EFFECT OF COTTONSEED CAKE IN LAMB DIETS IN FEEDLOT

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ABSTRACT

This study evaluated how different concentrations of this coproduct, in the diet, they affect consumption, digestibility, rumen pH, microbial synthesis, performance and carcass characteristics. The lambs were divided into five treatments receiving supplementation of 0, 7, 14, 21 and 28% of DM on intake, nutrient digestibility, ruminal characteristics, animal performance, feed efficiency and carcass characteristics. Five lambs rumen-cannulated were used by a 5x5 Latin square design to evaluate nutritional characteristics, and 40 lambs were used to evaluate animal performance and carcass characteristics. CSC levels did not affect nutrient intake, pH and ruminal ammonia, but the apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, non-fiber carbohydrates and total digestible nutrients decreased linearly. There was no effect of CSC on the excretion of purine derivative, urinary volume, and N microbial flow. Also, CSC levels did not affect average daily gain and dry matter intake or carcass weight, yield percentage, subcutaneous fat and meat pH, but a quadratic effect of CSC on shear strength was observed. It is concluded that despite the negative effects on digestibility, CSC can be included in diets for feedlot lambs up to 28% DM, not affecting intake and animal performance.

Keywords: biofuels, digestibility, feed conversion, intake, oilseeds.

NÍVEIS DE TORTA DE CAROÇO DE ALGODÃO EM DIETAS DE CORDEIROS CONFINADOS

RESUMO

Este estudo avaliou como diferentes concentrações deste coproduto, na dieta, afetam o consumo, a digestibilidade, o pH ruminal, a síntese microbiana, o desempenho e as características da carcaça. Os cordeiros foram divididos em cinco tratamentos recebendo suplementação de 0, 7, 14, 21 e 28% de MS sobre o consumo, a digestibilidade dos nutrientes, as características ruminais, o desempenho animal, a eficiência alimentar e as características da carcaça. Cinco cordeiros canulados no rúmen foram utilizados por um delineamento quadrado latino 5x5 para avaliar as características nutricionais, e 40 cordeiros foram utilizados para avaliar o desempenho animal e as características da carcaça. Os níveis de CSC não afetaram o consumo de nutrientes, o pH e a amônia ruminal, mas a digestibilidade aparente da matéria seca, matéria orgânica, proteína bruta, fibra em detergente neutro, carboidratos não fibrosos e nutrientes digestíveis totais diminuíram linearmente. Não houve efeito do CSC sobre a excreção de derivado de purina, volume urinário e fluxo microbiano de N. Além disso, os níveis de CSC não afetaram o ganho médio diário e a ingestão de matéria seca ou o peso da carcaça, a

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porcentagem de rendimento, a gordura subcutânea e o pH da carne, mas foi observado um efeito quadrático do CSC na resistência ao cisalhamento. Conclui-se que, apesar dos efeitos negativos na digestibilidade, o CSC pode ser incluído em dietas para cordeiros confinados até 28% MS, não afetando a ingestão e o desempenho animal.

Palavras-chave: biocombustíveis, digestibilidade, conversão alimentar, ingestão, oleaginosas.

NIVELES DE TORTA DE SEMILLA DE ALGODÓN EN DIETAS DE CORDEROS DE ENGORDE

RESUMEN

Este estudio evaluó cómo diferentes concentraciones de este coproducto, en la dieta, afectan el consumo, digestibilidad, pH ruminal, síntesis microbiana, desempeño y características de la canal. Los corderos se dividieron en cinco tratamientos que recibieron suplementación de 0, 7, 14, 21 y 28% de MS sobre el consumo, digestibilidad de nutrientes, características ruminales, desempeño animal, eficiencia alimenticia y características de la canal. Se utilizaron cinco corderos canulados en rumen mediante un diseño de cuadrado latino de 5x5 para evaluar las características nutricionales, y 40 corderos se utilizaron para evaluar el desempeño animal y las características de la canal. Los niveles de CSC no afectaron el consumo de nutrientes, el pH y el amoníaco ruminal, pero la digestibilidad aparente de la materia seca, materia orgánica, proteína cruda, fibra detergente neutra, carbohidratos no fibrosos y nutrientes digestibles totales disminuyeron linealmente. No hubo efecto de CSC sobre la excreción de derivado de purina, volumen urinario y flujo microbiano de N. Además, los niveles de CSC no afectaron la ganancia diaria promedio y el consumo de materia seca o el peso de la canal, el porcentaje de rendimiento, la grasa subcutánea y el pH de la carne, pero se observó un efecto cuadrático del CSC sobre la resistencia al corte. Se concluye que a pesar de los efectos negativos sobre la digestibilidad, el CSC puede incluirse en dietas para corderos de engorde hasta un 28% de MS, sin afectar el consumo y el rendimiento animal.

