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Research article

Performance, carcass yield and economic viability of sheep fed diets containing by-product of cotton agribusiness

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Abstract: The aim of this study was to evaluate the effect of replacing soybean meal with cottonseed cake in the diet offered to Santa Inês sheep on their performance, carcass yield and economic viability. Thirty-two uncastrated male sheep (27.48 ± 4.96 kg and 12 months old) were distributed in a completely randomized design, with 4 treatments (replacement of 0, 14, 28 and 42% of soybean meal with cottonseed cake in dry matter basis) and 8 replications. The inclusion levels of cottonseed cake in their diet provided a reduction in performance, thoracic perimeter, body capacity (measured *in vivo*), slaughter body weight, hot and cold carcass weights, external length, rump perimeter, thoracic perimeter, carcass compactness index, and leg compactness index ($p < 0.05$). The weights of the left half carcass, shoulder, neck, loin, and leg were reduced with increasing levels of cottonseed cake in their diet ($p < 0.05$). The quadratic effect was observed for the rib weight and the yield ($p < 0.05$). Diets with cottonseed cake had lower total feed costs/animal, revenue, gross margin, and leveling price, and had a higher benefit/cost. Under experimental conditions, despite reducing the feed costs and presenting an improvement in the cost ratio benefit, the use of levels up to 42% of cottonseed cake in sheep diets is not recommended, as it negatively affects the animal performance, carcass weights, and commercial cuts.

Keywords: commercial cuts; confinement; co-product; cottonseed cake; termination

1. Introduction

The intensification of biofuel production directly reflects the rise in prices of agricultural *commodities*, such as corn and soybeans. As a result, the use of alternative foods to compose the diets of small ruminants aims to offer the necessary nutrients to allow the animal to express its productivity, reduce feed costs and, in this way, make the activity sustainable [1].

Reducing the number of residues generated by agroindustry is a global trend aimed at mitigating the environmental impact. A large volume of agro-industrial residuals is annually produced in Brazil from the processing of oilseeds for biodiesel production, such as cotton (*Gossypium hirsutum*) [2]. Given the availability of these byproducts, research has been carried out on the use of this material in animal feed, with the strategy of reducing the environmental impacts of livestock production, in addition to boosting the industry's advancement towards models of a circular economy and sustainable production [3].

The industry extracts approximately 12.5% of linter, 15.2% of oil, 46.7% of cottonseed cake, 20.7% of husk, and 4.9% of processing residues from cottonseed. The oil is extracted through the hydraulic pressing of cottonseed, which can be followed by washing with chemical extractors to further extract the oil; the residue from this process is called cottonseed cake [4]. Cottonseed cake has a high nutritional value, with 230.1–360.0 g/kg of crude protein [2,5] and 710.80 g/kg of total digestible nutrients [6]; however, its composition varies depending on the efficiency of oil extraction [7]. This use can reduce the share of soybean meal in animal feed [5], thus reducing the costs of purchasing concentrates and improving the productive performance of cattle, as observed by Arcanjo et al. [8].

A characteristic that differentiates ruminants is the ability to efficiently transform fiber-rich foods with high levels of structural carbohydrates into products of animal origin [9]. Due to this characteristic, ruminants occupy an important niche in modern agriculture. Thus, although cottonseed cake has a high nutritional value, it also contains the anti-nutritional compound gossypol [10]. However, the ruminal environment promotes the binding of free gossypol to proteins, making it physiologically inactive [11]. Despite this tolerance to gossypol by ruminants, few studies have been carried out on the use of this food in the composition of diets for small ruminants; it has been observed that that cottonseed cake showed good acceptability by the animals when the levels of inclusion of cottonseed cake in the replacement of soybean meal in diets offered to goats and sheep were measured, thus providing similar weight gains between treatments [4,12].

With its high biological value, sheep meat is an excellent source of protein and is increasingly present in human food. Confinement systems have become more frequent, due to the possibility of developing nutritional management, thus guaranteeing meat production throughout the year [13]. To meet the growing demand for quality sheep meat, the sheep farming production chain has undergone changes in order to produce meat cuts with a satisfactory quality, thus generating a standardized product that can meet the demands of the consumer market. Therefore, the quantitative and qualitative characteristics of the carcasses are of a fundamental importance, as they are directly related to the final product [14].

The evaluation of the carcass yield is of great importance to determine the performance of the animal during its development, in addition to being a determining factor for the total cost of meat that will be passed on to the consumer [1]; this is a relevant reason to awaken the interest of sheep farmers into seeking alternative feeds of a low cost that meets the nutritional requirements of animals, with a view to a better final product [1,15]. A good diet offered to animals can contribute to improving the carcass characteristics, since animal nutrition is directly related to the quality of the final product, which is meat [14].

It is known that diet considerably affects the costs of a production system, representing between 60–70% of the sheep meat production costs [16]. Cottonseed cake has been used as follows: to ruminants feed as a protein supplement, due to the levels of crude protein and undegraded protein in the rumen; as a source of protected fat; and it has the potential to be used as roughage in forage-free diets due to its fiber levels [8]. When evaluating the intestinal digestibility of protein from biodiesel by-products in the forms of meal and cake, Couto et al. [17]found that, of the by-products evaluated, cottonseed cake had the highest intestinal digestibility coefficients, contributing to the greater availability of amino acids for absorption in the small intestine. When including sources of natural lipids protected from rumen degradation in the diet of cattle, Barducci et al. [18], they found that the use of cottonseed cake as a lipid source provided greater hot carcass weight, visceral fat, and increased the amount of unsaturated fatty acids in the meat.

