

## Article

# Performance, Carcass Traits and Meat Quality of Lambs Fed with Increasing Levels of High-Oleic Sunflower Cake

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**Abstract:** The aim of this study was to evaluate the effect of sunflower cake from high-oleic seeds on performance, carcass characteristics, meat quality, and intramuscular fatty acid composition of finishing lambs. Thirty-six crossbred ewe lambs were assigned to four treatments (nine lambs/treatment) in a completely randomized design: 0 (control), 150, 300 and 450 g/kg DM of high-oleic sunflower cake. The lambs were weighed weekly and slaughtered with  $42.3 \pm 0.18$  kg body weight and  $270 \pm 10.8$  days of old. The inclusion of sunflower cake did not affect weight gain, dry matter intake and metabolizable energy intake ( $p > 0.05$ ). There was an increase in neutral detergent fiber and EE intake ( $p < 0.01$ ) with the inclusion of sunflower cake in the diet of the lambs. The inclusion of sunflower cake reduced hot and cold carcass yields ( $p < 0.01$ ). Intramuscular fat content,  $L^*$ , oleic acid, rumenic acid and EPA fatty acids linearly increased ( $p < 0.01$ ) with the inclusion of high-oleic sunflower cake. The inclusion of high-oleic sunflower cake reduced saturated fatty acids ( $p < 0.01$ ), except stearic acid, which linearly increased ( $p < 0.01$ ). Up to 450 g/kg DM of high-oleic sunflower cake in the diet of lambs did not affect animal performance while providing a higher deposition of fat with better fatty acid composition for human consumption.

**Keywords:** ruminant nutrition; by-product; fatty acids; PUFA; saturated fatty acids; ovine



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## 1. Introduction

The search for more productive and sustainable food production systems, in addition to the elevated costs of animal feed, especially in the off-season, has been the fundamental driver for adhering to the use of alternative foods in animal feeding systems. By-products of the biodiesel industry have been prominent among the potential nutrient sources for animal feed, especially due to their lower price compared to traditional feeding ingredients and nutritious composition [1,2].

Sunflower (*Helianthus annuus* L.) is an oilseed with potential for biodiesel production due to its high oil concentration [3]. Currently, high-oleic sunflower oil is the most used in the food sector and as a raw material for non-food applications such as biofuels, due to its industrial properties, mainly high oxidative stability [4,5]. High-oleic sunflower has approximately 80% oleic acid, but some linoleic acid is still present in the achenes [4]. Unlike sunflower meal, sunflower cake is a by-product with a high fat content. It is produced when sunflower seeds are crushed for physical strength without the use of heat or solvent [6,7].

Meat fatty acid composition is one of the determining factors affecting fat quality [8], especially with regard to human health, emphasizing the recommendation of reduced intake of saturated fatty acids and increased intake of long-chain n3 polyunsaturated fatty acids (PUFA) in response to their effect in lipid-related diseases [9]. The inclusion of alternative animal feeds rich in unsaturated fatty acids is a strategy to reduce saturated fatty acids deposited in tissues and increase polyunsaturated fatty acids in red meat [9], primarily conjugated linoleic acid (CLA) isomers [10–12]. However, ruminal fatty acid biohydrogenation challenges the increase in PUFA content in the meat of ruminants through dietary intake, in addition to the activity of enzymes involved in fatty acid synthesis in the muscle (e.g.,  $\Delta 9$  desaturase and elongase) [13,14].

The application progress of sunflower cake has gained increased attention as a viable ingredient in ruminant nutrition due to its favorable nutrient profile and availability. High-oleic sunflower cake can be a high-energy feed ingredient in the diet of feedlot lambs and improve the profile of fatty acids in the meat, providing a direct deposition of monounsaturated fatty acids and, through biohydrogenation, convert to stearic fatty acid, which has a protective effect against cardiovascular disease [15]. However, due to the fat content associated with the amount of fiber in sunflower cake, the levels of inclusion of this by-product in the diet can reduce dry matter intake and animal performance [2,16] while others have shown that the inclusion of sunflower cake can support intake, nitrogen balance and ruminal fermentation parameters [17]. Given that, the objective of the present study was to assess the effects of increased levels of high-oleic sunflower cake (0, 150, 300 and 450 g/kg DM) in the diet of feedlot crossbred lambs on their performance, carcass traits, meat quality (pH, color, shear force and composition), and intramuscular fatty acid composition.

## 2. Materials and Methods

### 2.1. Facilities and Experimental Diets

This study was conducted in 2013 at the Sheep Sector in the Department of Animal Sciences of the Federal University of Lavras. Animal experimental procedures were approved and performed according to the Animal Care Guidelines (Protocol number 105/12).

Thirty-six female lambs ( $\frac{1}{2}$  Santa Inês  $\times$   $\frac{1}{2}$  Dorper, randomly selected from a larger herd), with an initial body weight of  $21.52 \pm 0.27$  kg and initial age of  $138 \pm 2.62$  days, were individually allocated to covered pens (1 m<sup>2</sup>). This experiment was carried out in a completely randomized design. Animals were allocated among the four treatments, totaling 9 replicates per treatment, which consisted of the inclusion of high-oleic sunflower cake (Table 1) in the proportions of 0, 150, 300, and 450 g/kg DM (Table 2). The diets were calculated to be isoproteic (181 g/kg DM), with a 20:80 roughage:concentrate ratio. The diets were formulated to meet the nutritional requirements of female lambs with 20 kg live weight and average daily weight gain of 250 g, according to the National Research Council [18]. Sunflower cake was obtained through cold pressing of high-oleic sunflower seeds from a Brazilian company (Parecis Alimentos S/A, Campo Novo do Parecis, Brazil).