Palabras clave: biocombustibles, digestibilidad, conversión alimenticia, consumo, oleaginosas.

INTRODUCTION

The significant contribution of fossil fuels to climate change is driven by using alternative energy matrices such as biofuels in several countries, whose production is based on oilseeds, a renewable source of biodiesel. The cottonseed used for biodiesel production also produces cottonseed cake (CSC), a by-product used in the diet of ruminants [1].

CSC has a peculiar composition concerning other dietary ingredients because it is a source of protein, energy, and fiber, in addition to its lower cost compared to soybean meal, for example, thus motivating its use in confinement diets. However, CSC should be used in the diet at levels that minimize adverse effects related to its high fiber content and moderate oil content (about 10%) to minimize adverse effects on ruminal fermentation, diet intake, and animal performance [2].

Although there are several published works related to cottonseed by-products in the literature, there is no consensus on the ideal level of inclusion of CSC in confinement diets, especially for feedlot lambs. To achieve this goal, this research evaluated dietary intake, nutrient digestibility, ruminal traits, microbial production, animal performance, and carcass traits of lambs fed levels of cottonseed cake in the diet.

MATERIALS AND METHODS

Location, animals, and experimental diets

Experimental procedures were carried out according to the Animal Experimentation Ethics Committee guidelines of the Federal University of Mato Grosso (protocol number 23108.193858/2017-62). The experiment was carried out at the Farm Experimental at the Federal University of Mato Grosso in Santo Antônio do Leverger, State of Mato Grosso, located at 15°47'5'' South Latitude, 56°04'00'' West Longitude, average altitude of 140 m, Central-South mesoregion of the State of Mato Grosso, microregion of Cuiabá. The climate, according to Köppen classification, is of type Aw, that is, climate tropical, megathermal, characterized by two well-defined seasons: dry (April to Sept) and rainy (Oct to Mar). The animals were identified, vaccinated against clostridiosis, dewormed, and housed in pens equipped with feed and water troughs.

Experimental diets consisted of 40% forage (corn silage) and 60% concentrate, which were formulated to be isonitrogenous (16% of crude protein), according to [3]. The concentrates were ground corn, soybean meal, urea/ammonium sulfate (9:1), mineral mix and cottonseed cake levels (0, 7, 14, 21 and 28% of the diet a dry matter basis), as indicated in Table 1. The cottonseed was supplied by a producing farm in partnership with the Federal University of Mato Grosso.

Table 1. Ingredients and chemical composition of experimental diets with cottonseed cake levels.

Ingredients	CSC levels (g/kg)				
	0	7	14	21	28
Corn silage	400	400	400	400	400
Ground corn	414	372	336	294	252
Soybean meal	162	132	102	72	42
Cottonseed cake	0	72	13.8	210	282
Urea/ammonium sulfate	6.0	6.0	6.0	6.0	6.0
Mineral mix	18	18	18	18	18
	Chemical composition (g/kg)				
DM	659.3	665.0	663.8	668.1	666.3
OM	920.9	920.7	920.8	920.6	920.5
CP	161.7	163.7	164.5	166.5	168.6
CF	33.6	38.1	42.5	47.0	51.6
TC	743.7	736.6	731.4	724.3	717.2
NDF	292.9	320.5	345.7	373.2	400.7
ADF	248.1	271.7	293.1	316.6	340.2
aNDFom(n)	255.0	281.5	305.8	332.2	358.7
NFC	488.7	455.2	425.6	392.0	358.5
TDN	743.2	736.1	724.6	711.0	697.9
iNDF	97.0	115.4	132.2	150.6	168.9

DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fat; TC: total carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; aNDFom(n): neutral detergent fiber analyzed with a heat-stable amylase and corrected for ash and nitrogenous compounds; NFC: non-fiber carbohydrates; TDN: total digestible nutrients; iNDF: indigestible neutral detergent fiber.