When testing different levels of cottonseed cake (0, 33, 66, 100%) as a replacement for soybean meal in lamb diets, Silva et al. [12] observed an increased dry matter intake as the levels of cottonseed cake increased in the diets. While testing levels of cottonseed cake (0, 33, 66, 100%) replacing soybean meal in goat diets, Assis et al. [5] observed that the highest intake of dry matter and crude protein occurred with the inclusion of 33% of cottonseed cake in diets. When testing cottonseed cake as a source of fiber to replace corn silage in cattle diets, Arcanjo et al. [19,20] found that despite the lower dry matter intake shown by animals that received cottonseed cake, they showed an increased weight gain and hot carcass yield in relation to animals that received a corn silage-based diet. Therefore, looking for alternative foods to compose the sheep diet is essential, not only to maximize performance, but to improve the quantitative and qualitative characteristics of the carcasses [21]. Thus, more studies are needed to elucidate the best level of inclusion of cottonseed cake in the diet of small ruminants and its impact on nutrient intake, performance, and carcass characteristics.

We hypothesize that the use of cottonseed cake as a replacement of soybean meal can improve the dry matter intake, weight gain, and carcass yield of sheep, thus reducing feed costs. Therefore, the aim of this study is to evaluate the performance, carcass characteristics of sheep, and the economic viability of diets containing different levels of cottonseed cake as a replacement for soybean meal.

2. Materials and methods

2.1. Study site

The experiment was carried out at the Animal Science Sector of the Universidade Federal do Mato Grosso (UFMT), Rondonópolis, Mato Grosso, Brazil, (16º 28’ S, 50º 34' W). The climate was Aw (Tropical climate with dry winter) according to the Köppen classification [22], which is tropical with well-defined dry and rainy seasons, hot and humid summers, and cold and dry winters. The relative humidity was 60% and the average rainfall was 1240 mm/year. The annual average temperature was 27.5 °C, ranging between 17 °C and 38 °C for the minimum and maximum averages, respectively.

2.2. Animals and experimental diets

Thirty-two intact Santa Inês rams (12 months old and 27.48 ± 4.96 kg of body weight) were distributed into individual stalls (2.50 m^2) equipped with feeding, drinking fountains, and salt troughs. The experimental design was a completely randomized design, with four treatments (diets) and eight animals per treatment.

This study was approved and certified by the Ethics Committee on the Use of Animals (CEUA) of the UFMT (Opinion no. 23108.046399/13-4).

The experiment lasted 60 days, preceded by 7 days of animal adaptation to the experimental diets. At the beginning of the adaptation period, the animals were identified, weighed, treated against endo- and ectoparasites through the application of an oral solution (200 µg/kg body weight; Ivomec, Merial, Campinas, Brazil), and vaccinated against clostridial disease (2 mL; Excell 10, Vencofarma, Villa Verde Agronegocios, Ribeirão Preto, Brazil). The animals were randomly allocated to the stalls as previously identified according to the treatments.

The treatments consisted of increasing levels of cottonseed cake (0, 14, 28, and 42% on a dry matter basis), partially replacing the soybean meal of concentrated feed. The experimental diets were formulated with a roughage: concentrate ratio of 50:50 on a dry matter basis (Table 1) and composed of corn silage, ground corn, soybean meal, cottonseed cake, and urea (Table 2), formulated for late maturing sheep according to the National Research Council (NRC) [23] requirements to contain 14% crude protein, prepared for sheep with a 25 kg body weight and an estimated daily weight gain of 200 g.

Since the concentration of crude protein in cottonseed cake is lower than the concentration of crude protein in soybean meal, urea was used in the composition of the diets so that the diets were isonitrogenous. Therefore, as the levels of cottonseed cake increased, the levels of urea in the diets also increased.

Table 1. Chemical composition of ingredients offered in the experimental diets.

Items	Ingredients			
	Corn silage	Ground corn	Soybean meal	Cottonseed cake
Dry matter (% fresh matter)	29.7	90.8	91.2	94.0
Mineral matter (% DM)	4.1	1.2	7.1	4.2
Crude protein (% DM)	5.4	8.2	45.0	30.2
Ether extract (% DM)	2.9	5.4	1.2	10.0
Acid detergent fiber (% DM)	25.4	3.0	9.6	38.5
NDFap (% DM)	45.1	10.8	10.2	47.3
iNDF (% DM)	21.7	1.5	1.6	27.0
Total carbohydrate (% DM)	87.6	85.2	46.7	55.5
Non-fibrous carbohydrates (% DM)	42.5	74.3	36.4	8.2
NDIN (%Total nitrogen)	16.7	10.2	3.2	17.1
ADIN (%Total nitrogen)	7.5	4.0	1.8	10.2

NDFap = Neutral detergent fiber corrected for ash and protein; iNDF = Indigestible neutral detergent fiber; NIDN = Neutral detergent insoluble nitrogen; ADIN = Acid detergent insoluble nitrogen; DM = Dry matter.

Table 2. Ingredient proportion and chemical composition (% dry matter) of the experimental diets.

