https://doi.org/10.17221/194/2020-VETMED

Health concerns of lambs fed cottonseed hulls combined with chitosan by examining the blood metabolic profile and histopathology of the kidney, liver, and rumen

Tamires da Silva Magalhaes[1], Gleidson Giordano Pinto de Carvalho[1], Edson Mauro Santos[2], Alessandra Estrela da Silva Lima[1], Jose Esler de Freitas Junior[1], Douglas dos Santos Pina[1], Stefanie Alvarenga Santos[1], Luis Fernando Batista Pinto[1], Gerson Barreto Mourao[3], Franklin Delano dos Santos Soares[4], Taiala Cristina de Jesus Pereira[1], Laudi Cunha Leite[5]*

[1]*Federal University of Bahia, Salvador, Bahia, Brazil*
[2]*Federal University of Paraíba, Areia, Paraíba, Brazil*
[3]*University of São Paulo, Piracicaba, São Paulo, Brazil*
[4]*State University of Southwest Bahia, Itapetinga, Bahia, Brazil*
[5]*Federal University of Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil*

**Corresponding author: gleidsongiordano@yahoo.com.br*

Citation: Magalhaes TS, Carvalho GGP, Santos EM, Lima AES, Freitas Junior JE, Pina DS, Santos SA, Pinto LFB, Mourao GB, Soares FDS, Pereira TCJ, Leite LC (2021): Health concerns of lambs fed cottonseed hulls combined with chitosan by examining the blood metabolic profile and histopathology of the kidney, liver, and rumen. Vet Med-Czech 66, 470–480.

Abstract: This study analysed the effect of cottonseed hulls and chitosan in diets for lambs by determining the blood metabolite profile, and the histopathology of the kidney, liver, and rumen. Eighty non-castrated Santa Inês lambs, approximately 120 days of age and a mean initial body weight (b.w.) of 22.6 (standard deviation ± 2.2 kg) were assigned to a completely randomised design, with a 2 × 2 factorial arrangement. Two chitosan levels and two cottonseed forms were evaluated. The experimental diets were following: diet containing whole cottonseed hulls (WC) without the addition of chitosan; diet containing WC with 136 mg/kg b.w. chitosan added; diet containing ground cottonseed hulls (GC) without the addition of chitosan; diet containing GC with 136 mg/kg b.w. chitosan added. The blood metabolites and hepatic enzymes ALT, AST, GGT were not significantly influenced in the treatment groups, except for the serum cholesterol concentration which was lower ($P < 0.05$) when the chitosan was combined with the whole cottonseed hulls. There were histopathological alterations ($P < 0.05$) in the liver and kidney tissue and moderate changes in the rumen samples in the animals fed cottonseed without chitosan, however, when chitosan was added, the changes were less marked. The combination of chitosan with cottonseed hulls (ground or whole) can be supplied safely to feedlot finishing lambs without compromising their health.

Keywords: clinical biochemistry; hepatocytes; hepatopathy; ruminal morphometry; small ruminants

Alternative feedstuffs in animal production may reduce feed costs, which can increase the profitability of the production system. However, new ingredients that could be used in animal diets

https://doi.org/10.17221/194/2020-VETMED

should be previously studied to ensure properties the health and prevent the accumulation of residual compounds in milk or meat, which may compromise human health (Costa et al. 2016).

Cottonseed is obtained from cotton processing to obtain fibres, which are destined for the textile industry. Also, this oilseed can be used for vegetable oil extraction or for animal feed. According to Valadares Filho et al. (2010), it contains high protein (23%), neutral detergent fibres (43.8%), and an ether extract (22.7%), which makes it an excellent protein-energetic ingredient. However, the nutritional quality of cottonseed is limited by the presence of free gossypol, a toxic compound that reacts with primary amines, and leads to the formation of gossypol imines or Schiff bases (Gadelha et al. 2014; Viana et al. 2021), which can interfere with the metabolism and morphophysiological parameters of the organs of animals that are submitted to diets containing this food.

The fatty acid profile of cottonseed is another important aspect. It is rich in linoleic (C18:2), palmitic (16:0) and oleic (C18:1) fatty acids (FA) at 53.2%, 25.3%, and 17.1%, respectively, while the stearic (C18:0) and linolenic (C18:3) fatty acids account for less than 3% of the total FA (Messana et al. 2009; Magalhaes et al. 2020). Therefore, more than 70% of the cottonseed oil consists of unsaturated fatty acids.

Certain fatty acids, especially polyunsaturated ones, are toxic to some ruminal bacteria. Thus, as a defence mechanism, biohydrogenation becomes a very important event in the rumen. In this sense, cottonseed hulls could be combined with a feed additive to interfere with ruminal biohydrogenation, once unsaturated fatty acids are desirable in the final products (milk and meat) of ruminants (Berchielli et al. 2006).

Chitosan is a non-toxic and biodegradable biopolymer, which can have a competitive cost compared to ionophores (Mingoti et al. 2016; Magalhaes et al. 2020; Pereira et al. 2020). Since it has a broad antimicrobial spectrum, is anti-inflammatory, is an antioxidant and has digestive modulatory on the ruminal metabolism (Khambualai et al. 2009; Goiri et al. 2010), it may influence the biohydrogenation and increase the supply of unsaturated fatty acids and nutraceutical compounds (Goiri et al. 2009a; Goiri et al. 2009b; Goiri et al. 2009c; Wencelova et al. 2014). Another benefit of its utilisation is the increase in the proportion of propionate and a decrease in the production of methane, which translates into greater energy efficiency in ruminants (Del Valle et al. 2017; Jimenez-Ocampo et al. 2019). However, its use as an additive in the ruminant diet is still poorly studied.

We hypothesised that diets containing cottonseed hulls change the liver condition of lambs. Thus, this study aimed to evaluate the influence of whole or ground cottonseed hulls, combined with chitosan in diets for lambs finished in the feedlot, through blood biochemistry and a histopathology.