Performance study

Forty crossbreed not castrated lambs, with an average initial body weight (BW) of 14.71 ± 2.25 kg, were allocated in a randomized block design to evaluate animal performance, feeding efficiency and carcass characteristics. The lambs were housed in covered pens (4.06 m^2), with two lambs per pen adjacent to pens used for the metabolism trial. The experiment lasted 89 days, of which the initial 15 days were designated for lamb adaptation and the last 74 days for data collection. Lambs were weighed at the start and end of the experiment to determine the body weight gain (g day^{-1}) after shrunk BW (SBW; 14h of solids fasting), as well as at every 28 days (no SBW) for weight gain monitoring. At the end of the experiment, lambs were transported to the commercial abattoir and slaughtered to evaluate carcass characteristics.
The shear force was measured according to the methodology described by [4], while fat thickness was measured from the *Longissimus lumborum* section, collected on the 3rd lumbar vertebra through the pachymeter after 48 hours of freezing. The loin eye area was obtained by drawing directly by overlaying tracing paper, then scanned and calculated with the help of a tablet (MDD model 1812 - DIGICOM) coupled to a computer equipped with the SPLAN software - digitized planimetry system program developed by the Faculty of Agricultural Sciences at the University of São Paulo (UNESP, Botucatu / SP).

Metabolism study

Five lambs rumen-cannulated wethers with an average body weight of 54.8 ± 7.11 kg were allocated in a 5×5 Latin square design. Each experimental period lasted 15 days, designated for the first 10 days for animal adaptation to the diets and the last 5 days were used for data collection. The animals were housed in individual covered pens (4.06 m^2) with concrete floors, feeding troughs and a water fountain. The wethers were weighed at the beginning and end of each experimental period.

The animals were fed twice daily (08h am and 04h pm) as a total mixed ration. Approximately 10% of orts were allowed. Daily orts of the previous day were collected and weighed throughout the experimental period to determine daily dry matter intake (DMI). In addition, digestibility coefficients were estimated from fecal samples collected twice daily (09h am and 03h pm), directly from the rectal ampoule of the wethers during the 11th to 13th days of each experimental period.

On days of fecal samples collection, samples of concentrate, corn silage and orts were also collected and, except for samples of concentrate, the others were pre-dried in a forced-air oven at 60 ± 5°C for 72 hours. Then, all samples were grounded in a Willey mill with a 1 mm sieve. Finally, composite samples by animal and experimental period were prepared for posterior determination of the chemical composition.

For rumen pH and rumen ammonia nitrogen (RAN) concentration measurement, the rumen fluid was collected manually on the 14th day of each experimental period before morning feeding (time zero) and 2, 4, 6 and 8 hours later, which was filtered immediately on cheesecloth and pH was measured by using a digital pH meter (Q400BD- Quimis®). After that, 40 mL of liquid was put in 50 mL cone tubes containing 1 mL of H_2SO_4 (1:1) and frozen for later analysis of RAN.

For the estimation of the urine volume and rumen microbial efficiency, on the 11th, 12th and 13th days of each experimental period, spot urine samples were collected approximately

04h after the morning feeding, and 10 mL was separated and diluted with 40 mL H_2SO_4 (0.036 N) and immediately frozen.

Blood samples were obtained from the ear vein on the 15th day of each experimental period before morning feeding and 1, 2, 3 and 4 hours later, which were used for measurement of glucose levels by using a digital glucometer (brand Accu-Chek®) equipped with fits-test (tape-reagent).

Chemical analysis

Samples of ingredients, orts and feces were analyzed for contents of dry matter (DM, method 967.03; [5]), organic matter (OM), ash (method 942.05; [5]), and crude fat (CF, method 2003.06; [6]). The micro-Kjeldahl procedure determined the crude protein (CP) (method 992.23; [5]; [7]). For neutral detergent fiber (NDF) analysis, samples were submitted to extraction with the neutral detergent solution and heat-stable alpha-amylase [8] and the residue was corrected for ash and nitrogenous compounds as described by [9]. Acid detergent fiber (ANF) was obtained as described by [10].

Total carbohydrates (TC) were calculated as the following equation: TC = 100 - (% CP + % ash + % CF), according to [11]. Due to the presence of urea in the diet, the content of non-fiber carbohydrates (NFC) was calculated as proposed by [12]: $NFC = 100 - [(\%\,CP - \%\,CP\ derived\ from\ urea + \%\,urea)] + \%NDF + \%\,EE + \%\,ash.$

Fecal excretion was estimated using indigestible NDF (iNDF) as an internal marker, which was determined by *in situ* incubation for 144 hours. All samples collected during the study were added [13] into the rumen of a rumen-fisttuled steer (500 kg of body weight) using tissue non-tissue bags (TNT-100 g m^{-2}).