Ingredients	Cottonseed cake (% DM)			
	0	14	28	42
Corn silage	50.0	50.0	50.0	50.0
Ground corn	29.0	28.8	28.4	28.0
Soybean meal	21.0	14.0	7.0	0.0
Cottonseed cake	0.0	7.0	14.0	21.0
Urea	0.0	0.2	0.6	1.0
Chemical composition				
Dry matter	60.34	60.56	60.79	61.02
Mineral matter	3.89	3.69	3.49	3.29
Crude protein	14.53	14.04	14.09	14.15
Ether extract	3.25	3.86	4.45	5.04
Acid detergent fiber	15.57	17.59	19.60	21.61
NDFap	27.84	30.42	32.97	35.52
iNDF	11.63	13.41	15.19	16.96
Hemicellulose	12.27	12.83	13.37	13.91
Total carbohydrates	87.33	78.78	79.06	79.34
Non-fibrous carbohydrates	50.48	48.36	46.09	43.81
NDIN (%Total nitrogen)	11.98	12.93	13.87	14.80
ADIN (%Total nitrogen)	5.28	5.86	6.43	7.01
Total digestible nutrients	65.57	64.03	60.78	58.34

NDFap = Neutral detergent fiber corrected for ash and protein; iNDF = Indigestible neutral detergent fiber; NIDN = Neutral detergent insoluble nitrogen; ADIN = Acid detergent insoluble nitrogen; DM = Dry matter. Mineral mixture provided *ad libitum* - Guaranteed levels (g/kg): 17.7 calcium, 8 phosphorus, 0.4 sodium, 2 sulfur, 5.5 copper, 6 iodine, 12 manganese, 0.15 selenium, 30 zinc and 8 fluoride (maximum).

2.3. Dry matter intake and productive performance

The feed was provided at 0730 hour and 1530 hour, and water and mineral salt (Coperphós® sheep) were offered *ad libitum*. The amount of feed offered was calculated according to the intake of the previous day, not allowing leftovers higher than 20% of the offered quantity. The daily dry-matter intake (DMI; in g/day; % body weight and g/kg body weight) was obtained by the difference between the total dry matter (DM) of the consumed diet and the total DM present in the leftovers.

Animals were weighed at the start and at the end of the experimental period after a solid-food deprivation period of 16-h (with water access), and the final body weight (FBW), total weight gain (TWG), and average daily gain (ADG) were determined. The feed conversion (FC) was calculated as the ratio between the DMI and the average daily weight gain of the animals during the entire experimental period.

2.4. Chemical analyses

Samples of ingredients, the diets offered, and leftovers were collected for chemical analyses. Samples were pre-dried in a forced-air oven (55 °C for 72 h) and ground to a 1-mm particle size (Wiley

Mill, Marconi, Arthur H. Thomas, PA, USA). Chemical analyses were performed using the procedures described by the Association of Analytical Chemists [24] of dry matter (method 967.03), ashes (method 942.05), crude protein (CP, method 981.10), ether extract (EE, method 920.29), and acid detergent fiber (ADF, method 973.18). The neutral detergent fiber (NDF) (treated using heat-stable alpha-amylase without sodium sulfite) was determined using the methods of Van Soest et al. [25]. The neutral detergent fiber corrected for ash and protein (NDFap) was determined using the methodology described by Licitra et al. [26] and Mertens [27]. The *in situ* NDF (iNDF) was obtained through the *in situ* incubation for 240-h [28], followed by an NDF analysis.

The fractions of hemicellulose (HEM), total carbohydrates (TC; [29]), non-fibrous carbohydrates (NFC; [30]), and total digestible nutrient (TDN; [31]) were estimated by the following equations:

$$\mathrm{HEM} = \mathrm{NDF} - \mathrm{ADF} \quad (1)$$

$$\mathrm{TC} = 100 - (\%\mathrm{CP} + \%\mathrm{Ash} + \%\mathrm{EE}) \quad (2)$$

$$\mathrm{NFC} = 100 - ((\%\mathrm{CP} - \%\mathrm{CP\ derived\ from\ urea} + \%\mathrm{urea}) + \%\mathrm{NDF} + \%\mathrm{EE} + \%\mathrm{Ash}) \quad (3)$$

$$\mathrm{TDN} = 88.9 - (0.779 \times \%\mathrm{ADF}) \quad (4)$$

The acid detergent insoluble nitrogen (ADIN) was obtained by treating the sample with an acid detergent solution for 1-h at 100 °C; subsequently, the nitrogen content in each residue [32], namely the neutral detergent insoluble nitrogen (NDIN) content, was determined as reported by Licitra et al. [26].

2.5. In vivo measurements

The *in vivo* measurements were carried out by a single evaluator, always on the right side of the animal, using a measuring tape and wooden square and the results were expressed in cm, taking the following measurements: body length, anterior height, posterior height, femur height, leg height, thoracic perimeter, rump width, and chest width. The body condition score (BCS) of the animals was assessed by palpation of the loin area, following the methodology described by Osório and Osório [33].

A five-point scale was adopted, with subdivisions of 0.5, with a value of 1 being excessively thin and devoid of fat coverage and 5 being excessively fat. The Frame Size Index (FSI) was obtained according to Souza Júnior et al. [34].

2.6. Slaughter

At the end of 60 experimental days, the animals were slaughtered. Before slaughter, the animals were deprived of solid food, in accordance with animal welfare standards. After this period, the animals were weighed to determine the slaughter body weight (SBW) and the fasting weight loss. The animals were stunned by cerebral concussion with a gun (Ctrade®, Tec 10 PP) with a penetrating captive dart, and immediately slaughtered by sectioning the carotid arteries and jugular veins, in accordance with the Regulations of the Health and Industrial Inspection Service of Products of Animal Origin [35].

With the information regarding the SBW and body length, the body capacity (BC; in kg/cm) was estimated according to Osório and Osório [33].