MATERIAL AND METHODS

This study was conducted according to the guidelines of the National Council for the Control of Animal Experimentation. The Committee on the Ethics of Animal Experiments of the Federal University of Bahia (UFBA), Brazil, approved the protocol (Permit No. 16-2016).

Animals, experimental design and diets

The experiment was conducted in the facilities of the Experimental Farm of the School of Veterinary Medicine and Animal Science – UFBA. The local environment is characterised by its humid climate, annual average precipitation of 1 900 mm, average relative humidity of 81%, and an average temperature of 25.3 °C, with a maximum of 28.1 °C and a minimum of 22.5 °C (CEI 1994).

Eighty non-castrated Santa Inês lambs, approximately 120 days of age and a mean initial body weight (b.w.) of 22.6 kg (standard deviation ± 2.2 kg) were used. Before the onset of the experiment, all the animals were dewormed (Ivomec Gold®; Merial, Salvador, Bahia, Brazil) and vaccinated against clostridia (Sintoxan®; Merial, Salvador, Bahia, Brazil). This experiment consisted of 15 days for the adaptation and 90 days for the sample collections.

The animals were housed individually in 1.0 × 1.0 m roofed stalls, with a slatted floor, equipped with individual feeding troughs and drinkers. The animals were distributed in a completely randomised design, with 2 × 2 factorial arrangements, with two doses of chitosan and two processing forms of cottonseed hulls. The treatments constituted:

https://doi.org/10.17221/194/2020-VETMED

1. Diet with whole cottonseed hulls (WC) without chitosan;
2. Diet with the inclusion of 136 mg/kg b.w. of chitosan and the WC;
3. Diet with ground cottonseed hulls (GC) without any chitosan;
4. Diet with the inclusion of 136 mg/kg b.w. of chitosan and the GC.

The chitosan used has 86.3% deacetylation, an apparent density of 0.33 mg/ml, and a pH of 7.9 (Polymar®; Fortaleza, Ceará, Brazil).

A standard experimental diet with 15% cottonseed [% dry matter (DM)] was used (Table 1). The diets were isonitrogenous (crude protein at 150 g/kg of DM) and formulated to allow a b.w. gain rate of 200 g/day as recommended by the National Research Council (NRC 2007) for lambs. The animals were fed *ad libitum*, and this diet contained approximately 50% forage (Tifton 85 hay) and 50% concentrate, thus allowing for refusals of 10% to 20% of fresh diet, twice daily meals at 9 a.m. and 4 p.m. Water was also supplied *ad libitum*. Samples of the dietary ingredients were collected weekly to constitute a composite sample, which was processed at the end of the experiment.

Table 1. Percentage and chemical composition of the ingredients of the experimental diet

Ingredient (g/kg dry matter)	Diet (%)
Tifton 85 hay	50.00
Ground maize	18.40
Soybean meal	14.50
Cottonseed	15.00
Urea	0.60
Mineral supplement[a]	1.50
Chemical composition	
Dry matter (DM)	86.45
g/kg of DM	
Organic matter	95.11
Ash	4.89
Crude protein	17.18
Ether extract	4.61
Non-fibre carbohydrates	31.15
NDFap[b]	41.74
Acid detergent fibre	20.85
Hemicellulose	20.90
Cellulose	17.88
Lignin	2.97
Total digestible nutrients	73.25

[a]Amount/kg of product: Calcium (max) 120 g; Phosphorus 87 g; Copper 590 mg; Iron 250 mg; Cobalt 40 mg; Iodine 80 mg; Manganese 1 300 mg; Selenium 15 mg; Zinc 3 800 mg; Fluorine (max) 710 mg; [b]Neutral detergent insoluble fibre corrected for ash and protein

Laboratory analysis and procedures

The samples were analysed for the dry matter [DM, Method 934.01 (AOAC 1990)], crude protein [CP, Method 981.10 (AOAC 1990)], and ether extract [EE, Method 920.29 (AOAC 1990)], neutral detergent fibre (NDF), and acid detergent fibre (ADF) as described by Detmann et al. (2012).

The neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were estimated from the residue of the extraction of the neutral and acid detergents, respectively, according to Licitra et al. (1996). The total digestible nutrient (TDN) was estimated using the formula proposed by Weiss (1999).

Approximately 10 ml of blood was individually collected in a sterile test tube from all the animals at the end of the experimental period. The samples were temporarily kept at room temperature until clot retraction occurred and then they were centrifuged at 3 500 *g* for 15 min to obtain the blood serum. Finally, the serum was stored at –20 °C until the analysis.

The serum samples were analysed for the total protein (TP), albumin (ALB), globulin (GLB), urea, total cholesterol, triglycerides, and creatinine concentrations, the activity of the enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), by using commercial kits (Doles Reagents, Goiânia, GO, Brazil). The data relative to the concentrations of the total proteins and albumin were used to calculate the globulin concentrations. The globulin concentrations were calculated as the mathematical difference between the total protein and the serum albumin levels, with values expressed in grams per decilitre.

On the last day of the trial period, the lambs were deprived of solids and subsequently transferred to a commercial slaughterhouse, following standard procedures. During the slaughter, samples of the kidneys and liver were collected and fixed

https://doi.org/10.17221/194/2020-VETMED

in 10% neutral buffered formalin for 24–72 hours. The fixed samples per standard procedures were embedded in paraffin wax, histological sections of 4-µm thickness were obtained and stained with haematoxylin and eosin (Prophet et al. 1992), and finally evaluated under a light microscope by a pathologist at the Laboratory of Pathology of the School of Veterinary Medicine and Animal Science of UFBA.

The morphometric analysis was run on the rumen, and rumen fragments from the ventral and dorsal sac were measured, which measured 1 cm^2/sample containing ruminal papillae with a preserved morphological structure. An ICC50W capture module (Leica®) coupled to a DM500 (Leica®) microscope was used to obtain the images.

The height, width, and length were determined in the medial region of the ruminal papillae according to the methodology proposed by Wang et al. (2009). These measurements were made with the ImageJ® software v2.0 (NIH, USA, 2009).