Total digestible nutrients (TDN) were calculated considering the intake and fecal excretion of nutrients using the following equation: $TDN\,(\%) = DTC + DCP + (2.25 \times DEE)$, in which DTC is total digestible carbohydrates; DCP, the digestible crude protein and DEE, the digestible ether extract [14].

The concentration of rumen ammonia nitrogenn in rumen fluid samples was determined by the micro-Kjeldahl system, without acid digestion and after distillation with 2N potassium hydroxide solution.

Urine samples were analyzed to determine the uric acid and creatinine concentration by enzymatic colorimetric method using a commercial kit. In contrast, allantoin, xanthine and hypoxanthine concentrations were obtained according to [15]. Total urine volume (UV) was estimated by the ratio of creatinine excretion per unit of body weight and creatinine concentration in urine as follows: UV = BW × CE / CC, with considered excretion of 10 mg kg^{-1} of body weight [16]. Where: CE is the daily creatinine excretion (mg kg^{-1} BW); BW, body weight, and CC, is creatinine concentration in urine.

Absorbed microbial purines (X, mmol day^{-1}) were estimated from the excretion of purine derivatives (Y, mmolday^{-1}) through the equation proposed by [15] for lambs: $Y = 0.84X + (0.150\ BW0.75e - 0.25x)$. Where: 0.84 = efficiency of exogenous purine absorption, 0.150 $BW^{0.75}$ = excretion of endogenous purine derivatives and -0.25 = rate of substitution "the new synthesis" by endogenous purines.

The intestinal flow of microbial nitrogen compounds (NMIC, g day^{-1}) was estimated from absorbed purines (X mmol day^{-1}), according to the equation of [15]: $NM\,(g/day) = X\,(mmol/day) \times 70/0.83 \times 116 \times 1000$. Considering the digestibility of 0.83 for

microbial purine and the relation of 0.116 purine N: total N and the total N content of purines 70 mg N $mmol^{-1}$. The microbial efficiency was calculated according to [2]: $ME = NMIC\ (g/day) \times 6.25\ /\ ITDN$, where ITDN is the intake of total digestible nutrients.

Statistical analysis

Both studies analyzed data using the MIXED procedures of SAS software (SAS Inst. Inc., Cary, NC, USA). For the metabolism assay, which used a Latin square design, the statistical models used were: $Yijk = \mu + ai + pj + \alpha k + eijk$, in which Y_{ijk} is the dependent variable, μ is the overall mean, a_i is the random effect of the animal, p_j is the random effect of the period, α_k is the fixed effect of the treatment (CSC levels) and e_{ijk} is the residual error. Data from rumen samples collected at each sampling interval were analyzed as repeated measurements using the PROC MIXED of SAS. According to the model: $Yijkl = \mu + ai + pj + \alpha k + \gamma l + (\alpha\gamma)kl + eijkl$, in which γ_l is time (hours), $(\alpha\gamma)_{kl}$ represents the interaction between α and γ and e_{ijkl} is the within units residual error.

For the performance test using a randomized complete block design, the statistical model used was: $Yij = \mu + \alpha i + bj + eij$, in which Y_{ij} is the dependent variable of performance and carcass and meat characteristic, μ is the overall mean, α_i is the fixed effect of the treatment (CSC levels), b_j represents the random effect of the block (body weight) and e_{ijk} is the residual error.

In both experiments, CSC levels analyzed were using linear regression for the first (linear), second (quadratic) and third (cubic) degrees. Results were presented as means and standard error (SE). The p-values ≤0.05 were considered significant, while tendency was considered when $0.05 \leq p \leq 0.15$.

RESULTS

Metabolism study

The CSC used in this study presented 302.0 g kg^{-1} of CP, 100.0 g kg^{-1} of CF and 505.5 g kg^{-1} of NDF as main components, while for corn silage, the chemical composition was 291.1 g kg^{-1} of DM, 62.3 g kg^{-1} of CP, 23.2 g kg^{-1} of CF, 555.5 g kg^{-1} NDF and 341.1 g kg^{-1} NFC. The inclusion of ingredients and the chemical compositions of diets with different levels of CSC are presented in Table 1.

The CSC levels did not affect the intake of DMI and other nutritional components, except for the intake of CF and NDF, which showed a linear increase (p=0.071) and decrease (p=0.088) trend, respectively (Table 2).