2.7. Carcass characteristics

The carcasses were weighed to obtain the hot carcass weight (HCW) and the hot carcass yield (HCY). The carcasses were transferred to the cold room (4 °C for 24-h), where they were suspended by hooks, maintaining a distance of 17 cm between the tarsal and metatarsal joints. After this period, the carcasses were weighed to obtain the cold carcass weight (CCW), cold carcass yield (CCY), and cooling losses (CL). The kidneys and pelvic-renal fat were removed from the chilled carcasses, weighed, and the values obtained were subtracted from the HCW and the HCY [36].

After the refrigeration period, the following were measured (in cm): external carcass length (ECL), internal carcass length (ICL), leg length, leg perimeter, rump width, rump perimeter, chest width, chest depth, and chest perimeter [33]. The measurements were used to calculate the carcass compactness index (CCI, kg/cm) and the leg compactness index (LCI, cm).

Conformation, carcass finishing and pelvic-renal fat were subjectively evaluated. Conformation was evaluated on a five-point scale, with a value of 1 for a very poor conformation and 5 for an excellent conformation. For the evaluation of finishing, a value of 1 was assigned for an excessively lean carcass devoid of fat coverage and 5 for an excessively fat carcass. For pelvic-renal fat, grades were assigned from 1 (little fat) to 3 (a lot of fat) [36].

2.8. Commercial cuts, fat thickness and rib eye area

The carcasses were longitudinally sectioned and the left half carcass was weighed (LHCW) and divided into five commercial cuts: shoulder, neck, rib, loin, and leg. The cuts were weighed separately and the yield of each cut was calculated in relation to the LHCW. After obtaining the cuts, the legs were identified, stored in plastic bags, and frozen for later dissection.

A transverse incision between the 12th and 13th ribs of the left half carcass was made to expose the *Longissimus dorsi* muscle to measure the minimum (C) and maximum (GR) covering fat thickness on the muscle. In this same area, the maximum width (A) and maximum depth (B) of the muscle were measured to determine the rib eye area (REA) [37]. Additionally, the loins were subjectively evaluated and classified according to color (1-light pink; 2-pink; 3-light red; 4-red; 5-dark red); texture (1-very thick; 2-thick; 3-medium; 4-fine; 5-very fine) and marbling (1-non existent; 2-little; 3-medium; 4-very; 5-excessive) [33].

2.9. Muscularity index

The legs were thawed (8 °C for 24-h) and subsequently dissected. The tissues were separated into bone, muscle, fat (subcutaneous and intermuscular), and other tissues (composed of tendons, glands, nerves and blood vessels), which were individually weighed and expressed as a percentage, in relation to the leg weight. The muscle:bone and muscle:fat ratios were determined according to Osório and Osório [33].

The five main muscles that involve the femur (*Biceps femoris*, *Semimembranosus*, *Semitendinosus*, *Quadriceps femoris* and *Adductor*) were individually weighed to determine the leg muscularity index (LMI; g/cm). The femur bone was weighed and its length (in cm) was measured with a measuring tape. The LMI was determined according to Urbano et al. [38].

2.10. Economic analysis

The economic analysis was carried out to verify the feasibility of replacing soybean meal with cottonseed cake in diets. Therefore, the fixed and operational costs related to labor, health, and miscellaneous expenses were not considered. Market prices applied during the execution period of the experiment were considered for the ingredients of the diet (Table 3).

Table 3. Description of the costs of experimental diets (USD$/kg of natural matter), in absolute values, of the confinement of sheep fed diets containing cottonseed cake replacing soybean meal.

Items	Cost	Cottonseed cake (% DM)			
		0	14	28	42
Corn silage	0.08	3.89	3.89	3.89	3.89
Ground corn	0.16	9.50	9.43	9.30	9.17
Soybean meal	0.48	20.20	13.47	6.73	0.0
Cottonseed cake	0.18	0.0	2.46	4.93	7.39
Urea	1.23	0.0	0.49	1.47	2.46
Total cost	---	33.59	29.74	26.32	22.91

The variables related to the economic cost of the experiment were analyzed according to Pinho et al. [39]. With the cost of each feed, the amount of feed ingested, the dry matter intake of each animal, and the feeding costs (FC) were calculated as follows:

$$\mathrm{FC} = [(\mathrm{iDMI} \times \mathrm{Confinement\ period}) \times \mathrm{CED}] \quad (5)$$

where: iDMI = individual dry matter intake (g/day); Confinement period = 60 days; CED = cost of the experimental diet, in USD$/kg.

Revenues were calculated by multiplying the carcass weights of each animal (kg) by the market price paid per kg of carcass (USD$ 4.28). The assessment of the gross margin was evaluated through the difference between the revenue values (animal sales) and the expense values (feed costs). The benefit/cost ratio (B/C; >1, indicates economic viability) was used to compare the values of the carcass weight multiplied by the carcass price in kg, in relation to the feed cost.

The leveling price (USD$/kg) was calculated by dividing the feed costs by the price per kg of the meat, aiming to adopt a more practical approach to the gross margin from the use of the by-product from the cottonseed agroindustry in the diet of finishing sheep.

2.11. Statistical analyses

The results obtained were analyzed using PROC GLM from the Statistical Analysis System Software (SAS 9.1; [40]) and subjected to an analysis of variance and regression at a 5% probability. The initial bodyweight was used in the statistical model as a covariate when significant. Moreover, the subjective evaluations also included the Levene test to verify the variance homogeneity using the "HOVTEST" command. P-values less than 0.05 were considered significant. The following statistical model was used:

$$\mathrm{Yij} = \mu + \mathrm{TAi} + \mathrm{Aj} + \mathrm{eijk} \quad (6)$$

where: Yij = observation referring the animal j in the content of soybean meal substitution by cottonseed cake; μ = overall average; TAi = effect of the content of soybean meal substitution by cottonseed cake i (i = 1, 2, 3 and 4); Aj = effect of the animal j (j = 1, 2, 3, 4, 5, 6, 7, 8, ..., 32); eijk = random error at each observation.