Statistical analysis

The data were tested by an analysis of variance (ANOVA) in a completely randomised design. To the effect of the treatment, the data were analysed using the PROC MIXED procedure of the SAS software v9.1 (SAS, 2005), according to the model below:

$$Y_{ijk} = \mu + s_i + T_{ej} + (s_i \times T_{eij}) + e_{ijk} \quad (1)$$

where:

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

A 2 × 2 factorial arrangement (whole or ground cottonseed, with or without chitosan) was adopted. The effects of the cottonseed processing form, chitosan addition, and the interaction between these two factors were tested.

The treatment means were obtained by the least-squares means (LSMEANS) procedure, and a significance level of 5% was adopted for all the variables.

RESULTS

It was observed that the cottonseed (whole or ground) by itself or combined with the chitosan in the diet for the feedlot lambs had no influence ($P > 0.05$) on the blood serum metabolites, except for the serum cholesterol, for which there was interaction effect ($P > 0.002$). The cholesterol was lower when the chitosan was combined with the WC (Table 2). No effect of all diets was detected on the creatinine in blood plasma. The protein metabolism was also not affected by the cottonseed (whole or ground) by itself or combined with the chitosan in the diet for the feedlot lambs (Table 2).

The total protein, albumin, and globulin concentrations, whose respective mean values were 0.07, 0.03, and 0.04 g/l, remained within the range described as ideal (Kaneko et al. 2008; Batista et al. 2009). The glucose and triglycerides, whose respective mean values were 0.67 and 0.36 g/l, remained within the ideal range (Kaneko et al. 2008). The hepatic enzymes, such as ALT and AST (1–4.7 µkat/l), remained within the normal range in all the groups evaluated (Kaneko et al. 2008; Batista et al. 2009). Only the mean values of the GGT were in the upper limit in the CG group with chitosan and were slightly higher from the normal range in the other groups (0.3–0.9 µkat/l) (Kaneko et al. 2008).

The morphological evaluation of the hepatic parenchyma revealed steatosis and a periportal inflammation, the intensity of which ranged from moderate to mild (Table 3). The evaluation of the histological fragments of the liver parenchyma revealed the presence of discrete, predominantly periportal microvacuolar steatosis, with greater intensity in the animals that received the ground cottonseed with chitosan. We also observed a mononuclear and multifocal inflammatory infiltrate, composed of lymphocytes and plasma cells, located predominantly in the periportal region (Table 3). However, the inflammation intensity was lower (mild) in the sheep fed chitosan and higher (mild) in the animals fed diets without the chitosan. Multifocal congestion ranging from moderate to mild was also observed in all the analysed groups (Table 3).

In the histological sections of the kidneys, stained with haematoxylin and eosin, a moderate multifocal mononuclear inflammatory process was observed in the interstitium with the predominance of lymphocytes, sometimes plasmocytes and macrophages (Figure 1).

https://doi.org/10.17221/194/2020-VETMED

Table 2. Values of the blood metabolites and liver enzymes in the lambs fed cottonseed by itself or combined with chitosan (136 mg/kg b.w.)

Parameter	Without chitosan		With chitosan		SEM	*P*-value		
	WC	GC	WC	GC		P	Q	P × Q
Urea (g/l)	0.39	0.40	0.40	0.39	1.08	0.93	0.75	0.98
Creatinine (g/l)	0.008	0.008	0.008	0.008	0.01	0.21	0.17	0.46
Total proteins (g/l)	0.07	0.07	0.07	0.07	0.06	0.18	0.33	0.14
Albumin (g/l)	0.03	0.03	0.03	0.03	0.05	0.30	0.44	0.69
Globulin (g/l)	0.04	0.04	0.04	0.04	0.06	0.20	0.43	0.23
Cholesterol (g/l)	0.693	0.689	0.752	0.678	1.17	0.88	0.03	0.002
Triglycerides (g/l)	0.357	0.355	0.365	0.347	1.43	0.31	0.50	0.94
Glucose (g/l)	0.685	0.705	0.688	0.701	0.96	0.95	0.53	0.20
ALT (μkat/l)	0.32	0.30	0.32	0.30	0.01	0.29	0.68	0.72
AST (μkat/l)	1.36	1.35	1.36	1.36	0.04	0.14	0.14	0.13
GGT (μkat/l)	0.92	0.90	0.94	0.88	0.02	0.93	0.06	0.24

Interaction breakdown		
	chitosan	
Cottonseed	0	0.136
	cholesterol	
Whole	63.4^{Bb}	75.2^{Aa}
Ground	70.1^{Aa}	67.8^{Bb}
SEM	2.17	2.17

ALT = alanine-aminotransferase; AST = aspartate-aminotransferase; GC = ground cottonseed; GGT = gamma-glutamyl-transferase; SEM = standard error of the mean; WC = whole cottonseed

Probability value for processing effects (P), chitosan (Q) and interaction between P × Q; Means followed by different letters differ statistically ($P < 0.05$) from each other; Lower and upper case letters correspond to the rows and columns respectively

Table 3. Liver and kidney histopathological alterations in the lambs fed diets containing cottonseed by itself or combined with chitosan (136 mg/kg b.w.)