**Table 1.** Chemical and main fatty acid composition of the sunflower cake.

Chemical Composition	g/kg DM
Dry matter, g/kg as fed	968.8
Organic matter	943.3
Crude protein	276.0
Neutral detergent fiber	440.0

**Table 1.** *Cont.*

Acid detergent fiber	357.0
Ether extract	160.0
Ash	57.0
Non fibrous carbohydrates	72.0
Metabolizable energy <sup>a</sup> , (Mcal/kg)	2.77
Fatty acid composition	g/100 g total fatty acids
C12:0 (Lauric)	0.00
C16:0 (Palmitic)	4.40
C18:0 (Stearic)	2.21
C18:1c9 (Oleic)	81.02
C18:2 n6 (Linoleic)	5.30
C18:3 n3 ( $\alpha$ -Linolenic)	0.23
Saturated fatty acids	7.90
Unsaturated fatty acids	91.30
Monounsaturated fatty acids	85.64
Polyunsaturated fatty acids	5.66

<sup>a</sup> Estimated according to [18], where metabolizable energy = 0.82  $\times$  digestible energy.

**Table 2.** Diet composition, chemical analysis and main fatty acids composition of the experimental diets.

	High-Oleic Sunflower Cake (g/kg DM)			
	0	150	300	450
Ingredients (g/kg DM)				
Tifton 85 hay ( <i>Cynodon</i> spp.)	200.0	200.0	200.0	200.0
Ground corn grain	560.0	440.0	380.0	310.3
Soybean meal	210.0	180.0	90.0	7.70
Sunflower cake	0.00	150.0	300.0	450.0
Premix mineral-vitamin supplement <sup>a</sup>	20.0	20.0	20.0	20.0
Limestone	10.0	10.0	10.0	10.0
Chemical composition (g/kg DM)				
Dry matter, g/kg as fed	947.7	950.0	952.2	955.5
Organic matter	933.3	932.2	934.4	933.3
Crude protein	181.5	182.0	180.2	180.5
Neutral detergent fiber	357.4	388.1	418.6	449.0
Acid detergent fiber	130.3	163.6	196.8	229.8
Ether extract	30.0	49.8	69.5	89.1
Ash	67.2	68.6	66.6	68.6
Non fibrous carbohydrates	364.0	311.5	265.4	212.8
Metabolizable energy <sup>b</sup> , (Mcal/kg)	2.70	2.72	2.67	2.62
Fatty acid composition (g/100g total fatty acids)				
C12:0 (Lauric)	0.04	0.02	0.02	0.01
C16:0 (Palmitic)	18.51	11.63	8.50	6.80
C18:0 (Stearic)	3.07	2.76	2.61	2.61
C18:1c9 (Oleic)	25.60	52.77	63.40	68.80
C18:2 n6 (Linoleic)	41.22	25.41	17.00	12.00
C18:3 n3 ( $\alpha$ -Linolenic)	1.08	0.83	0.63	0.42
Saturated fatty acids	26.23	17.50	12.65	11.46
Unsaturated fatty acids	74.95	83.13	86.67	88.00
Monounsaturated fatty acids	32.40	56.61	68.90	75.40
Polyunsaturated fatty acids	42.56	26.52	17.84	12.60

<sup>a</sup> Composition per kg of mixture: 122 g Calcium, 87 g Phosphorus, 18 g Sulfur, 147 g Sodium, 3800 mg Zinc, 590 mg Copper, 2000 mg Manganese. <sup>b</sup> Estimated according to [18], where metabolizable energy = 0.82  $\times$  digestible energy.

## 2.2. Animal Performance, Slaughter and Carcass Traits

The lambs were subjected to 15 days of an adaptation protocol to adapt to facilities and experimental diets. The total mixed ration was offered *ad libitum* twice daily (07:00 and 15:00 h). The amount of diet provided everyday was adjusted according to the recorded intake of the previous day, calculated to achieve 5% refusals. Feeding leftovers were weighed daily

to determine the average daily intake. One sample of the feed refusals from each animal was collected daily and combined into a weekly sampling and taken to a dry-forced ventilation oven for drying to determine daily dry matter intake. The samples were ground using a Wiley-type mill with a sieve size of 1 mm to determine the concentrations of total dry matter (DM) (method 967.03), ash (method 942.05), crude protein (CP) (method 981.10), and ether extract (EE) (method 920.29) [19]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedure described by Van Soest et al. [20], with an NDF correction for ash (NDFa). Non-fibrous carbohydrate (NFC) concentration was calculated using the following equation:  $NFC = 100 - (CP + NDF + EE + \text{ash})$ .