3. Results

3.1. Performance and in vivo measurements

There was no effect of diets on dry matter intake and feed conversion ($p > 0.05$). A reduction in the final weight ($p = 0.011$), total weight gain ($p = 0.010$), and average daily gain ($p = 0.010$) were observed in sheep as the levels of cottonseed cake in the diets were increased (Table 4). In relation to the *in vivo* measurements, only the thoracic perimeter ($p = 0.022$) and body capacity ($p = 0.019$) of the sheep were influenced by the diets, which were reduced with the increase in cottonseed cake in the composition of the diets tested. There was no effect of the diets on the BCS, body length, back height, hind leg height, femur height, leg height, rump width, chest width, and FSI ($p > 0.05$) (Table 4).

Table 4. Performance and *in vivo* measurements of Santa Ines rams fed diets containing levels of cottonseed cake.

Items	Cottonseed cake (% DM)				SEM	P-Value	
	0	14	28	42		Linear	Quadratic
Initial BW, kg	27.64	27.61	27.34	27.35	-	-	-
Dry matter intake, g/day	1391.0	1247.7	1244.1	1163.8	114.03	0.206	0.792
Dry matter intake, % BW	4.07	3.74	3.74	3.69	4.29	0.434	0.696
Dry matter intake, g/kg $BW^{0.75}$	98.18	89.62	89.59	87.20	9.51	0.360	0.717
Final BW[1], kg	43.50	41.18	40.28	39.19	2.39	0.011	0.567
Total weight gain[2], kg	15.86	13.56	12.94	11.84	1.00	0.010	0.561
Average daily gain[3], g	0.264	0.226	0.216	0.197	0.02	0.010	0.561
Feed conversion	11.40	10.87	10.40	10.17	2.14	0.625	0.525
In vivo measurements							
Body condition score	2.75	3.00	2.63	2.75	0.198	0.609	0.620
Body length, cm	93.19	91.00	91.56	89.75	1.961	0.128	0.892
Anterior height, cm	65.44	63.06	65.19	64.25	1.233	0.808	0.412
Posterior height, cm	67.25	64.94	66.75	66.13	1.235	0.784	0.345
Femur height, cm	21.38	21.94	21.44	21.69	0.482	0.778	0.720
Leg height, cm	60.13	58.56	58.50	58.06	1.146	0.140	0.523
Thoracic perimeter[4], cm	80.69	78.50	78.63	77.75	1.728	0.022	0.382
Rump width, cm	11.19	11.00	10.81	11.06	0.296	0.570	0.205
Chest width, cm	16.00	15.69	15.44	15.00	0.486	0.053	0.848
Body capacity[5], kg/cm	0.455	0.440	0.426	0.423	0.019	0.019	0.500
Frame Size Index	67.06	64.75	65.62	64.53	1.594	0.102	0.457

BW = Body weight; SEM = Standard error of the mean; Significant at 5% probability level. Equations: $\hat{Y}^1 = 43.08 - 0.095x$, $R^2 = 0.93$; $\hat{Y}^2 = 16.725 - 1.27x$, $R^2 = 0.93$; $\hat{Y}^3 = 0.279 - 0.0212x$, $R^2 = 0.93$; $\hat{Y}^4 = 81.063 - 0.869x$, $R^2 = 0.79$; $\hat{Y}^5 = 0.4627 - 0.0107x$, $R^2 = 0.93$.

3.2. Carcass characteristics, morphometric evaluation and commercial cuts

The inclusion of cottonseed cake levels in the diets reduced the slaughter body weight ($p = 0.007$), hot carcass weight ($p = 0.018$), cold carcass weight ($p = 0.016$), and rib eye area ($p = 0.029$) of the sheep. There was no effect of cottonseed cake levels on the fasting weight loss, hot and cold carcass yields, cooling losses, carcass conformation, carcass fattening, minimum and maximum fat thickness, and the pelvic-renal fat assessment ($p > 0.05$) (Table 5).

Table 5. Carcass weights and yields, rib eye area, fat thickness, and perirenal fat of Santa Ines rams fed diets containing levels of cottonseed cake.

Items	Cottonseed cake (% DM)				SEM	P-Value	
	0	14	28	42		Linear	Quadratic
Slaughter body weight[1], kg	42.36	40.01	38.99	38.00	2.791	0.007	0.508
Fasting weight loss, kg	1.22	1.33	1.40	1.53	0.469	0.679	0.707
Hot carcass weight[2], kg	19.26	19.13	18.60	15.53	2.460	0.018	0.338
Cold carcass weight[3], kg	19.01	18.90	18.37	15.35	2.389	0.016	0.402
Hot carcass yield, %	44.65	43.68	43.12	40.47	8.192	0.566	0.249
Cold carcass yield, %	44.57	43.21	42.62	40.26	7.814	0.578	0.278
Cooling losses, %	1.29	1.24	1.20	1.17	1.564	0.530	0.226
Carcass conformation	3.50	3.25	3.19	3.00	0.334	0.178	0.901
Carcass fattening	3.06	2.75	2.81	2.75	0.242	0.417	0.579
Rib eye area[4]	13.02	12.63	11.15	10.86	0.841	0.029	0.706
Minimum fat thickness	1.7	1.6	1.6	1.6	0.027	0.258	0.111
Maximum fat thickness	4.1	4.0	3.8	3.6	0.044	0.615	0.056
Pelvic-renal fat assessment	2.50	2.00	2.00	2.25	0.224	0.451	0.08

SEM = Standard error of the mean; Significant at 5% probability level. Equations: $\hat{Y}^1 = 41.95-0.100x$, $R^2 = 0.94$; $\hat{Y}^2 = 19.89-0.083x$, $R^2 = 0.74$; $\hat{Y}^3 = 19.63-0.082x$, $R^2 = 0.73$; $\hat{Y}^4 = 13.11-0.056x$, $R^2 = 0.92$.