Histopathology	Without chitosan		With chitosan	
	WC	GC	WC	GC
		liver		
Moderate to mild congestion	+	+	+	+
Microvacuolar steatosis (periportal)	+	+	+	++
Mononuclear infiltrate (periportal)	++	++	+	+
		kidney		
Corticomedullary and spinal cord congestion	+	+	+	+
Cortical tubular necrosis	+	+	+	+
Mononuclear infiltrate	++	++	+	+

(+) present; (++) the inflammatory reaction in the region was more intense and evident; GC = ground cottonseed; WC = whole cottonseed

The inflammatory reaction in the renal pelvis region was more intense and evident in the diets without the chitosan (not in the Tables). Cortical tubular necrosis and mild to moderate corticomedullary and medullary congestion were also observed in all the treatments (Figure 1 and Table 3), which

https://doi.org/10.17221/194/2020-VETMED

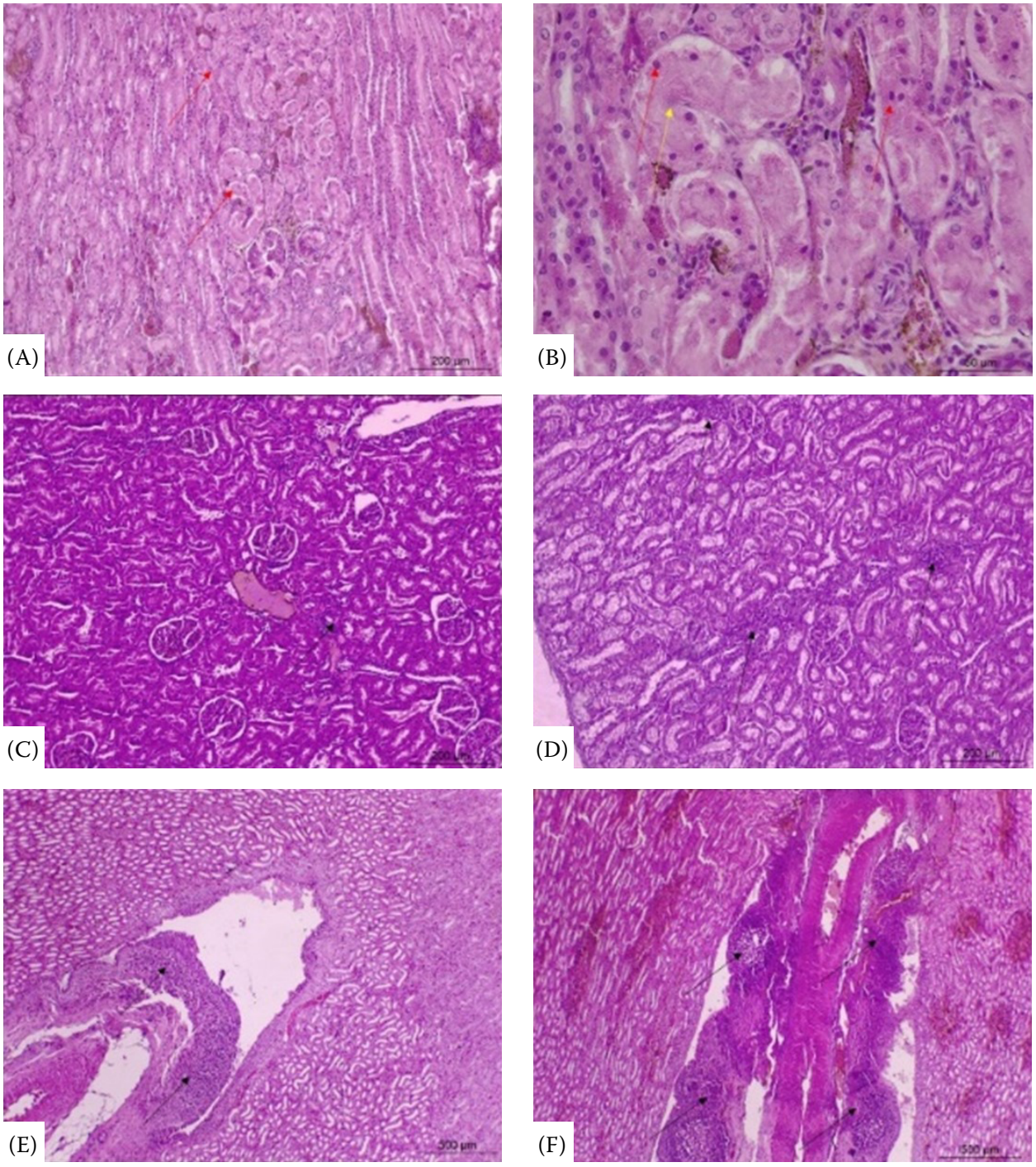

Figure 1. Photomicrographs of the renal parenchyma of the Santa Inês lambs submitted to the cottonseed diet with and without the chitosan addition; haematoxylin-eosin (H&E)
(A and B) Renal tubule coagulation necrosis (red arrows). Note in (B), nuclei in pyknosis (red arrows) and Karyorrhexis (yellow arrow). Obj. × 10, × 4, respectively. (C and E) Group with the chitosan addition. Discreet interstitial inflammatory infiltrates (interstitial nephritis – black arrow) and renal pelvis. Obj. × 10, × 4, respectively. (D and F) The group without the added chitosan. Mild interstitial inflammatory infiltrates (interstitial nephritis – black arrows) and intense inflammatory infiltrates in the renal pelvis. Obj. × 10, × 4, respectively

may have been the result of the presence of the gossypol pigment in the cottonseed.

The diet containing GC with 136 mg/kg b.w. chitosan added promoted the same changes, however,

