamb performance was determined by individually weighing the animals at the start of the experiment and then at every 24 days to measure their average daily gain (ADG). Lambs were weighed always in the morning, after a 16 h fast. For the calculation of ADG, lambs were weighed before the 16 h fast to determine their final BW (or pre-slaughter weight). ADG was calculated as follows:

$$\text{ADG} = \frac{\text{Final body weight post-fast} - \text{Initial body weight post-fast}}{\text{Days in the feedlot}}$$

After the total daily DM intake (total DMI) and ADG data were obtained, it was possible to calculate the animals' feed conversion (FC) as well as its opposite variable, feed efficiency (FE), using the formulae below:

$$\text{FC} = \text{total DMI}/\text{ADG}$$

$$\text{FE} = \text{ADG}/\text{total DMI}$$

where FC = feed conversion (kg of DM intake per kg of weight gain); total DMI = total daily dry matter intake; ADG = average

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daily gain (kg/day); and FE = feed efficiency (kg of weight gain per kg of dry matter intake).

Statistical analyses

Data were subjected to analysis of variance in a completely randomized design. To test the effect of treatments, the data were analysed by using the PROC MIXED procedure of SAS software (version 9.1) (SAS, 2005), according to the model below:

$$Y_{ijk} = \mu + s_i + T_{ej} + (s_i \times T_{eij}) + e_{ijk}$$

where μ = mean; s_i = fixed effect of cottonseed processing form; T_{ej} = random effect of chitosan addition; $s_i \times T_{eij}$ = interaction effect between cottonseed processing form and chitosan addition; and e_{ijk} = error.

A 2 × 2 factorial arrangement (whole or ground cottonseed, with or without chitosan) was adopted. The effects of cottonseed processing form, chitosan addition and the interaction between these two factors were tested. Treatment means were obtained by the LSMEANS procedure, and the significance level of 5% was adopted for all variables.

Results

No differences were found for the intakes of nutritional components. The ADCs of DM ($P = 0.006$) and OM ($P = 0.011$) was higher in the animals fed the diets containing the whole cottonseed. The chitosan-containing diets provided higher ($P = 0.025$) EE ADC. There was an interaction effect ($P = 0.011$) on aNDFom ADC, which increased with the use of chitosan associated with whole cottonseed (Table 2).

In the evaluation of production performance (Table 3), no interaction effect between the treatments was detected for ADG, FC or FE.

The animals that received the treatments containing the whole cottonseed showed higher microbial protein synthesis (in g micCP/day and g micCP/kg TDN) ($P < 0.01$). No differences were observed in nitrogen balance, intake or excretion in faeces and urine. However, chitosan addition led to increased N retention ($P = 0.037$). Lambs fed the chitosan-containing diets also showed higher microbial protein synthesis (in g micCP/day and g micCP/kg TDN) ($P < 0.01$ and $P = 0.045$, respectively). There was an interaction effect for microbial protein synthesis (in g micCP/day and g micCP/kg TDN) ($P < 0.01$) (Table 4).

Discussion

The intake of nutritional components was not influenced by the treatments, possibly because the diets were similar in nutritional composition. Further, the slow release of fat in the rumen may have allowed for hydrogenation of unsaturated fatty acids, thus reducing the inhibitory effect of fat on nutrient digestibility (Geron *et al.*, 2012). The ether extract in ground cottonseed, which is probably released more effectively than in chitosan, may have contributed to a better ruminal fermentation due to its potential modulating effect. As a result, nutrient intake was not compromised (Goiri *et al.*, 2010).

Dry matter ($P = 0.001$) and OM ($P = 0.001$) ADCs were lower in the groups fed diets with ground cottonseed (Table 2). However, these variables were expected to be higher or similar to those obtained with the whole cottonseed, since, according to Nocek and Tamminga (1991), reducing the grain particle size increases the surface contact area, passage rate and degradation rate. This was confirmed by Teixeira *et al.* (2002), who evaluated the effective potential degradability and degradation rate of whole and ground cottonseed. In this way, the higher rates of passage and degradation and the likely more effective release of fat from ground cottonseed might have contributed to reducing the ADC of these nutrients, since the diet did not have a fat content that might compromise digestibility (over 5%) (Palmquist and Jenkins, 1980).