CSC levels caused a linear decrease in DM digestibility as well as of OM (p<0.001), CP (p=0.007), NDF (p=0.011), NFC (p<0.001), TC (p<0.001) and TDN (p<0.001), except CF digestibility which was not affected (p>0.05).

Nevertheless, CSC levels did not affect rumen pH values, and no interaction diet × sampling time was observed (Table 3). However, a quadratic reduction in pH values due to sampling time was identified (p=0.003), where the minimum value of 6.12 was estimated at 04 h after morning feeding.

Table 2. Effects of CSC levels on nutrient intake (kg/day) and digestibility (g/kg of DM).

Item	CSC levels					SE	*p*-value	
	0	7	14	21	28		L	Q
Intake, kg/day								
DM	1.078	0.919	1.194	1.022	1.026	0.145	0.998	0.830
OM	0.995	0.845	1.100	0.942	0.943	0.134	0.989	0.831
CP	0.172	0.149	0.198	0.169	0.169	0.024	0.865	0.737
CF	0.029	0.027	0.045	0.044	0.045	0.007	0.071	0.646
NDF	0.317	0.293	0.414	0.411	0.412	0.050	0.088	0.703
NFC	0.510	0.407	0.489	0.360	0.358	0.061	0.103	0.968
TC	0.808	0.685	0.875	0.748	0.746	0.104	0.857	0.850
Digestibility, g/kg DM								
DM	703.5	692.6	613.2	535.9	542.1	34.0	<0.001	0.714
OM	742.0	736.5	656.9	577.2	590.1	31.0	<0.001	0.678
CP	705.0	710.0	649.4	596.8	611.5	34.0	0.007	0.079
CF	627.1	661.9	671.1	838.4	744.4	81.0	0.149	0.747
NDF	461.1	501.5	387.1	344.3	363.4	46.0	0.011	0.823
NFC	923.9	882.6	880.0	781.0	810.4	25.0	<0.001	0.532
TC	758.5	752.1	664.2	567.4	584.9	31.0	<0.001	0.643
TDN	720.1	722.2	654.7	600.7	601.1	29.0	<0.001	0.907

SE: standard error. L: linear effect; Q: quadratic effect. DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fat; NDF: neutral detergent fiber; NFC: Non-fiber carbohydrate; TC: total carbohydrate; TDN: total digestible nutrients.

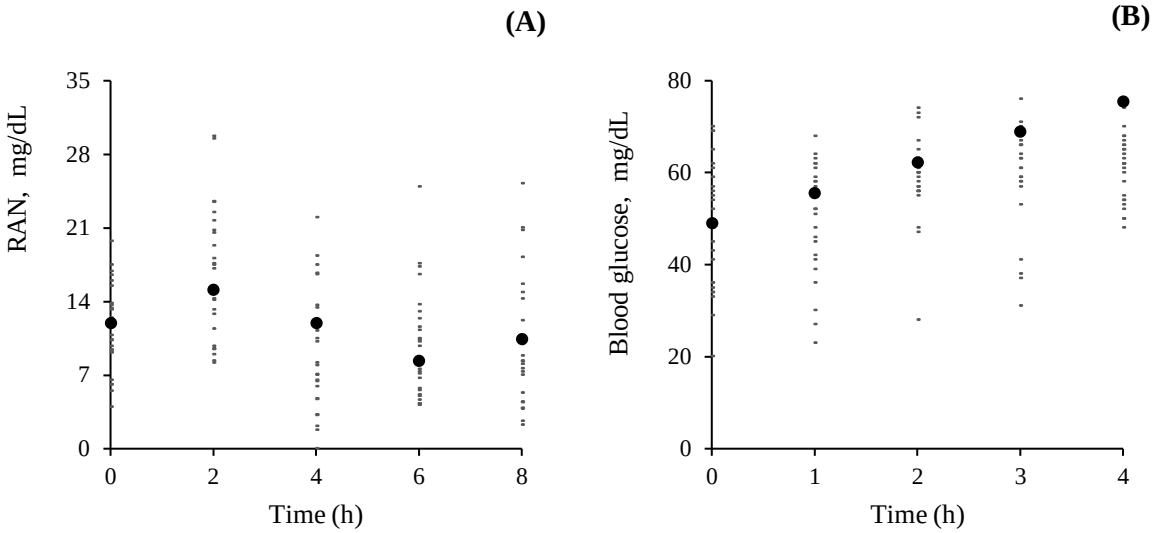
Table 3. Effects of CSC levels and of sampling time on rumen pH.