https://doi.org/10.17221/194/2020-VETMED

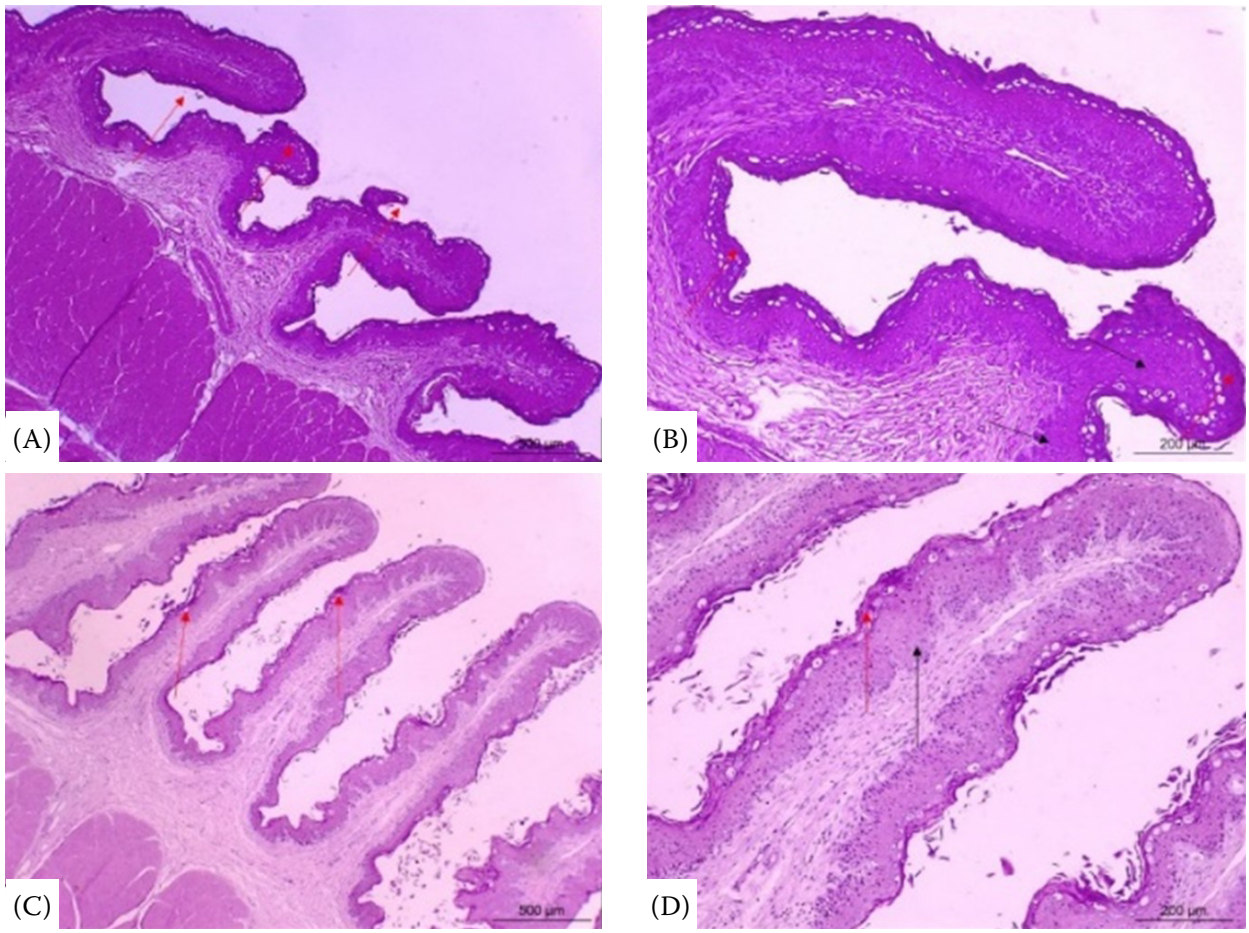

Figure 2. Photomicrographs of the rumen of the Santa Inês lambs submitted to the cottonseed diet with and without the chitosan addition; haematoxylin-eosin (H&E)

(A and B) The group without the added chitosan. Ruminal villi with moderate acanthosis (black arrows) and parakeratosis hyperkeratosis (red arrows). Note vacuolated epithelial cells (hydropic-vacuolar degeneration). Obj. × 4, × 10, respectively. (C and D) The group with the addition of chitosan. Ruminal villi with mild acanthosis (black arrow) and parakeratosis hyperkeratosis (red arrows). Note vacuolated epithelial cells (hydropic-vacuolar degeneration). Obj. × 4, × 10,respectively

Table 4. Histopathological examination of the rumen of the lambs submitted to the experimental diets

Histopathology	Without chitosan		With chitosan	
	WC	GC	WC	GC
Acanthosis	++	++	++	+
Hyperkeratosis	++	++	++	+
Multifocal hydropic degeneration	++	++	++	+

(+) present; (++) the inflammatory reaction in the region was more intense and evident; GC = ground cottonseed; WC = whole cottonseed

Table 5. Mean measurement of the rumen papillae in relation to the treatments

Treatments	Length (mm)	Width (mm)	Area (mm^2)
Whole cottonseed	1.51 ± 0.28	0.44 ± 0.07	6.33 ± 1.24
Ground cottonseed	1.65 ± 0.42	0.38 ± 0.07	7.18 ± 3.04
With chitosan[1]	1.54 ± 0.37	0.45 ± 0.11	7.12 ± 2.27
Without chitosan	1.21 ± 0.41	0.38 ± 0.03	4.83 ± 2.17
P-value[2]	0.360	0.303	0.265

[1]Chitosan used at 0.136 mg/kg b.w.; [2]Probability value

to a lesser extent (Figure 2 and Table 4). Despite the changes observed in the rumen morphological analysis, the morphometric evaluation of the rumen papillae showed no difference between the treatments regarding the length, width, and area (Table 5).

https://doi.org/10.17221/194/2020-VETMED

DISCUSSION

The histomorphometric and blood metabolites are important tools to check the adequacy of the nutrients' absorption, the functionality of vital organs, as well as to observe any possible harm to the animal (Sa et al. 2014). The levels of urea demonstrated that the relationship between the protein and energy in the experimental diets remained balanced because the blood concentration is closely related to the interaction between these factors (Sauberlich et al. 1981; Payne and Payne 1987). Similarly, no effect of diet was detected on the creatinine in the blood plasma. When the urea and creatinine values are within the normal range, it allows one to infer the absence of an impaired renal function in relation to the diets offered in the experimental period (Table 2).