Lambs were weighed weekly until they reached a body weight of  $42.3 \pm 0.18$  kg. The animals were submitted to feed and water fasting for 16 h and weighed to assess the pre-slaughter weight (SW). Then, the animals were transported to a commercial slaughterhouse, where they were slaughtered according to the slaughterhouse regulations. Non-carcass body parts were removed and weighed to determine the non-carcass traits (NCTs). Stomach and intestinal contents were weighed to determine gastrointestinal tract content (GITC) weight, and empty body weight (EBW) was estimated according to the equation:  $EBW = SW - GITC$ . The carcasses were weighed to obtain hot carcass weight (HCW) and hot carcass yield ( $HCY = HCW/SW \times 100$ ); subsequently, the carcasses were cooled for 24 h at 4 °C. After cooling, the carcasses were weighed again, obtaining the cold carcass weight (CCW) and cold carcass yield ( $CCY = CCW/SW \times 100$ ). The cooling loss was calculated according to the equation:  $CL = (HCW - CCW)/HCW \times 100$ . Then, the carcasses were split longitudinally, and an incision was made in the left half carcass, between the 12th and 13th ribs for exposure of the longissimus muscle. Subcutaneous fat thickness (SFT) was measured with a digital caliper. At the same point, the eye muscle area (EMA) of the muscle was drawn on a transparency with the aid of a permanent pen and measured using the software Universal Desktop Ruler (AVPSoft®). Percentage yields of carcass cuts (loin, ribs, leg, shoulder, rack and neck) were calculated relative to the cold carcass weight of the lambs.

### 2.3. Meat Quality Analysis

The longissimus thoracis et lumborum muscle was sampled and divided into three steak samples, vacuum-packed with polyethylene packages and kept in refrigerator at 2 °C for 3 days before further analyses. One steak was used for the measurement of meat color. After 30 min of opening the vacuum bags at room temperature, color measurements were taken using a CM-700 Minolta spectrophotometric colorimeter (Konica Minolta, Osaka, Japan). Three readings were recorded on each steak to calculate the average values of the lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ). The saturation index (Chroma) and hue angle were calculated according to MacDougall [21], using the following equations:  $\text{Chroma} = ((a^*)^2 + (b^*)^2)^{0.5}$  and  $\text{hue} = \arctan(b^*/a^*)$ . Meat pH was measured in triplicate using a digital pH meter (TESTO-205 pH meter, Campinas, Brazil). Additionally, the same steak sample was also analyzed for moisture, CP, EE, and ash concentration, according to the AOAC [19]. The second steak was used to measure cooking loss. In summary, the weight of the samples was recorded, and the samples were placed on a rack above a glass baking dish. A digital thermometer was inserted into the geometric center of the samples to monitor their internal temperature. The steaks were cooked in an electric oven, and once the internal temperature of the sample reached 40 °C, the steak was flipped and cooked further until the internal temperature reached 71 °C, as previously described [22]. After cooking, the samples were kept at room temperature for 15 min, weighed again to determine cooking loss, and cooled at 4 °C for 24 h. After refrigeration, for determination of shear force (SF) three subsamples measuring 1 cm<sup>2</sup> each were cut parallel to the muscle

fiber. The subsamples were sectioned using a texturometer (ModleTA-TX2, Stable Micro Systems Ltd., Godalming, Surrey, UK) attached to a Warner–Bratzler slide and calibrated using a weight of 2 kg with an adjusted speed of 200 mm/min. The results were presented in kgf/cm<sup>2</sup> and five replicate measurements were taken per steak.

The third steak was used for the quantification of intramuscular fatty acid composition according to Hara and Radin [23]. The fatty acid profile was assessed using the methods reported by Rodrigues-Ruiz et al. [24]. After extraction and methylation, each sample was injected into a gas chromatograph (model Focus CG, Finnigan) equipped with a flame ionization detector and a 100 m long CP-Sil 88 capillary column (Varian) with an internal diameter of 0.25 µm and a film thickness of 0.20 µm. Hydrogen was used as the carrier gas at a 1.8 mL/min flow rate. The oven temperature program started at 70 °C with a 4 min wait time. Subsequently, the temperature increased to 175 °C at 13 °C/min, with a 27 min wait time, followed by an increase to 215 °C at 4 °C/min, with a 9 min wait time. The temperature was increased by 7 °C/min until it reached 230 °C, which was held for 5 min, totaling 65 min. The vaporizer temperature was set at 250 °C, and the detector temperature was set at 300 °C. The fatty acids were identified by comparing the retention times of methyl esters with a predefined pattern, and then quantified by area normalization of the methyl esters, and were expressed as percentages of total methylated fatty acids.

The  $\Delta 9$ -desaturase and elongase enzymatic activity indices were calculated as reported by Malau-Aduli et al. [25], using the following equations:  $\Delta 9$ -Desaturase 16 = 100 [(C16:1cis-9)/(C16:1cis-9 + C16:0)];  $\Delta 9$ -Desaturase 18 = 100 [(C18:1cis-9)/(C18:1cis-9 + C18:0)]; and Elongase = 100 [(C18:0 + C18:1cis-9)/(C16:0 + C16:1cis-9 + C18:0 + C18:1cis-9)]. The atherogenicity and thrombogenicity indices were calculated according to Ulbricht and Southgate [26], as indicators of the risk for cardiovascular disease: Atherogenicity = {(C12:0 + 4 × (C14:0) + C16:0) / (MUFA + n3 + n6)} and Thrombogenicity = {(C14:0 + C16:0 + C18:0) / [(0.5 × MUFA) + (0.5 × n6) + (3 × n3) + (n3/n6)]}.