The positive effect of chitosan inclusion on the ADC of ether extract partially explains its effect as a modulator of fermentation and the increased efficiency of utilization of the energy generated in the ruminal system. In a study with sheep, Goiri *et al.* (2009) did not observe differences in nutrient digestibility except for the digestibility of NDF, which decreased, suggesting an effect on cellulolytic bacteria. Changes in ruminal fermentation may be a consequence of the decrease in DM intake when chitosan is added to the animal diet, which may in turn be related to the higher lipid content of chitosan (Bassi *et al.*, 2012; Garcia-Rodriguez *et al.*, 2015).

de Paiva *et al.* (2016) worked with increasing levels of chitosan (50, 100 and 150 mg/kg) and Del Valle *et al.* (2017) tested the levels of 0 and 4 g/kg in the diet of cattle and both researchers observed positive effects on nutrient digestibility, which they attributed to alterations in ruminal fermentation. These two studies involved diets containing soy grain, i.e. a similar protocol to that tested in the present study except for the processing type evaluated.

ADG was similar across the treatment groups, and the similar intakes and digestibilities of the nutritional components explain this finding. Cunha *et al.* (2008) evaluated Santa Inês sheep fed cottonseed and observed a lower ADG than that found in the present experiment. However, this may be a consequence of the other diet ingredients, whose digestibility may be more severely affected by the lipid level than by the physical form of cottonseed.

The ADC of NDFap and microbial protein synthesis were higher in the animals fed whole cottonseed and chitosan. The association between ground cottonseed and chitosan reduced the absorption of this nutritional fraction and, partially, microbial production.

Chitosan has been shown to be effective on animal production. In several studies with ruminants, e.g. beef cattle (Araújo *et al.*, 2015; Dias *et al.*, 2017) and dairy cattle (Garcia-Rodriguez *et al.*, 2015; de Paiva *et al.*, 2016; Gandra *et al.*, 2016; Del Valle *et al.*, 2017), chitosan was effective in improving nutrient digestibility, microbial protein synthesis and, in some cases, feed efficiency and milk yield.

The influence of ground cottonseed on the lower microbial protein synthesis may be related to the higher rates of passage and degradation and the more effective release of fat, which also has a toxic effect on the rumen microorganisms (Bassi *et al.*, 2012). These facts are associated with the lesser action of chitosan on the fibrous fraction of the diet (Wencelová *et al.*, 2014), since half of it was composed of Tifton-85 grass hay, which probably influenced ruminal fermentation and, consequently, contributed to the reduced NDFap digestibility and microbial protein synthesis

In the current experiment, it can be stated that the uptakes of nitrogen and energy were balanced, allowing the development of

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Table 2. Daily intake and apparent digestibility coefficient in Santa Inês lambs fed diets with cottonseed (ground or whole) with/without chitosan addition (136 mg of chitosan/kg of BW)

	Cottonseed		Chitosan			*P* value*		
Item	Whole	Ground	0	136	S.E.M.	P	C	P × C
Feed intake (kg/day)								
DM	1.09	1.11	1.09	1.12	0.020	0.942	0.135	0.730
OM	1.07	1.08	1.04	1.11	0.070	0.757	0.165	0.849
CP	0.18	0.19	0.18	0.19	0.003	0.497	0.723	0.538
EE	0.06	0.06	0.06	0.06	0.007	0.396	0.569	0.247
aNDFom	0.41	0.40	0.39	0.41	0.007	0.446	0.207	0.983
NFC	0.32	0.33	0.32	0.33	0.005	0.466	0.438	0.189
TDN	0.70	0.63	0.83	0.57	0.022	<0.001	0.239	0.007
Feed intake (g/kg $BW^{0.75}$)								
DM	82.88	81.48	83.92	82.27	0.914	0.409	0.625	0.946
Feed intake (g/kg BW)								
DM	35.1	34.7	36.0	34.1	0.04	0.019	0.966	0.402
NDF	13.2	12.5	13.2	12.7	0.02	0.098	0.731	0.814
Apparent digestibility coefficient								
DM	0.69	0.63	0.65	0.68	0.007	0.006	0.222	0.129
OM	0.72	0.67	0.68	0.71	0.007	0.011	0.098	0.227
CP	0.76	0.76	0.73	0.80	0.006	0.050	0.155	0.267
EE	0.86	0.86	0.84	0.88	0.005	0.992	0.025	0.142
aNDFom	0.46	0.36	0.39	0.41	0.004	0.427	0.563	0.011
NFC	0.85	0.81	0.82	0.84	0.006	0.070	0.201	0.292
TDN	0.83	0.76	0.87	0.75	0.011	0.005	0.332	0.126
Evaluation of interaction effect								
Cottonseed	Chitosan							
	0				136			
Content of digestible aNDFFom (g/kg)								
Whole	468^{Bb}				523^{Aa}			
Ground	527^{Aa}				491^{Bb}			
S.E.M.	16.8				16.7			

S.E.M., standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; EE, Ether extract; aNDFom, Neutral detergent fibre corrected for ash and protein; NFC, non-fibrous carbohydrates; TDN, total digestible nutrients.
*Probability value for the effects of processing (P), chitosan (C) and interaction between P × C. Means followed by different letters (lowercase in the row and uppercase in the column) differ statistically ($P < 0.05$) according to the F test.