Similar values were reported by Batista et al. (2009) when they tested healthy animals, and they obtained an average of 0.008 g/l for the creatinine and 0.373 g/l for the urea, values considered feasible for animals under Brazilian climate characteristics. Menezes et al. (2012) fed sheep with detoxified castor meal and a similar protein intake and observed no significant influence of the diet on the blood plasma urea levels. However, the values were above those suggested for the species (0.683 g/l). Therefore, it is believed there was an imbalance in the energy/protein ratio due to inadequate energy intake. This hypothesis may be supported by the hepatic gluconeogenesis from the amino acids, which increases the serum ammonia, which becomes urea. Thus, there is an increase in the circulating urea, as well as the energy expenditure for the hepatic metabolism (Huntington and Archibeque 2000).

The evaluations of the glucose and triglycerides are confirmed by other authors, e.g., Rodrigues et al. (2010), who mentioned the importance of evaluating glucose and triglyceride levels, stressing that it is essential to include such parameters in the biochemical analyses not only to measure the energy deficiency, but also to detect lesions in the liver due to excessive mobilisation of the fat reserve.

Enzymes, such as ALT and AST, are used as markers for determining liver injury resulting in increased hepatocyte permeability. This corroborates Gonzalez and Silva (2017), who showed that the activity of these enzymes makes it possible to make inferences about the site and the degree of the cell damage, given that their increase is directly linked to hepatic lesions of diverse origins – liver, skeletal muscle, heart, and kidneys. Hence, the GGT values also confirm the integrity of the hepatic function, considering that, Gonzalez and Silva (2017), stated that only the GGT from the hepatic origin is found in the blood. According to Meira et al. (2009), this enzyme may also be related to other organs such as the pancreas, kidneys, and intestines.

The steatosis and periportal inflammation are not enough to cause clinical or functional organ alterations, but sufficient stimulation could increase the GGT levels, which is related to the bile duct integrity. Franciscato et al. (2006) demonstrated an elevation of the GGT enzyme even in subclinical levels of hepatic dysfunction. However, according to Tennant (1997), its activity is relatively high in the liver of cattle, horses, sheep, and goats.

The diets significantly influenced the serum cholesterol level, however, the values found are within the range considered normal for sheep (0.5–0.76 g/l) (Kaneko et al. 2008). This suggests that there were probably no deficiencies in the energy metabolism, and no excess body fat reserves being mobilised (Homem et al. 2010; Rodrigues et al. 2010). A reduction in the serum cholesterol levels was also observed in the animals that received only the whole cottonseed.

Hepatic steatosis is a reversible degenerative injury characterised by the accumulation of triglycerides in the hepatocyte cytoplasm. The macrovascular form is characterised by the presence of a large fat vacuole, which pushes the nucleus to the cell periphery, in a signet ring. The microvacuolar steatosis is represented by numerous small cytoplasmic vacuoles with a centrally located nucleus; and both forms of steatosis can coexist (Cal et al. 2009).

The processing of cottonseed results in the exposure of the grain kernel, which contains the largest portion of the fat of the seed. Consequently, the high body fat availability over prolonged periods can increase its absorption rate and its metabolism in the liver. This may have contributed to the development of the microvacuolar steatosis in the animals fed ground cottonseed and chitosan when compared to the other diets. The bioavailability of an ether extract may be slower when the whole cottonseed was consumed with the absence of grain disintegration, which would decrease the fat availability. Thus, the protection promoted by the seed coat was important at this point.

https://doi.org/10.17221/194/2020-VETMED

In this context, it can be affirmed that diets with a 50 : 50 forage -to-concentrate ratio, when supplied with cottonseed by themself or combined with chitosan, probably result in the hepatic steatosis of the feedlot lambs. This corroborates the study of Abou-Donia et al. (1970), which pointed out that gossypol, the polyphenolic pigment found in cottonseed, could be harmful to the liver. However, these liver injuries observed in the present study were with slight intensity and did not result in any apparent clinical signs or in macroscopic alterations that impaired the carcass commercialisation.

The inflammatory process observed in the periportal region of the hepatic parenchyma of all the animals may be the result of gossypol toxicity. According to Carvalho et al. (2013), the gossypol leads to an alteration in the reduced glutathione-redox cycle and in the cellular metabolism of the rat liver, stimulating a hepatocyte inflammation as well as degeneration. This redox cycle has the function of minimising cell damage that occurs when it is under oxidative stress (Kumar et al. 2020). This inflammatory process would already be a sufficient stimulus to cause an increase in the GGT, an enzyme related to bile duct integrity.

However, a less intense inflammatory process was found in the individuals fed chitosan. Studies showed that a chitosan treatment in rats with osteoarthritis caused an increase in the interleukin 10 (IL-10) production and the inhibition of nitric oxide synthetase (iNOS), thus characterising the anti-inflammatory properties of this oligosaccharide (Kong et al. 2017).

The results of this study corroborate Jing et al. (1997) and Chou et al. (2015), who observed that chitosan has the ability to reduce inflammation in patients with kidney disease, which may prove the anti-inflammatory character of chitosan. In the evaluation of the rumen histological fragments of the sheep fed whole cottonseed added with chitosan, some important findings were observed: moderate multifocal acanthosis mucosa, moderate multifocal parakeratotic hyperkeratosis and vacuolisation of the epithelial cells (hydropic-vacuolar degeneration) with focally extensive distribution in the region of the rumen villi. This finding might mean this diet facilitates the absorption of volatile fatty acids and improves the availability of energy for the animal compared to other diets.

Acanthosis is characterised by an increase in the cell number in the basal and spinous layers of the epithelium, whereas hyperkeratosis is epithelial hyperplasia, characterised by an increase in the thickness of the corneal layer (Maxie et al. 2007). Both acanthosis and hyperkeratosis are common in cattle. However, acanthosis and hyperkeratosis were not expected in the rumen of the sheep in the present study, since the forage to concentrate ratio was 50 : 50, and since there was 15% cottonseed in the concentrate, which has a high NDF content. However, it is noteworthy, there was a reduction in the intensity of these characteristics in the animals fed chitosan diets. Cottonseed hulls combined with chitosan in the diets for lambs proved to be an alternative feed source. This combination did not affect the blood metabolites, liver histopathological profile, and rumen papillae morphometry in lambs during 90 days in the feedlot.