#### 2.4. Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Version 9.1, SAS Institute, Cary, NC, USA) to determine the significant effects of the inclusion of high-oleic sunflower cake in the diet. The initial body weight of the animals was included as a covariate, according to the following model:  $Y_{ij} = \mu + T_i + P_j + e_{ij}$ ; where  $Y_{ij}$  is the observed value,  $\mu$  is the constant associated with each observation,  $T_i$  is the effect of using sunflower cake ( $i = 0$  to 450 g/kg DM),  $P_j$  is the effect of the covariate initial live weight and  $e_{ij}$  is the experimental error. When significant effects were detected ( $p < 0.05$ ), linear regression models were fitted using the REG procedure of SAS, testing linear and quadratic models ( $\alpha = 0.05$ ).

### 3. Results and Discussion

#### 3.1. Growth Performance and Carcass Characteristics

The inclusion of high-oleic sunflower cake up to 450 g/kg DM in the diet of the lambs did not affect dry matter intake, crude protein intake, daily weight gain, or gain/feed ratio ( $p > 0.05$ ) (Table 3). The intake of EE and NDF linearly increased ( $p < 0.01$ ) with the inclusion of high-oleic sunflower cake due to the high concentrations of these nutrients in the by-product. Conversely, a reduction in the NFC intake was observed. These outcomes were anticipated, as including sunflower cake in the diet reduced the proportion of soybean meal, thereby maintaining isoproteic condition. Sunflower cake contains higher levels of EE and NDF but lower levels of NFC compared to soybean meal. The primary rationale for incorporating fat into ruminant diets is to enhance energy intake without utilizing rapidly fermentable feeds. However, reductions in dry matter intake and NDF digestibility are commonly reported due to the inhibitory effect of fatty acids on ruminal microorganisms [27].



These fatty acids include medium-chain (10 to 14 carbons) and long-chain polyunsaturated fatty acids [18,28]. However, there was an elevated EE intake, especially in the diet with 450 g/kg DM of high-oleic sunflower cake inclusion (89 g/kg of DM). The by-product used in the present study is composed mostly of oleic acid (81.0 g/100 kg total fatty acids), a monounsaturated fatty acid which is less toxic to ruminal microorganisms [29]. Previous studies have shown that antiprotozoal and antibacterial effects from feed oils are associated with the degree of unsaturation of their fatty acids [30,31]. A reduction in the detrimental effects of unsaturated fatty acids on digestibility through the use of oleic acid was also observed by Weld and Armentano [32] using plenary high-oleic soybeans, which moderately increased milk fat content compared with conventional soybeans. Therefore, the performance of lambs fed with increased levels of high-oleic sunflower cake was not greatly affected apart from an increase in EE and NDF intake due to their superior concentration in the diet.

**Table 3.** Performance of lambs fed diets with increased levels of high-oleic sunflower cake.

	High-Oleic Sunflower Cake (g/kg DM)				SEM	p-Value			Equation	R <sup>2</sup>
	0	150	300	450		T	L	Q		
Intake										
Dry matter, kg/day	1.07	1.15	1.11	1.09	0.03	0.42	0.86	0.14	Y = 1.11	-
Crude protein, g/day	189.00	197.23	188.12	180.44	3.90	0.06	0.08	0.06	Y = 188.70	-
Neutral detergent fiber, g/day	361.41	426.63	453.83	496.13	12.00	<0.01	<0.01	0.07	Y = 370.64 + 2.85x	0.95
Ether extract, g/day	35.63	64.07	86.36	105.91	2.51	<0.01	<0.01	0.07	Y = 38.12 + 15.5x	0.98
NFC, g/day	406.51	389.28	332.28	251.78	8.22	<0.01	<0.01	0.05	Y = 422.97 – 34.4x	0.94
Weight gain, kg	22.35	22.15	21.61	21.68	0.37	0.40	0.73	0.86	Y = 21.95	-
Daily weight gain, g	176.87	200.09	206.21	184.41	8.90	0.09	0.45	0.08	Y = 191.90	-
Gain: feed, kg/kg	6.29	5.75	5.38	5.92	0.23	0.14	0.31	0.07	Y = 5.84	-
Gastrointestinal tract content, kg	3.63	4.29	4.78	5.20	0.21	<0.01	<0.01	0.46	Y = 3.71 + 0.03x	0.90
Empty body weight, kg	39.06	38.01	37.26	36.90	0.34	<0.01	<0.01	0.28	Y = 38.88 – 0.048x	0.73
Slaughter weight, kg	43.00	42.41	41.70	42.15	0.36	0.10	0.12	0.20	Y = 42.32	-

NFC: non-fibrous carbohydrates; SEM: standard error of the mean; R<sup>2</sup>: coefficient of determination; T: treatment effect; L: linear effect; Q: quadratic effect.

A significant linear decrease in empty body weight ( $p < 0.01$ ) and a linear increase in gastrointestinal tract content ( $p < 0.01$ ) were observed with the inclusion of high-oleic sunflower cake (Table 3). Diets containing higher levels of NDF can prolong rumen retention time and increase total digesta volume [33], ultimately leading to a proportional decrease in empty body weight once gastrointestinal contents are removed [34].