Table 3. Performance of Santa Inês lambs fed diets with cottonseed (ground or whole) with/without chitosan addition (136 mg of chitosan/kg of BW)

	Cottonseed		Chitosan			*P* value[a]		
Item	Whole	Ground	0	136	S.E.M.	P	C	P × C
ADG	0.20	0.19	0.19	0.19	0.003	0.413	0.624	0.626
FE (DMI kg/ADG kg)	0.18	0.18	0.18	0.18	0.002	0.511	0.630	0.992
FC (ADG kg/DMI kg)	5.7	5.7	5.7	5.8	0.13	0.742	0.721	0.309

S.E.M., standard error of the mean; ADG, average daily gain; FE, food efficiency; FC, food conversion.
[a]Probability value for the effects of processing (P), chitosan (C) and interaction between P × C.

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Table 4. Nitrogen balance and microbial protein synthesis of Santa Inês lambs fed diets with cottonseed (ground or whole) with/without chitosan addition (136 mg of chitosan/kg of BW)

	Cottonseed		Chitosan			*P* value*		
Item	Whole	Ground	0	136	S.E.M.	P	C	P × C
Nitrogen (g/day)								
Nitrogen intake	30.8	28.8	28.5	31.1	0.74	0.165	0.060	0.083
Faecal nitrogen	7.1	7.6	7.6	7.1	0.29	0.356	0.400	0.605
Urinary nitrogen	1.0	1.1	1.0	1.1	0.12	0.563	0.816	0.709
Retained nitrogen	22.4	20.0	19.9	22.5	0.65	0.055	0.037	0.127
Microbial protein synthesis								
g micCP/day	92	59	67	84	3.8	<0.001	<0.001	<0.001
g micCP/kg TDN	145	107	116	136	3.7	<0.001	0.045	0.008
Evaluation of interaction effect								
Cottonseed	Chitosan							
	0			136				
Microbial synthesis (g micCP/day)								
Whole	74.85^{Ab}			109.4^{Aa}				
Ground	59.1^{Ba}			59.2^{Ba}				
S.E.M.	3.79			3.79				
Microbial synthesis (g micCP/kg TDN)								
Whole	118^{Ab}			145^{Aa}				
Ground	107^{Ba}			103^{Bb}				
S.E.M.	5.3			5.2				

S.E.M., standard error of the mean; g micCP/day, grams of microbial crude protein per day; g micCP/kg TDN, grams of microbial crude protein/kg TDN.
*Probability value for the effects of processing (P), chitosan (C) and interaction between P × C. Means followed by different letters (lowercase in the row and uppercase in the column) differ statistically ($P < 0.05$) according to the *F* test.

the rumen microbiota. A positive balance indicates a relationship between the amounts of protein and energy in the diet (Silva *et al*., 2016). Chitosan can improve nitrogen utilization by reducing deamination, thus allowing a larger amount of amino acids to reach the duodenum to be absorbed, which explains the improved N retention in the animals fed the chitosan-containing diets (de Paiva *et al*., 2016).

Microbial protein increased (by 33%) with the use of whole cottonseed and chitosan. This response might have been due to the better synchronism between the release of lipids and protein resulting from the use of cottonseed. With respect to chitosan, the result may be due to the ionic interaction between its amine group and the bacterial surface (Kong *et al*., 2010), coupled with the decreased methane production and increased propionic acid production provided by the use of chitosan (Belanche *et al*., 2016). In a review on the properties and mode of action of chitosan, Kong *et al*. (2010) observed that it increased the amount of N excreted in milk without changing N intake and improved N utilization efficiency.

ADG was similar between the evaluated experimental diets, which is explained by the similar intakes and digestibilities of nutritional components across the treatment groups. Cunha *et al*. (2008) conducted an experiment in which they fed cottonseed to Santa Inês sheep and found lower ADG than those observed in the current experiment. However, this effect may be due to other dietary ingredients whose digestibility might have been more affected by the lipid level than by the physical form of cottonseed.

Conclusion

Whole cottonseed associated with 136 mg chitosan in sheep diets increases ether extract ADC and microbial protein synthesis. However, it is necessary to determine the best level of chitosan in diets with the whole cottonseed.

Cottonseed processing form and the use of chitosan do not affect the performance of feedlot-finished lambs.

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Ethical standards. The study was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (EMEVZ-UFBA) (permit number 16/2016) and was carried out on the Experimental Farm of EMEVZ-UFBA, located at 12°23′57.51″ South latitude and 38°52′44.66″ West longitude, in São Gonçalo dos Campos, Bahia, Brazil.

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