Acknowledgement

The authors gratefully acknowledge CNPq and CAPES for the scholarships awarded.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abou-Donia MB, Luman CM, Dieckert JW. Metabolite fate of gossypol: The metabolism of ^{14}C-gossypol in rats. Lipids. 1970 Nov;5(11):938-46.

AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 15th ed. Washington, DC: The Association of Official Analytical Chemists; 1990.

Batista MCS, Castro RS, Rego EW, Carvalho FAA, Silva SMMS, Carvalho CCD, Riet-Correa F. Hemograma, proteinograma, ionograma e dosagens bioquimicas e enzimaticas de ovinos acometidos por conidiobolomicose no Nordeste do Brasil [Hematology and blood biochemistry in sheep with conidiobolomycosis in northeastern Brazil]. Pesq Vet Bras. 2009 Jan;29(1):17-24. Portuguese.

Berchielli TT, Pires AV, Oliveira SG. Ruminant nutrition. 1st ed. Jaboticabal: FUNEP; 2006. 583 p.

Cal L, Borteiro C, Benech A, Rodas E, Abreu MN, Cruz JC, Gonzalez Montana JR. Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia. Arq Bras Med Vet Zootec. 2009 Apr;61(2):306-12.

https://doi.org/10.17221/194/2020-VETMED

Carvalho WL, Maioli MA, Mendes LCN, Rozza DB, Mingatto FE. Mecanismos da intoxicacao do figado de rato causada pelo gossipol [Mechanisms of the intoxication of rat liver caused by gossypol]. Pesq Vet Bras. 2013 Mar; 33(3):339-44. Portuguese.

CEI. Basic information on Bahian municipalities. 14th ed. Paraguaçu, Bahia, Brazil: Statistics and Information Center; 1994.

Chou CK, Li YC, Chen SM, Shih YM, Lee JA. Chitosan prevents gentamicin-induced nephrotoxicity via a carbonyl stress-dependent pathway. Biomed Res Int. 2015; 2015:675714.

Costa JB, Oliveira RL, Silva TM, Ayres MC, Estrela-Lima A, Carvalho ST, Ribeiro RD, de Cruz GA. Liver metabolic and histopathological profile in finishing lambs fed licuri (Syagrus coronata(Mart.)Becc.) cake. Trop Anim Health Prod. 2016 Mar;48(3):501-7.

Del Valle TA, Paiva PG, Jesus EF, Almeida GF, Zanferari F, Costa AGBVB, Bueno ICS, Renno FP. Dietary chitosan improves nitrogen use and feed conversion in diets for mid-lactation dairy cows. Livest Sci. 2017 Jul;201:22-9.

Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG. Methods for food analysis – National Institute of Science and Technology of Animal Science, NIST. Viscount of Rio Branco, MG: Supreme; 2012. 214 p.

Franciscato C, Lopes STA, Veiga APM, Martins DB, Emanuelli MP, Oliveira LSS. AST, CK and GGT enzymes serum activities in Crioulo horses. Pesq Agropec Bras. 2006 Oct;41(10):1561-5.

Gadelha IC, Fonseca NB, Oloris SC, Melo MM, Soto-Blanco B. Gossypol toxicity from cottonseed products. Sci World J. 2014 May: [11].

Goiri I, Garcia-Rodriguez A, Orengui LM. Effects of chitosan on in vitro rumen digestion and fermentation of maize silage. Ani Feed Sci Tech. 2009a Apr;148(2-4):276-87.

Goiri I, Garcia-Rodriguez A, Orengui LM. Effects of chitosan on mixed ruminal microorganism fermentation using the rumen simulation technique (Rusitec). Ani Feed Sci Tech. 2009b Apr;152(1-2):92-102.

Goiri I, Orengui LM, Garcia-Rodriguez A. Dose response effects of chitosan on in vitro rumen fermentation mixtures differing in forage to concentrate rations. Ani Feed Sci Tech. 2009c May;151(3-4):215-27.

Goiri I, Oregui LM, Garcia-Rodriguez A. Use of chitosans to modulate ruminal fermentation of a 50 : 50 forage-to-concentrate diet in sheep. J Anim Sci. 2010 Feb;88(2): 749-55.

Gonzalez FHD, Silva SC. Introduction to veterinary clinical biochemistry. 3rd ed. Porto Alegre: Federal University of Rio Grande do Sul Publisher; 2017. 538 p.

Homem Jr AC, Ezequiel JMB, Galati RL, Gonçalves JS, Santos VC, Sato RA. Sunflower grains or protected fat in diets with high concentrate and compensatory gain of feedlot lambs. R Bras Zootec. 2010 Mar;39(3):563-71.

Huntington GB, Archibeque SL. Practical aspects of urea and ammonia metabolism in ruminants. Proceedings of the American Society of Animal Science. Sci J Anim Sci. 2000 Jan;77(E-Suppl): [11].

Jimenez-Ocampo R, Valencia-Salazar S, Pinzon-Diaz CE, Herrera-Torres E, Aguilar-Perez CF, Arango J, Ku-Vera JC. The role of chitosan as a possible agent for enteric methane mitigation in ruminants. Animals (Basel). 2019 Nov 9;9(11):942-54.

Jing SB, Li L, Ji D, Takiguchi Y, Yamaguchi T. Effect of chitosan on renal function in patients with chronic renal failure. J Pharm Pharmacol. 1997 Jul;49(7):721-3.

Kaneko JJ, Harhey IW, Bruss ML. Clinical biochemistry of domestic animal. San Diego: Academic Press; 2008. 928 p.

Khambualai O, Yamauchi K, Tangtaweewipat S, Cheva-Isarakul B. Growth performance and intestinal histology in broiler chickens fed with dietary chitosan. Br Poult Sci. 2009 Sep;50(5):592-7.

Kong Y, Zhang Y, Zhao X, Wang G, Liu Q. Carboxymethylchitosan attenuates inducible nitric oxide synthase and promotes interleukin-10 production in rat chondrocytes. Exp Ther Med. 2017 Dec;14(6):5641-6.