Hot and cold carcass weights and their yield percentage linearly decreased ( $p < 0.01$ ) as a result of the observed increase in gastrointestinal tract content, even though there was no change in slaughter weight nor in the sum of non-carcass components with the inclusion of high-oleic sunflower cake in the lambs' diet ( $p > 0.05$ ) (Table 4). Lima et al. [2] also reported a reduction in hot and cold carcass yields with the inclusion of up to 300 g/kg of sunflower cake in the diet of male Santa Ines lambs. Additionally, Lima et al. [2] observed a decrease in weight gain and slaughter weight of the lambs with the inclusion of sunflower cake in the diet, which is not supported by our findings. In diets with up to 9.5% EE in which cottonseed meal was replaced by sunflower cake, Junior et al. [35] did not observe effects on weight gain and empty body weight of confined lambs despite reductions in hot and cold carcass yields. This effect can be attributed to the varying levels of sunflower cake included in the diet, which can increase gastrointestinal fill. As a result, a larger proportion of the animal's live weight consists of digesta, leaving a smaller share for carcass tissue once the gastrointestinal tract is emptied at slaughter. Consequently, both hot and cold carcass weights are reduced.

**Table 4.** Carcass characteristics of lambs fed diets with increased levels of high-oleic sunflower cake.

	High-Oleic Sunflower Cake (g/kg DM)				SEM	p-Value			Equation	R <sup>2</sup>
	0	150	300	450		T	L	Q		
Non-carcass traits, kg	21.07	20.83	21.41	20.60	0.49	0.74	0.69	0.32	Y = 20.98	-
Hot carcass weight, kg	23.52	22.44	21.80	21.45	0.34	<0.01	<0.01	0.21	Y = 23.64 – 0.05x	0.65
Hot carcass yield, %	54.70	52.93	52.39	50.97	0.77	0.02	<0.01	0.16	Y = 23.17 – 0.04x	0.73
Cold carcass weight, kg	23.03	22.06	21.40	21.00	0.31	<0.01	<0.01	0.17	Y = 54.43 – 0.075x	0.64
Cold carcass yield, %	53.57	52.02	51.33	49.91	0.74	<0.01	<0.01	0.23	Y = 53.39 – 0.078x	0.72
Cooling loss, %	2.06	1.82	1.82	2.08	0.15	0.54	0.84	0.07	Y = 1.94	-
Eye muscle area, cm <sup>2</sup>	17.96	17.01	17.00	16.94	0.76	0.70	0.34	0.58	Y = 17.23	-
Subcutaneous fat thickness, mm	2.51	2.74	2.93	3.11	0.11	0.01	<0.01	0.90	Y = 2.54 + 0.013x	0.99
Kidney fat, kg	1.14	1.13	1.15	1.34	0.05	0.04	0.02	0.08	Y = 1.09 + 0.004x	0.66
Gastrointestinal tract fat, kg	2.52	2.77	2.85	3.39	0.10	<0.01	<0.01	0.17	Y = 2.48 + 0.017x	0.90
Cut yield, %										
Loin	6.42	6.44	6.33	6.61	0.15	0.68	0.47	0.30	Y = 6.45	-
Ribs	13.31	13.48	14.47	13.70	0.34	0.15	0.42	0.09	Y = 13.74	-
Leg	31.17	31.36	30.52	31.03	0.48	0.70	0.45	0.55	Y = 31.02	-
Shoulder	17.30	17.85	16.63	17.47	0.36	0.15	0.44	0.94	Y = 17.31	-
Rack	22.50	21.68	22.45	22.53	0.46	0.52	0.52	0.26	Y = 22.29	-
Neck	5.70	6.94	5.31	6.12	0.43	0.08	0.88	0.83	Y = 6.02	-

SEM: standard error of the mean; R<sup>2</sup>: coefficient of determination; T: treatment effect; L: linear effect; Q: quadratic effect.

The inclusion of up to 450g/kg DM of high-oleic sunflower cake in the diet of the lambs did not affect eye muscle area and cooling loss ( $p > 0.05$ ) (Table 4). Eye muscle area is an indicator of muscular tissue development in animals and is highly correlated with valuable commercial cuts. In the present study, the yields of commercial cuts were not affected by the treatment effect ( $p > 0.05$ ) (Table 4). Similar findings have been reported in the literature with the use of sunflower cake in the diet of male Santa Ines lambs and male Boer goats [35,36]. The inclusion of high-oleic sunflower cake in the diet linearly increased subcutaneous fat thickness ( $p < 0.01$ ), reaching 3.11 mm in lambs fed 450 g/kg DM of high-oleic sunflower cake (Table 4). Regardless of treatment, the fat thickness was presumably insufficient to affect the percentage of cooling loss. Hristov et al. [37] did not observe effects on eye muscle area or fat thickness when adding 5% of high-oleic acid safflower oil to the diet of beef cattle. Lima et al. [2] also did not observe an effect on subcutaneous fat thickness of lambs fed up to 6% EE from sunflower cake. Kidney fat also increased with the inclusion of high-oleic sunflower cake in the diet of the lambs ( $p < 0.01$ ) (Table 4). Therefore, the increase in subcutaneous fat thickness and kidney fat can be attributed to a higher energy intake from increased levels of lipids in the high-oleic sunflower cake diets which support fat storage in the body [38]. However, these dietary changes did not significantly alter eye muscle area or cooling loss, suggesting that the inclusion of high-oleic sunflower cake in the diet of the lambs appears to affect fat deposition more than muscle growth due to the high-fat content and energy-dense nature of the sunflower cake.

### 3.2. Meat Quality

Meat pH was not significantly affected by the experimental diets ( $p > 0.05$ ), with a mean value of 5.56 (Table 5). The observed pH values indicate that the glycogen concentration available in the animals was satisfactory at slaughter and was unaffected by the increased EE intake, which can otherwise prevent the pH from lowering to optimal levels [39]. Similar results were observed by Junior et al. [35] when sunflower cake was included in lamb diets containing up to 9.5% EE, and by Oliveira et al. [40] in the goat diets.