In the evaluations carried out on the carcass, the inclusion of cottonseed cake levels in the diets reduced the external length ($p = 0.018$), rump perimeter ($p = 0.008$), thoracic perimeter ($p = 0.002$), carcass compactness index ($p = 0.039$), and leg compactness index ($p = 0.040$), according to the increased levels of cottonseed cake in the diets offered to sheep. There was no effect of cottonseed cake levels on the internal length, leg length, leg perimeter, rump width, thorax width, and thorax depth ($p > 0.05$) (Table 6).

The weights of the left half carcass ($p = 0.007$), shoulder ($p = 0.021$), neck ($p = 0.029$), loin ($p = 0.026$), and leg ($p = 0.009$) cuts were reduced according to the increase of cottonseed cake levels in diets. The quadratic effect was observed for the rib weight ($p = 0.015$) and yield ($p = 0.036$). There was no effect of the diets on the shoulder, neck, loin, and leg yields ($p > 0.05$) (Table 7).

Table 6. Morphometric evaluation and compactness index of Santa Ines rams fed diets containing levels of cottonseed cake.

Items	Cottonseed cake (% DM)				SEM	P-Value	
	0	14	28	42		Linear	Quadratic
External length[1], cm	62.65	59.37	60.02	59.25	1.111	0.018	0.139
Internal length, cm	65.31	63.31	63.71	63.41	1.221	0.209	0.325
Leg length, cm	43.70	44.60	43.33	43.84	0.915	0.869	0.791
Leg perimeter, cm	33.41	33.91	32.38	32.71	1.176	0.532	0.946
Rump width, cm	26.63	26.18	26.36	25.36	0.515	0.140	0.592
Rump perimeter[2], cm	69.60	67.66	66.83	66.20	1.229	0.008	0.438
Thorax width, cm	25.19	23.99	24.76	23.53	0.772	0.130	0.967
Thorax depth, cm	28.14	28.00	28.04	28.08	0.644	0.950	0.843
Thoracic perimeter[3], cm	79.11	76.35	76.73	73.64	1.689	0.002	0.866
CCI[4], kg/cm	0.29	0.30	0.29	0.24	0.015	0.039	0.250
LCI[5], cm	0.61	0.59	0.61	0.58	0.014	0.040	0.943

CCI = Carcass compactness index, LCI = Leg compactness index; SEM = Standard error of the mean; Significant at 5% probability level. Equations: $\hat{Y}^1 = 62.713 - 0.955x$, $R^2 = 0.60$; $\hat{Y}^2 = 70.331 - 1.1037x$, $R^2 = 0.93$; $\hat{Y}^3 = 78.01 - 0.170x$, $R^2 = 0.87$; $\hat{Y}^4 = 0.312 - 0.001x$, $R^2 = 0.71$; $\hat{Y}^5 = 0.623 - 0.0008x$, $R^2 = 0.73$.

Table 7. Weights and yields of carcass cuts of Santa Ines rams fed diets containing levels of cottonseed cake.

Variables	Cottonseed cake (% DM)				SEM	P-Value	
	0	14	28	42		Linear	Quadratic
Left half carcass weight[1], kg	8.34	7.62	7.31	7.35	0.507	0.007	0.117
Shoulder[2], kg	1.62	1.50	1.48	1.44	0.095	0.021	0.485
Shoulder, %	19.47	19.75	20.32	19.56	0.260	0.411	0.069
Neck[3], kg	0.78	0.67	0.70	0.65	0.047	0.029	0.430
Neck, %	9.33	8.78	9.60	8.80	0.281	0.571	0.679
Rib[4], kg	2.61	2.33	2.16	2.33	0.182	0.020	0.015
Rib[5], %	31.31	30.66	29.59	31.70	0.608	0.974	0.036
Loin[6], kg	0.72	0.66	0.63	0.62	0.048	0.026	0.357
Loin, %	8.69	8.61	8.62	8.43	0.262	0.582	0.980
Leg[7], kg	2.60	2.45	2.33	2.31	0.153	0.009	0.364
Leg, %	31.20	32.20	31.86	31.52	0.374	0.702	0.103

SEM = Standard error of the mean; Significant at 5% probability level. Equations: $\hat{Y}^1 = 8.4708 - 0.3277x$, $R^2 = 0.79$; $\hat{Y}^2 = 1.657 - 0.0578x$, $R^2 = 0.89$; $\hat{Y}^3 = 0.7888 - 0.0361x$, $R^2 = 0.66$; $\hat{Y}^4 = 3.1644 + 0.6529x - 0.1103x^2$, $R^2 = 0.97$; $\hat{Y}^5 = 34.235 + 3.4358x - 0.6891x^2$, $R^2 = 0.75$; $\hat{Y}^6 = 0.7426 - 0.0341x$, $R^2 = 0.87$; $\hat{Y}^7 = 2.6694 - 0.0981x$, $R^2 = 0.91$.