Kumar V, Abbas A, Aster J. Robbins and Cotran pathologic bases of disease. 10th ed. Cambridge: Elsevier; 2020. p. 1392.

Licitra G, Hernandez TM, Van Soest PJ. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Tech. 1996 Mar;57(4):347-58.

Magalhaes TS, Santos EM, de Freitas Jr JE, Santos SA, Dos Santos Pina D, Cirne LGA, Pinto LFB, Mourao GB, Dos Santos Soares FD, Leite LC, Ruiz Alba HD, Tosto MSL, Carvalho GGP. Chitosan and cottonseed processing method association on carcass traits and meat quality of feedlot lambs. PLoS One. 2020 Nov 23;15(11):e0242822.

Maxie MG, Jubb KVF, Kennedy PC, Palmer N. Jubb, Kennedy, and Palmer's pathology of domestic animals. 5th ed. Edinburgh; New York: Elsevier Saunders; 2007. 899 p.

Meira Jr EBS, Rizzo H, Benezi FJ, Gregory L. Influence of age and sex on total serum protein and albumin and aspartato-aminotransferase and gama-glutamiltransferase serum activities on Santa Inês sheep. Braz J Vet Res An Sci. 2009 Oct;46(6):448-54.

Menezes DR, Costa RG, Araujo GGL, Pereira LGR, Oliveira PTL, Silva AEVN, Voltolini TV, Moraes SA. Parametros sanguineos, hepaticos e ruminais de ovinos alimentados com dietas com farelo de mamona destoxificado [Blood,

https://doi.org/10.17221/194/2020-VETMED

liver and ruminal parameters of sheep fed diets with detoxified castor bean meal]. Pesq Agropec Bras. 2012 Jan; 47(1):103-10. Portuguese.

Messana JD, Berchielli TT, Arcuri PB, Reis RA, Pires AV, Malheiros EB. Valor nutritivo do residuo do processamento do caroco de algodao suplementado com levedura e avaliado em bovinos [Nutritive value of cottonseed processing residue supplemented with yeast assessed with cattle]. R Bras Zootec. 2009 Oct;38(10):2031-7. Portuguese.

Mingoti RD, Freitas Jr JE, Gandra JR, Gardinal R, Calomeni GD, Barletta RV, Vendramini THA, Paiva PG, Renno FP. Dose response of chitosan on nutrient digestibility, blood metabolites and lactation performance in Holstein dairy cows. Livest Sci. 2016 Feb;187:35-9.

NRC – National Research Council. Nutrient requirements of small ruminants: Sheep, goats, cervids, and New World camelids. Washington, DC: The National Academies Press; 2007. 362 p.

Payne JM, Payne S. The metabolic profile test. New York: Oxford University Press; 1987. 179 p.

Pereira TL, Fernandes ARM, Oliveira ER, Consolo NRB, Marques OFC, Maciel TP, Pordeus NM, Barbosa LCGS, Buarque VLM, Padilla ARH, Colnago LA, Gandra JR. Serum metabolomic fingerprints of lambs fed chitosan and its association with performance and meat quality traits. Animal. 2020 Sep;14(9):1987-98.

Prophet EM, Mills B, Arrington JB, Sobin LH. Laboratory methods in histotechnology. 1st ed. Washington DC, USA: American Registry of Pathology; 1992. 279 p.

Rodrigues MRC, Rondina D, Araujo AA, Arruda IJ, Silva LM, Nunes-Pinheiro DC, Fernandes AAO. Utilizacao do bagaco de caju (Anacardium Occidentale) naalimentacao de cordeiros do desmame a puberdade: Respostas metabolicas, hormonais e sexuais [Use of cashew bagasse (Anacardium occidentale) in the feeding of lambs from weaning to puberty: Metabolic, hormonal and sexual responses]. Cienc Anim. 2010;20(1):17-26. Portuguese.

Sa HM, Teles TL, Borges I, Macedo Jr GL, Silva SP. Perfil metabolico em ovinos alimentados com inclusoes crescentes da torta do babacu na dieta [Metabolic profile in sheep fed increasing inclusions of babassu pie in the diet]. Vet News. 2014;20(2):48-56. Portuguese.

Sauberlich HE, Skala JH, Dowdy RP. Laboratory tests for the assessment of nutritional status. Boca Raton, FL, USA: CRC Press, Inc.; 1981. 136 p.

Tennant BC. Hepatic function. In: Kaneko JJ, Harvey JW, Bruss ML, editors. Clinical biochemistry of domestic animals. 5th ed. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto: Academic Press; 1997. p. 327-52.

Valadares Filho SC, Silva FF, Rocha Jr VR, Cappelle ER. Brazilian tables of feed composition for cattle. 3rd ed. Viçosa: University Press; 2010. 502 p.

Viana PT, de Carvalho GGP, Viana MCP, de Assis DYC, de Figueiredo MP, Cirne LGA, Figueredo JS, Sousa LS, de Santana Junior HA, Pina DDS, Alba HDR. Effect of high-concentrate diets with calcium lignosulfonate and cottonseed processing method on quantitative traits and non-carcass components of feedlot cull ewes. Arch Anim Breed. 2021 Sep 2;64(2):355-63.

Wang YH, Xu M, Wang FN, Yao JH, Zan LS, Yang FX. Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. Livest Sci. 2009 May;122(1): 48-52.

Weiss WP. Energy prediction equations for ruminant feeds. In: Cornell Nutrition Conference For Feed Manufacturers, Rochester. Rochester: New York State College of Agriculture & Life Sciences, Cornell University; 1999. p. 176-85.

Wencelova M, Varadyova Z, Mihalikova K, Kisidayova S, Jalc D. Evaluating the effects of chitosan, plant oils, and different diets on rumen metabolism and protozoan population in sheep. Turk J Vet Anim Sci. 2014;38(1):26-33.

Received: October 1, 2020
Accepted: June 23, 2021