**Table 5.** Meat quality of lambs fed diets with increased levels of high-oleic sunflower cake.

	High-Oleic Sunflower Cake (g/kg DM)				SEM	p-Value			Equation	R <sup>2</sup>
	0	150	300	450		T	L	Q		
pH	5.57	5.57	5.60	5.56	0.02	0.79	0.86	0.86	Y = 5.58	-
Cooking loss, %	21.56	21.85	20.23	20.81	0.87	0.55	0.32	0.32	Y = 21.11	-
Shear force, kgf/cm <sup>2</sup>	3.93	4.09	3.49	3.99	0.23	0.29	0.61	0.61	Y = 3.88	-
L*	37.66	37.54	37.96	38.87	0.19	<0.01	<0.01	0.03	Y = 37.47 + 0.025x	0.61
a*	21.76	21.12	20.87	20.91	0.48	0.55	0.17	0.17	Y = 21.17	-
b*	13.22	13.13	13.20	13.59	0.26	0.62	0.37	0.37	Y = 13.29	-
Chrome	25.47	24.88	24.71	24.96	0.48	0.70	0.37	0.37	Y = 25.00	-
Hue	31.32	31.90	32.31	33.08	0.59	0.21	0.04	0.87	Y = 32.15	-
Moisture, %	73.15	72.90	72.60	72.08	0.14	<0.01	<0.01	0.40	Y = 73.18 - 0.022x	0.95
Crude protein, %	21.18	20.95	19.80	21.59	0.57	0.09	0.74	0.07	Y = 20.88	-
Intramuscular fat, %	4.52	4.52	4.95	5.37	0.13	<0.01	<0.01	0.13	Y = 4.39 + 0.020x	0.89
Ash, %	1.18	1.19	1.17	1.15	0.02	0.73	0.31	0.77	Y = 1.17	-

SEM: standard error of the mean; R<sup>2</sup>: coefficient of determination; T: treatment effect; L: linear effect; Q: quadratic effect.

The inclusion of high-oleic sunflower cake did not significantly affect cooking loss or shear force values ( $p > 0.05$ ) (Table 5). Shear force is commonly used as an index of meat toughness and is associated with higher concentrations of intramuscular fat [41]. Although no difference was observed in shear force, there was a linear increase in intramuscular fat concentration and  $L^*$  with the inclusion of up to 450 g/kg DM of high-oleic sunflower cake in the diet of lambs ( $p < 0.01$ ) (Table 5). The increase in  $L^*$  is related to the increase in fat content, as this component contributes to higher luminosity in the meat [42]. This effect on luminosity through the addition of fat in the meat was also reported by Brito et al. [43] and Holman et al. [44]. Higher nutritional levels during the finishing period are associated with increased subcutaneous and intramuscular fat. Elevated intramuscular fat concentration has been shown to reduce the toughness of beef, which Nakamura et al. [45] suggested is due to changes in collagen architecture. Additionally, this elevation could affect the consumer's perception of toughness by improving meat juiciness and flavor. There was no treatment effect on  $a^*$ ,  $b^*$ , Chroma and hue values ( $p > 0.05$ ) (Table 5). The inclusion of high-oleic sunflower cake lamb diets linearly decreased meat moisture content ( $p < 0.01$ ). This result is associated with the increase in fat as lipid content in meat is negatively associated with moisture content [46]. There was no treatment effect on ash and crude protein concentrations of lamb meat ( $p > 0.05$ ) (Table 5). Oliveira et al. [40] observed the same pattern in goats fed sunflower cake. A significant increase in intramuscular fat concentration was observed by Qwele et al. [6] with the inclusion of 170g of sunflower cake in goats' diet.

### 3.3. Fatty Acid Profile

Inclusion of up to 450 g/kg DM of high-oleic sunflower cake in the diet of lambs did not significantly affect lauric (C12:0) and myristic (C14:0) fatty acids ( $p > 0.05$ ) (Table 6). Chikwanha et al. [12] showed that even-chain fatty acids are frequently found in ovine meat and their adverse effects on human health have been widely debated. Fatty acids with 12 to 16 C are undesirable for human health because they raise the serum concentration of low-density lipoprotein, contributing to increased coagulation, inflammation, and insulin resistance [47]. In the present study, palmitic acid (C16:0) concentration decreased ( $p < 0.01$ ) with the inclusion of high-oleic sunflower cake; on the other hand, an increase in stearic acid (C18:0) concentration ( $p < 0.01$ ) was observed. These results are beneficial to human health as palmitic acid is associated with the development of cardiovascular diseases (CVDs) [48], and stearic acid is associated with protection against CVDs [15].



**Table 6.** Fatty acid composition (g/100g fatty acid methyl esters) of the longissimus muscle of lambs fed diets with increased levels of high-oleic sunflower cake.