Item	CSC levels					SE	*p-values*		
	0	7	14	21	28		CSC	Time	CSC*vs*Time
pH	6.26	6.19	6.25	6.32	6.25	0.120	0.960	0.005	0.604
	Time					SE	Regression		
	0	2	4	6	8		L	Q	C
pH	6.57	6.23	6.12	6.20	6.15	0.093	0.001	0.003	0.092

SE: standard error; L: linear effect; Q: quadratic effect; C: cubic effect.

There was no effect of CSC levels (p=0.674) on RAN concentrations, with an average value of 11.53 ± 0.93, as well as no interaction effect (p=0.668) among CSC levels × sampling time, whereas a cubic effect (p<0.001) of sampling time was observed (Figure 1A).

Blood glucose was not affected (p=0.871) by CSC levels, with the observed mean value of 55.66 ± 1.54 mg dL^{-1}, and neither was not observed an interaction effect among CSC levels × sampling time (p=0.112). However, sampling time caused a linear increase in glucose levels (p<0.001) (Figure 1B).



Regression	*p*-value	*p*-value
Linear	*<0.001*	*<0.001*
Quadratic	*0.492*	*0.194*
Cubic	*<0.001*	*0.255*

Figure 1. Rumen ammonia nitrogen (RAN; panel a) and blood glucose (panel b) in relation to time after morning feeding. Linear models: NAR = 11.95 + 4.19(Time) - 1.55(Time)2 + 0.126(Time)3 and Blood glucose = 48.96 + 3.32(Time).

The CSC levels did not affect (p>0.05) urinary volume and total daily excretion of purine derivatives (p>0.05), which presented average values of 1.48 L day^{-1} and 8.12 mmol day^{-1}, respectively. However, there was a trend for a quadratic effect (p=0.062) on rumen microbial N production (Table 4).

Table 4. Effects of CSC levels on urinary volume, urinary excretion of the purine derivatives, and microbial synthesis.

Variable	CSC levels					SE	*p*-value	
	0	7	14	21	28		L	Q
UV	1.31	1.39	1.75	1.33	1.66	0.259	0.392	0.745
PDE	8.76	7.53	8.00	6.45	9.86	2.126	0.863	0.344
MN	6.37	5.47	5.81	4.69	9.23	1.501	0.230	0.062

SE: standard error. L: linear effect, Q: quadratic effect, UV = urinary volume (L/day), PDE = purine derivative excretion (g/day), MN = microbial N (g/day).

Performance study

There were no effects of CSC levels on final body weight (p>0.05), average daily gain (p>0.05), and feed intake (p>0.05); however, a trend for the quadratic effect (p=0.058) of the CSC levels was observed on feed efficiency (Table 5).

Also, there were no effects of CSC levels on carcass weight (p>0.05), dressing percentage (p>0.05), subcutaneous fat (p>0.05) and meat pH (p>0.05), while a trend for linear reduction (p=0.099) was found on the loin eye area and a quadratic (p=0.013) effect on shear strength (Table 6).

Table 5. Effects of CSC levels on animal performance and feed conversion.

Components	CSC levels					SE	*p*-value	
	0	7	14	21	28		L	Q
IBW (kg)	14.87	14.40	15.01	14.38	14.91	0.857	0.979	0.812
FBW (kg)	31.38	30.87	29.26	28.98	31.09	1.582	0.612	0.265
ADG (g)	229	228	198	203	225	14.1	0.420	0.126
DMI (g/d)	892	996	907	917	963	46.56	0.687	0.920
FC (kg/kg)	3.89	4.41	4.59	4.55	4.30	0.229	0.204	0.058

IBW: Initial body weight; FBW: final body weight; ADG: average daily gain; DMI: dry matter intake; FC: feed conversion; SE: standard error; L: linear effect; Q: quadratic effect.

Table 6. Effects of CSC levels on carcass traits

Components	CSC levels					SE	P value	
	0	7	14	21	28		L	Q
CW (kg)	12.61	12.87	11.40	13.89	12.62	0.848	0.680	0.915
DY (%)	40.28	39.28	39.06	40.05	39.00	0.982	0.977	0.631
LEA (cm^2)	12.45	12.59	11.18	10.71	11.10	0.878	0.099	0.659
SBF (mm)	4.46	4.20	3.15	3.06	3.55	0.595	0.115	0.262
pH	5.75	5.82	5.82	6.05	5.85	0.090	0.109	0.316
SF (kgf/cm^2)	4.97	4.64	3.67	3.98	4.30	0.290	0.023	0.013

CW: carcass weight, DY: dressing yield, LEA: loin eye areas, SBF: subcutaneous fact, SF = shear force, SE: standard error; L: linear effect, Q: quadratic effect.