3.3. Qualitative characteristics of the loin, tissue composition and leg muscularity index

There was no effect of cottonseed cake levels on the qualitative characteristics in the loins evaluated ($p > 0.05$). Additionally, there was no effect of cottonseed cake levels on the tissue composition, muscle:bone and muscle:fat ratios, and the leg muscularity index ($p > 0.05$) (Table 8).

Table 8. Qualitative characteristics of the loin, tissue composition and leg muscularity index (LMI) of Santa Ines rams fed diets containing levels of cottonseed cake.

Items	Cottonseed cake (% DM)				SEM	P-Value	
	0	14	28	42		Linear	Quadratic
Color	2.62	2.37	2.75	2.62	0.332	0.748	0.844
Marbling	3.37	2.87	2.87	2.62	0.367	0.155	0.710
Texture	3.25	3.00	3.25	3.25	0.251	0.856	0.585
Weights, kg							
Reconstituted leg	2.56	2.42	2.30	2.29	0.126	0.070	0.240
Bone	0.35	0.34	0.32	0.32	0.013	0.451	0.076
Muscle	1.83	1.74	1.63	1.62	0.098	0.069	0.411
Total fat	0.31	0.27	0.26	0.28	0.023	0.140	0.592
Other tissues	0.07	0.07	0.08	0.07	0.006	0.613	0.059
Yield, %							
Bone	13.71	13.88	14.13	14.02	0.181	0.669	0.591
Muscle	71.33	71.90	70.87	70.66	0.552	0.350	0.481
Total fat	12.27	11.12	11.43	12.31	0.601	0.059	0.430
Other tissues	2.70	3.10	3.57	3.01	0.359	0.128	0.892
Ratios							
Muscle:bone	5.20	5.18	5.01	5.04	0.095	0.524	0.217
Muscle:fat	5.81	6.47	6.20	5.74	0.341	0.140	0.592
LMI, g/cm	0.58	0.56	0.56	0.56	0.010	0.155	0.710

SEM = Standard error of the mean; Significant at 5% probability level.

3.4. Economic evaluation

In the economic evaluation, the costs in percentage values of the ingredients that made up each experimental diet were determined (Table 9), taking the price paid per kg of the ingredients into account, according to trade in Rondonópolis-MT, Brazil. There was an increase in the contribution of ground corn, representing around 30.07, 34.05, 38.85, and 45.45% of diet costs, as the levels of replacement of soybean meal with cottonseed cake increased.

Table 9. Percentage values of feed costs in confinement of Santa Ines rams fed diets containing levels of cottonseed cake.

Items	Cottonseed cake (% DM)			
	0	14	28	42
Corn silage	30.47	34.41	38.87	44.67
Ground corn	31.98	36.48	41.45	48.21
Soybean meal	68.01	52.09	30.01	0.0
Cottonseed cake	0.0	9.53	21.97	38.87
Urea	0.0	1.90	6.57	12.91

The diet with a higher share of cottonseed cake resulted in lower total feed/animal costs, revenue, gross margins, and leveling prices. On the other hand, a greater benefit/cost ratio was observed (Table 10).

Table 10. Feed costs and financial return (in USD$) in relation to meat production from sheep fed diets containing cottonseed cake.

Costs	Cottonseed cake (% DM)			
	0	14	28	42
Confinement days	60	60	60	60
Total feed/animal costs[1]	2.01	1.72	1.51	1.15
Revenue[2]	81.45	80.97	78.72	65.79
Gross margin[3]	64.47	65.92	60.66	50.99
Benefit/cost[4]	1.06	1.22	1.37	1.52
Meat price/kg (USD$)[1]	4.28	4.28	4.28	4.28
Leveling price (kg)[5]	0.77	0.66	0.58	0.44

[1]Average value per animal; [2]Carcass weight of each animal (kg) multiplied by the market price paid per kilogram of carcass of USD$ 4.28; [3]Total revenue (USD$) – Total cost (USD$); [4]Carcass weight x USD$ 4.28 ÷ Total cost (USD$); [5]Total cost (USD$) ÷ Meat price/kg (USD$).

4. Discussion

4.1. Performance, and in vivo measurements

Although it was observed that the diets provided the animals with similar dry matter intakes, daily gains were reduced with the replacement of soybean meal with cottonseed cake. Moreover, it was observed that only the sheep that received the diet containing 42% cottonseed cake showed daily gains below the estimated 200 g/day [23], obtaining an ADG of 197 g/day.

Although the diets were isonitrogenous, possibly the low degradability of the crude protein in cottonseed cake, due to the high ADIN content, limited the microbial protein production in the rumen environment, causing a reduction in weight gain. Furthermore, urea has a rapid degradation in the rumen due to its high solubility, thus making synchronicity between the availability of ammonia and energy difficult, which is exactly what is required for the growth and development of the microbiota and the consequent increase on the sheep performance [41]. Another point that should be noted is that the TDN content in diets decreased with increased cottonseed cake levels, which directly affected the nutrient intake. According to Oliveira et al. [42], the reduction in TDN intake may be caused by the lower energy input via the reduction in the concentration of succinate in the animal's liver tissue, which is related to the change in the microbial profile in the ruminal environment; this resulted from the reduction of gram-negative bacteria to the detriment of gram-positive bacteria, with a consequent change in the concentration of volatile fatty acids, that is, with a reduction in the concentration of propionic and butyric acids in relation to acetic acid and with a reduction in the supply of amino acids of potentially digestible feed origin in the small intestine.

