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Meat Science

Soybean molasses increases subcutaneous fat deposition while reducing lipid oxidation in the meat of castrated lambs

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Abstract

This study aimed to evaluate the effect of including soybean molasses (**SM**) on performance, blood parameters, carcass traits, meat quality, fatty acid, and muscle (longissimus thoracis) transcriptomic profiles of castrated lambs. Twenty Dorper × Santa Inês lambs (20.06 ± 0.76 kg body weight [**BW**]) were assigned to a randomized block design, stratified by BW, with the following treatments: CON: 0 g/kg of SM and SM20: 200 g/kg of SM on dry matter basis, allocated in individual pens. The diet consisted of 840 g/kg concentrate and 160 g/kg corn silage for 76 d, with the first 12 d as an adaptation period and the remaining 64 d on the finishing diet. The SM20 diet increased blood urea concentration ($P = 0.03$) while reduced glucose concentration ($P = 0.04$). Lambs fed SM showed higher subcutaneous fat deposition ($P = 0.04$) and higher subcutaneous adipocyte diameter ($P < 0.01$), in addition to reduced meat lipid oxidation ($P < 0.01$). SM reduced the quantity of branched-chain fatty acids in longissimus thoracis ($P = 0.05$) and increased the quantity of saturated fatty acids ($P = 0.01$). In the transcriptomic analysis, 294 genes were identified as differentially expressed, which belong to pathways such as oxidative phosphorylation, citric acid cycle, and monosaccharide metabolic process. In conclusion, diet with SM increased carcass fat deposition, reduced lipid oxidation, and changed the energy metabolism, supporting its use in ruminant nutrition.

Lay Summary

This study investigated the effects of incorporating soybean molasses (**SM**) into the diet of castrated lambs on various aspects of their performance and meat quality. Twenty lambs were divided into two groups: one was fed a control diet without SM whereas the other was fed a similar diet but containing 20% of SM. The feeding trial lasted for 76 d. Results showed that the SM inclusion in the diet led to increased blood urea levels and decreased glucose concentrations. SM inclusion also resulted in lambs with higher levels of subcutaneous fat and larger adipocytes, while reducing meat lipid oxidation. Moreover, SM altered fatty acid composition in the meat, decreasing branched-chain fatty acids and increasing saturated fatty acids. In agreement with these findings, transcriptomic analysis revealed a significant change in the expression of genes related to energy metabolism in the muscle of lambs fed SM. In conclusion, incorporating SM in lamb's diet increased fat deposition, improved meat quality, and induced a transcriptomic change in the muscle energy metabolism, supporting its potential use in ruminant nutrition.

Key words: by-product, fat, soluble carbohydrates, transcriptome

Abbreviations: *a**, red intensity; AIND, ash insoluble in neutral detergent; ADF, acid detergent fiber; ADG, average daily gain; *b** yellow intensity; BCFA, branched-chain fatty acid; BWi, body weight initial; BWf, body weight final; CeTF, coexpression and transcription factor; CLA, conjugated linoleic acids; CKL, cooking loss; CON, control treatment; CP, crude protein; DEG, differential gene expression; DM, dry matter; EE, ether extract; $FADH_2$, flavin adenine dinucleotide; FE, feed efficiency; *L**, lightness; LMA, longissimus muscle area; MDA, malondialdehyde; MM, mineral matter; MUFA, monounsaturated fatty acid; NADH, nicotinamide adenine nucleotide; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; PCA, principal component analysis; PUFA, polyunsaturated fatty acid; RIN, RNA integrity number; SFA, saturated fatty acid; SFT, subcutaneous fat thickness; SM20, soybean molasses treatment; SM, soybean molasses; TMR, total mixed ration; WHC, water holding capacity

Introduction

Finishing lambs in a feedlot system using high-concentrate diets aims to enhance the value of lamb carcasses by accelerating muscular development and fat deposition to produce higher-quality meat products (Van der Merwe et al., 2020). The high digestibility of starch allows efficient energy

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utilization from the diet, along with a significant amount of glucose via propionate, leading to intramuscular fat synthesis (Gilbert et al., 2003; Moharrery et al., 2014). However, high inclusions of corn can lead to metabolic problems such as ruminal acidosis due to a decrease in ruminal pH, resulting in inflammatory responses of the ruminal epithelium and poor fiber utilization (Gozho et al., 2006; Nagaraja and Lechtenberg, 2007).