DISCUSSION

CSC is a fascinating ingredient for feeding ruminants, with a peculiar composition. It differs from all other foods as it contains high CP, oil and fiber [17]. It is a strategic ingredient in confinement diets because of cost reduction, associated with lower demand for soybean meal, increasing energy content and providing rumen health [18].

The CSC evaluated in this study presented CP, CF, and NDF contents within the range observed in the literature, which ranged from 23.01 to 36.5%, from 6.2 to 16.3%, and from 20.8 to 51.91%, respectively [1]; [17]; [19]; [20]; [21]. The cotton cultivar can influence the variation in the chemical composition of CSC, the climatic conditions of the region where it is planted, and the industrial oil extraction process. The extraction method is another important factor, considering the laboratory analysis, focusing on CF and NDF, which show more significant variation than the method used for CP analysis.

The replacement of ground corn and soybean meal by CSC caused a significant increase in CF and NDF contents in the diets, which can be expected to promote effects on DMI, considering the adverse effects of high levels of NDF and CF on the DMI. However, in this study, NDF and CF contents, even in CSC diets, were not high enough to decrease DMI by rumen fill or physiological effects [22]. The absence of effects of CSC in the diets on the dry matter was also observed in goats [20], dairy cows [21] and lambs [1]; [17], [23] once CF levels were maintained below the upper limit of 6% [24]. Contrarily, [19] observed that CSC promoted an increase in DMI in dairy cows when replacing 50% of soybean meal in the diet.

However, the increase of CSC levels reduced the digestibility of DM, OM, CP, NDF, TC and TDN, which can be attributed to the harmful effects of lipids and gossypol, mainly unsaturated fatty acids, on rumen microorganisms and their digestive capacity as well as by higher NDF (8.15%) and iNDF (14.90%) content according to CSC levels, [17] found a linear

decrease in the digestibility of nutrients (DM, OM, NDF, and TDN) according to the levels of CSC (zero to 120g kg^{-1} DM) in lambs diets. They were attributed to the higher lignin content in the CSC diets than in the diet control and a possible effect on the rate of digestion passage through the gastrointestinal tract. [25] noted that cottonseed cake also reduced DM digestibility, even though it had a lower EE content than whole cottonseed, but that the inclusion of cottonseed cake increased the EE content and reduced the NFC intake, in addition to increasing the gossypol content of the diets, which can also decrease digestibility. These same authors observed that the inclusion of cottonseed cake also increased the ADF and lignin content of the diet, which also contribute to reduced diet digestibility.

Contrarily, [20] did not find the effect of partially replacing ground corn and soybean meal by CSC levels (zero to 120 g kg^{-1} DM) on nutrient digestibility in goat diets. [26] reported that feed conversion as kg DMI/kg gain in fattening lambs was affected by different protein supplementations.

NDF represents the fraction of slowly and incompletely digested feeds, while iNDF is entirely unavailable in the digestive tract. Thus, the increased concentration of these fractions in feeds or diets has been negatively associated with their nutritive value. Contrarily, the NFC fraction is rapidly and wholly digested in the digestive tract, positively related to the nutritive value of feeds and diets [27]; [28].

The reduction of RAN concentrations at 4 and 6 hours after morning feeding on diets containing CSC compared to control diet (zero CSC) might be associated with lower rumen degradable protein content on CSC as well as a slower digestion rate of its CP, compared to soybean meal [19]. However, considering that CSC diets present lower NFC content and higher NDF and iNDF content than the control diet.

[29] suggested that rumen microbial growth might be impaired by RAN concentration lower than 5 mg dL^{-1}, while [30] suggested 10 mg dL^{-1}. Thus, practically all diets containing CSC can note a RAN lower than 10 mg/dL after feeding, which might have affected the rumen microbial population, considering that digestion in the rumen contributes at least 65% of all digestion in the gastrointestinal tract of ruminants. Ammonia is an essential source of N for many ruminal microorganisms, especially those associated with fiber digestion [10]. The shortage of RAN in the rumen is expected to cause a decrease in total digestion [31], which can be used to explain the decrease in nutrient digestion according to CSC levels.