Possibly, the reduction in digestible fractions intake, mainly CP and NFC, resulted in lower absorption and deposition of nutrients in tissues. Furthermore, we observed that replacing soybean meal with cottonseed cake resulted in an increase in the EE and NDF contents and a reduced NFC, which could result in a reduction in voluntary intake by animals. Since the NFC is the main source of energy readily available in the rumen, these carbohydrates affect the metabolism and microbial multiplication depending on the availability of nitrogen, and consequently the feed digestion, microbial protein production, and the amount of amino acids and peptides available for absorption in the small intestine [43,44]. As NDF is slowly fermented by the rumen microbiota, there is a decreased production of volatile fatty acids, the main source of energy for ruminant metabolism, compared to NFC [45,46]. This reduction in animal weight gain negatively influenced the hot and cold carcass weights and commercial cuts, as these were directly affected by the total weight gain and slaughter body weight [47].

The reduction in weight gain can also be observed by the reduction in the animals' body capacity according to the increase in cottonseed cake levels in the diets offered, thus indicating that the animals had a smaller body size with the ingestion of diets containing 42% of cottonseed cake in its composition. Furthermore, we observed that the smallest chest perimeter was presented by animals that had a lower body capacity, which directly influenced the rib weight and yield. This fact was also observed by Lima et al. [48] when they tested diets that contained different levels of sunflower cake in the diets of Santa Inês sheep. According to Grandis et al. [49], the chest perimeter is a variable measured *in vivo* that can be used to predict the animal weight, as it is related to the animals' body volume. These results are important to create a database that allows one to estimate the weight of animals according to body measurements through equations. However, information about *in vivo* measurements and their possible correlations with performance and carcass variables is scarce for adult sheep [50].

4.2. Carcass characteristics

The measurements carried out on the carcasses, such as external length, rump perimeter, and thoracic perimeter, accompanied the weight gain of the animals, with decreased measurements alongside increased levels of cottonseed cake in the diets. Likewise, the CCI and LCI were also influenced by the diets offered, thus indicating a reduction in tissue deposition in the carcass per unit of length. According to Corrêa et al. [51], the CCI makes it possible to estimate muscle deposition in the carcass, making it possible to have a perspective on the financial return on the invested capital. The LCI quantifies the capacity for deposition of meat in the leg, indicating either a greater or lesser specialization of meat production by lambs; therefore, with longer and lighter legs, there will be less compactness [52].

Combined with the previously mentioned measurements, the rib eye area is a measurement that reflects the amount of muscle in the carcass and has been performed *in vivo* by ultrasound, as it is efficient in monitoring meat gain during animal growth and development [3]. It was already expected that the rib eye area would be reduced according to the increase in cottonseed cake levels in the diets tested, as this measure is related to the decrease in body capacity and observed slaughter and carcass weights. With the inclusion of cottonseed cake in diets, due to the higher concentration of ADIN, which is bound to the fibers and cannot be degraded by microorganisms or absorbed by the small intestine, the possible reduction in the digestibility of CP results in losses of N that would be directed to the muscular development, corroborating the behavior observed for CCW and weight of commercial cuts, as verified by Machado et al. [53].

When evaluating levels of replacing soybean meal with cottonseed cake in feedlot sheep diets, Silva et al. [12] found no effect on the body weights, carcass weights, and rib eye area, which is different from our findings. However, these authors used young animals (4 months old) in their experiment, which were in the growth and development stage, differing from our animals, which were mature animals (12 months old). Hammond [54] emphasizes that, as the animal grows, changes occur in its body proportions. Thus, Benaglia et al. [55] determined that late-maturing muscles are more appropriate to represent the development and volume of muscle tissue, which is why the *Longissimus dorsi* is the most appropriate, in addition to being easy to measure. However, in the present study, increasing levels of cottonseed cake in the diets reduced the animals' weight gain, meaning that the sheep did not reach muscular maturity due to a lower growth performance, a fact that contributed to the reduction in deposition of muscle tissue, and consequently the REA of the *L. dorsi*.

Therefore, future studies with younger animals are necessary to determine whether the levels of cottonseed cake tested here can provide beneficial effects on carcass weights and yields.

4.3. Economic evaluation

Replacing soybean meal with cottonseed cake resulted in a reduction in the cost of diets. However, due to the negative effect of the replacement levels of soybean meal with cottonseed cake on the average daily gain, there was a reduction of 0.088, 0.222, and 0.208 USD$ for each percentage unit of replacement of soybean meal with cottonseed cake on the cost of total feed/animal, revenue, and gross margin, respectively.

The increase observed in the benefit:cost ratio is a reflection of the feed conversion of animals and the prices of soybean meal and cottonseed cake; during the experimental period, the by-product had a competitive price in relation to the price of soybean meal. There was a reduction of 0.006 USD$ in the leveling price for each percentage unit of replacement of soybean meal with cottonseed cake in the concentrated feed. With this result, it is possible to provide the producer with a price that is possible to cover the costs of feeding the animals and obtain a profit.

However, although replacing soybean meal with cottonseed cake reduced diet costs and daily cost per animal, revenue also decreased as a result of lower total weight gains. This result shows that the economic viability of using cottonseed cake depends on the prices of the by-product and soybean meal, because although the replacement of the by-product reduced the price per kg of concentrate, this reduction was insufficient to compensate for the increase in conversion to feed.

5. Conclusions

Under experimental conditions, the use of levels of up to 42% of cottonseed cake in the sheep's diet, replacing soybean meal, is not recommended. By providing similar dry matter intake, it reduces the animal weight gain, carcass weight, and commercial cuts.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflicts of interest.

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