	High-Oleic Sunflower Cake (g/kg DM)				SEM	p-Value			Equation	R <sup>2</sup>
	0	150	300	450		Q				
						T	L	Q		
C12:0, lauric	0.07	0.08	0.08	0.07	0.003	0.13	0.98	0.23	Y = 0.08	-
C14:0, myristic	1.97	2.04	2.07	2.06	0.065	0.70	0.59	0.68	Y = 2.04	-
C16:0, palmitic	23.94	22.65	22.09	21.70	0.290	<0.01	<0.01	0.09	Y = 29.15 – 0.70x	0.99
C17:0, margaric	1.28	0.96	0.88	0.70	0.035	<0.01	<0.01	0.06	Y = 1.44 – 0.18x	0.93
C18:0, stearic	12.80	12.55	13.91	14.80	0.412	<0.01	<0.01	0.18	Y = 8.48 + 0.73x	0.87
∑ Saturated	41.06	39.11	39.28	39.09	0.245	<0.01	<0.01	0.03	Y = 42.96 – 0.57x	0.80
C16:1c9, palmitoleic	2.46	2.43	2.14	2.06	0.092	<0.01	<0.01	0.77	Y = 3.73 – 0.15x	0.99
C17:1, heptadecenoic	0.92	0.71	0.57	0.47	0.022	<0.01	<0.01	0.02	Y = 1.43 – 0.15x	0.98
C18:1c9, oleic	46.53	48.88	49.60	49.40	0.490	<0.01	<0.01	0.08	Y = 46.92 + 0.90x	0.78
∑ Monounsaturated	53.97	55.46	55.88	56.31	0.484	0.01	<0.01	0.27	Y = 56.34 + 0.75x	0.99
C18:2 n6, linoleic	2.31	2.23	1.86	1.88	0.118	<0.01	<0.01	0.70	Y = 2.90 – 0.16x	0.74
C18:2 c9 t11 rumenic	0.22	0.26	0.26	0.28	0.012	0.01	<0.01	0.43	Y = 0.18 + 0.01	0.77
C18:3 n6, γ-linolenic	0.03	0.03	0.04	0.04	0.002	<0.01	<0.01	0.61	Y = –0.006 + 0.004	0.99
C18:3 n3, α-linolenic	0.07	0.06	0.07	0.07	0.008	0.65	0.50	0.55	Y = 0.07	-
C20:4 n6, araquidonic	0.560	0.536	0.532	0.583	0.027	0.553	0.77	0.36	Y = 0.553	-
C20:5 n3, EPA	0.004	0.004	0.007	0.007	0.0008	0.01	<0.01	0.80	Y = –0.013 + 0.001x	0.99
C22:5 n3, DPA	0.06	0.07	0.06	0.05	0.004	<0.01	<0.01	0.10	Y = 0.03 – 0.006x	0.64
C22:6 n3, DHA	0.007	0.008	0.006	0.009	0.001	0.43	0.47	0.55	Y = 0.007	-
∑ Polyunsaturated	3.38	3.30	2.79	2.95	0.147	0.02	0.01	0.45	Y = 3.50 – 0.18x	0.69
∑ Unsaturated	57.29	59.00	59.00	59.23	0.365	<0.01	<0.01	0.05	Y = 58.42 + 0.58x	0.81
∑ UFA/∑ SFA	1.37	1.49	1.50	1.51	0.015	<0.01	<0.01	0.02	Y = 1.29 + 0.04x	0.99
∑ n3	0.36	0.37	0.33	0.31	0.008	<0.01	<0.01	0.19	Y = 0.37 – 0.02x	0.81
∑ n6	3.01	2.90	2.37	2.38	0.077	<0.01	<0.01	0.51	Y = 2.95 – 0.24x	0.71
n6: n3	8.54	8.00	6.96	7.32	0.314	<0.01	<0.01	0.09	Y = 8.43 – 0.45x	0.66
Δ9-desaturase 16	9.91	9.80	9.46	8.50	0.112	<0.01	<0.01	0.04	Y = 0.37 – 0.02x	0.78
Δ9-desaturase 18	79.18	79.30	78.07	76.89	0.353	<0.01	<0.01	0.07	Y = 79.70 – 0.81x	0.59
Elongase	0.69	0.71	0.72	0.72	0.003	<0.01	<0.01	0.03	Y = 0.64 + 0.01x	0.95
Atherogenicity	0.54	0.53	0.53	0.51	0.004	<0.01	<0.01	0.87	Y = 0.56 – 0.008x	0.81
Thrombogenicity	1.32	1.30	1.24	1.24	0.019	0.02	<0.01	0.64	Y = 1.50 – 0.03x	0.63

SEM: standard error of the mean; R<sup>2</sup>: coefficient of determination; T: treatment effect; L: linear effect; Q: quadratic effect.

Ruminal biohydrogenation can be negatively affected by the high inclusion of polyunsaturated fatty acids in the diet [49]. Additionally, the conversion of C18:1 to C18:0 is impaired by the reduction in rumen pH, resulting from the high intake of rapidly fermentable carbohydrates [50]. In the present study, the fat source and the supply of fibrous carbohydrates, both provided by sunflower cake, contributed to the maintenance of rumen metabolism, favoring complete biohydrogenation and the formation of stearic acid, which was subsequently deposited in the meat. Furthermore, the significant increase in C18:1 dietary intake with the elevated levels of high-oleic sunflower cake may have potentiated the final stage of the ruminal biohydrogenation process by continuously providing a high concentration of the C18:0 precursor.