Although the linear reduction in rumen pH has been observed as a function of time after morning feeding, all pH values were maintained within physiological limits considered adequate for the activity of ruminal microorganisms, particularly fiber digestors, which require rumen pH above 6.0 [10]. [24] suggested that fiber digestion would decline when rumen pH is below 6.2, indicating that this reduction could be responsible for the decrease in fiber digestibility associated with supplementing non-fiber carbohydrates on ruminant diets.

The decrease in pH values as a function of sampling time may be associated with increased microbial activity in the rumen. This fact can be promote an increase in the production of volatile fatty acids (VFA) during nutrient fermentation, buffered by the absorption of the ruminal epithelium, saliva, secretion, and passage into the lower digestive tract to maintain pH values in the physiologically normal range [32]. Considering the pH values observed in the present study, it can be inferred that the buffered mechanisms were adequate to maintain the physiological conditions in the rumen, or the VFA production rate was not so high as to promote a drop in pH values.

CSC levels did not affect purine derivative excretion and rumen microbial protein synthesis. Indicates that RAN concentrations and other ruminal characteristics were not limiting in lambs fed CSC diets to sustain microbial growth as in the control diet. Dietary carbohydrates are the primary energy source for the maintenance and growth of rumen microorganisms. At the same time, lipids are not fermented in the rumen and thus are not an energetic source for

the rumen microbial population [33]. Thus, replacing carbohydrates with lipids is expected to decrease microbial protein synthesis while increasing microbial efficiency, but it was not observed in this study.

Also, lipid supplementations have been associated with adverse effects on rumen protozoa, which theoretically would reduce the bacteria predation by protozoa and, thus, might increase microbial efficiency [34]. Likewise, lipids supplementation for ruminants is cited for harming rumen fermentation by preventing microbial attachment in forage particles, negatively affecting fiber digestion, and by toxic effects of polyunsaturated fatty acids on the rumen microbial population.

The increasing levels of CSC did not promote changes in lamb blood glucose, indicating that glucose precursors in blood were unaffected by CSC levels. However, the content of the total carbohydrates, particularly the non-fiber carbohydrates, the main precursors of propionic acid in the rumen, was reduced by the increment of CSC. However, blood glucose concentration as a function of time was increased, probability due to carbohydrate fermentation in the rumen, which increased the glucose production precursors in the rumen (propionic acid).

Although CSC levels decreased nutrient digestibility, animal performance (final body weight and ADG) was not affected by CSC levels, which the lack of effects of CSC on dry matter intake can explain. Since DMI is the main factor affecting animal performance [22], similar to this study, other authors also found no effect of dietary carbohydrates on animal performance [17]; [20]; [21].

However, according to digestibility data, it would expect a negative effect of CSC levels on ADG. However, it should be emphasized that the DMI expressed as a percentage of BW is completely different among the two trials, where in the metabolism study, the average DMI was 1.9%. In contrast, in the performance trial, it was 4.15%, which promoted some effect on the rumen passage rate (kp). Thus, for the kp estimation of forage and concentrate, using the equations suggested by [35], a kp value of 3.01 and 3.81 for forage and concentrated, respectively, on the digestibility trial, and 3.93 and 5.25, respectively, on the performance study.

Using the kp estimative to calculate the rumen retention time (RRT; 1 kp^{-1}), an RRT of 33.20 and 25.20 h for forage and concentrate for animals used in the digestibility trial could be estimated, and 25.40 and 19.03 h for animals kept on the performance trial. So, it could be inferred that on animals at the performance trial, the negative effects of CSC in the rumen environment can be associate reduce by a faster digesta passage rate compared to animals used in the digestibility trial.

The trend for the quadratic effect of CSC (Table 6) in the loin eye area can be associated with lower numeric values observed for ADG in animals fed with CSC diets, especially with 14 and 21% of CSC, compared to the control diet. However, no statistical difference was found since low ADG can affect the loin eye area due to a lower muscle deposition.

The quadratic effect of CSC levels on shear strength can be associated with higher lipid levels in diets containing CSC and its effects on muscle composition. Thus, CSC supplementation improves meat tenderness, associated with its taste, the primary factor affecting the lamb's meat quality.

CONCLUSION

Despite causing a reduction in digestibility of dietary nutrients, cottonseed cake may be included in up to 28% of dry matter diets for feedlot lambs since it does not cause negative effects on nutrient intake, rumen microbial protein synthesis, animal performance and carcass traits.

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