The inclusion of high-oleic sunflower cake in the diet significantly decreased the sum of the SFA ( $p < 0.01$ ). There was an increase in oleic acid (C18:1 c9) concentration in meat with the inclusion of high-oleic sunflower cake ( $p < 0.01$ ) as a result of the high concentration of this fatty acid in the by-product used. A significant decrease in enzymatic activity of Δ9-desaturase 18 ( $p < 0.01$ ) and Δ9-desaturase 16 ( $p < 0.01$ ) was observed with the inclusion of high-oleic sunflower cake. The reduction in Δ9-desaturase 18 is associated with the modulatory effect of C18:1c9 on sterol regulatory element-binding protein-1c (SREBP-1c), the transcription factor that encodes genes for the enzyme Δ9-desaturase (SCD1) [51]. Choi et al. [52] reported that oleic acid downregulated SCD1 expression in bovine subcutaneous and intramuscular preadipocytes.

The inclusion of up to 450 g/kg DM of high-oleic sunflower cake in the diet reduced the concentration of palmitoleic acid (C16:1c9) in the meat ( $p < 0.01$ ). This monounsaturated fatty acid is associated with increased insulin resistance [53]. This occurrence may have

caused a linear increase in stearic acid ( $p < 0.01$ ), which is the fatty acid with the highest ruminal flow for absorption in the small intestine [54].

The essential fatty acids series n6 (linoleic) and n3 (linolenic) are the main fatty acids that contribute to human well-being and health [55]. In the present study, the inclusion of high-oleic sunflower cake reduced the concentration of these fatty acids in the diets (Table 2), which resulted in a decrease in n6 concentration in meat ( $p < 0.01$ ) (Table 6). Although there was a reduction in linoleic acid concentration in the diets with the inclusion of high-oleic sunflower cake, this result did not affect linolenic acid concentration in the meat ( $p > 0.05$ ). A study with cattle fed sunflower cake up to 27% in DM supports the results for linolenic acid in the meat; however, there were opposite findings for the concentration of linoleic acid and EPA [56]. The inclusion of high-oleic sunflower cake in the diet reduced the sum of PUFA in the meat ( $p < 0.02$ ) as a result of the intrinsic characteristics of the by-product used, which presented a small amount of PUFA (5.66 g/100 g).

The inclusion of high-oleic sunflower cake in the diet resulted in a linear increase in rumenic acid (C18:2 c9 t11) and conjugated linoleic acid (CLA), achieving an approximate 27% increase compared to the control diet. The CLA is a fatty acid associated with the prevention of diseases such as cancer, atherosclerosis, alterations in protein and energy metabolism, and reduced immune response [12,57]. The CLA can be formed through the ruminal biohydrogenation process or by the action of  $\Delta 9$ -desaturase on vaccenic acid in the tissues [58]. In the present study, there was a reduction in  $\Delta 9$ -desaturase activity with the inclusion of high-oleic sunflower cake in the diet, which suggests that the greatest contribution to the increase in CLA in the meat was due to the biohydrogenation process. Possibly, a higher lipid concentration in the diets with the inclusion of high-oleic sunflower cake compared to the control diet increased the supply of fatty acid intermediates of ruminal biohydrogenation in the small intestine. During FA biohydrogenation, including the CLA intermediate, FA continually leaves the rumen, is absorbed across the small intestine, and can be deposited in muscle tissue [59].

The inclusion of high-oleic sunflower cake in the diet of lambs did not affect DHA ( $p > 0.05$ ); however, it increased EPA ( $p < 0.01$ ) and reduced DPA ( $p < 0.01$ ). The conversion of C18: 2 and C18: 3 to PUFA is dependent of the n6/n3 ratio in the diet [60]. Elongase activity increased with the inclusion of high-oleic sunflower cake in the diet ( $p < 0.01$ ); however, it did not increase the concentration of DPA and DHA. This result is associated with greater proportions of C18: 0 and a lower proportion of C16: 0 in lamb meat fed with high-oleic sunflower cake compared to the control diet. The sum of n3 and n6 fatty acids decreased ( $p < 0.01$ ) with the inclusion of sunflower cake, reducing the ratio n6/n3 ( $p < 0.01$ ). Omega-3 fatty acids are precursors to a series of bioactive fatty acids important in reducing the risk of cardiovascular, cancer, and Alzheimer's disease [61]. Desirable values for the n6/n3 ratio in foods for human consumption are between 2 and 6 [62,63]. Although the inclusion of high-oleic sunflower cake reduced the concentration of n3 and n6 fatty acids in the meat, a linear decrease in the atherogenicity and thrombogenicity indices was observed ( $p < 0.01$ ). Lower values of atherogenicity and thrombogenicity indices are associated with a greater amount of anti-atherogenic fatty acids [64]. The inclusion of high-oleic sunflower cake in the diet of the lambs improved the fatty acid profile, as evidenced by a reduction in saturated fatty acids in the meat.

#### 4. Conclusions

Up to 450 g/kg DM dietary supplementation of feedlot lambs with sunflower cake rich in oleic acid did not affect animal performance, even in those lambs that consumed diets containing approximately 9% ether extract. Although the increased levels of high-oleic sunflower cake in lambs diet decreased carcass yield and increased fat deposits, the

inclusion of up to 450 g/kg DM of high-oleic sunflower cake in the diet of the lambs significantly reduced the concentration of hypercholesteremic fatty acids (C14:0 and C16:0), increased the concentration of fatty acids with neutral or protective effects on cardiovascular diseases (C18:0 and C18:1), and that of EPA and CLA in the meat. This study demonstrates the potential use of up to 450 g/kg DM of high-oleic sunflower cake in feedlot diets for lambs to improve nutritional properties of red meat without compromising animal performance.

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