The inclusion of soybean molasses (**SM**) in diets with a high starch content may represent an alternative to mitigate the aforementioned metabolic problems. For instance, previous studies have observed that the replacement of starch with SM provided greater stability in ruminal pH (Pereira-Junior et al., 2019). This can be explained by the large amount of soluble carbohydrates present in SM (e.g., sucrose and fructose), which can lead to a higher influx of simple sugars into the intestine as they are readily solubilized in water. Indeed, Weisbjerg et al. (1998) have reported that soluble carbohydrates escape from the rumen at a rate similar to water, preventing their ruminal fermentation. With the increased supply of soluble carbohydrates in the intestine, SM may lead to greater lipogenic activity in reserve tissues, including subcutaneous and intramuscular tissue. Accordingly, simple carbohydrates as fructose present in SM have been shown to have a high lipogenic activity (Samuel, 2011). Similarly, diets with inclusion of up to 30% SM increased intramuscular fat by 44% in lambs (Rodrigues et al., 2020).

Therefore, the objective of this study was to investigate the effects of SM inclusion on meat quality of castrated finishing lambs. Castrated animals were considered here given their earlier fat deposition, which could enhance the SM effect. To assess the effects of SM we carried out several analyses related to carcass traits and meat quality, including fatty acid and transcriptome profiles of the longissimus thoracis muscle. Our main hypothesis was that SM inclusion in the diet of castrated lambs improves carcass traits and meat quality by enhancing fat deposition in castrated feedlot-finished lambs.

Materials and Methods

The trial was conducted at São Paulo State University, Jaboticabal campus, Brazil (21°14'05" S latitude, 48°17'09" W longitude, and 615 m altitude). All experimental procedures in this study adhered to the Ethical Principles for Animal Experimentation regulated by the National Council for the Control of Animal Experimentation and approved by the Ethics Committee on Animal Use of the institution (approval number 002583/2020).

Animals and experimental diets

The study was conducted with twenty castrated male lambs (Dorper × Santa Inês; initial body weight of 20.06 ± 0.76 kg; 98 d of age) housed in individual pens (1.2 m²) with elevated slatted flooring inside a covered shed and provided with ad libitum water access from communal water troughs. Prior to the experiment, the lambs underwent preventive health management, including deworming (Closantel 0.8%, Calbos, Curitiba, PR, Brazil), vitamin supplementation (Vit ADEK Calbos, Curitiba, PR, Brazil), and vaccination against clostridiosis (Bovilis Poli-Star T, Kenilworth, NJ, USA).

For the adaptation program established for the finishing diet (840 g/kg concentrate), the animals received, respectively, two adaptation diets containing 480 g/kg and 660 g/kg concentrate for 6 d each. Therefore, on day 13, the assessments of intake and performance were initiated. The diets were formulated to be similar in energy and protein content (Tables 1 and 2), following the NRC (2007) recommendations, differing only in the inclusion of SM (CON: without soybean molasses; SM20: with 200 g/kg soybean molasses on dry matter [**DM**] basis). The composition of the used SM was DM: 548.0 g/kg; mineral matter: 107.5 g/kg; ether extract (**EE**): 13.7 g/kg; crude protein (**CP**): 63.0 g/kg; neutral detergent fiber (**NDF**): 0.0 g/kg; and, non-fiber carbohydrates (**NFCs**): 815.8 g/kg.

Due to physical limitations imposed by pen size, the experiment was ended when lambs achieved an average body weight of 35 kg. Once every 2 wk, animals were weighed without fasting to monitor body weight and determine feeding endpoint and harvesting date. Thus, the experimental period consisted of 12 d on adaptation and 64 d on the finishing diet, totaling 76 d.

Feed intake and performance

The feed provided to the animals was individually weighed daily (corn silage, concentrate, and SM) and manually mixed to homogeneity in the form of a total mixed ration (**TMR**) to prevent selection by the animals. Half of the ration was offered at 0700 hours, while the other half was offered at 1600 hours. Daily, before the next morning meal, the adjustment was made by feed bunk scores, and DM intake was

Table 1. Proximate and chemical composition of the experimental diets containing or not SM

Item	Diets[1]	
	CON	SM20
Proximate composition, g/kg		
Corn silage	160.0	160.0
Corn	640.0	440.0
Wheat bran	57.0	44.0
Citrus pulp	55.0	42.0
Soybean molasses	–	200.0
Soybean meal	53.0	76.0
Urea	8.0	11.0
Mineral salt	17.0	17.0
Sodium bicarbonate	10.0	10.0
Chemical composition, g/kg		
Dry matter	662.4	664.5
Mineral matter	42.8	55.4
Crude protein	140.2	132.8
Ether extract	36.7	53.8
NDFap	222.1	159.6
NFC[2]	573.3	618.9
Starch	522.6	376.0
Soluble carbohydrates[3]	35.1	230.8
Pectin	15.6	12.1
Metabolizable energy, Mcal/kg	3.28	3.33

[1]CON: control diet without the addition of SM; SM20: diet with the addition of 200 g/kg of SM on the DM basis.
[2]Non-fiber carbohydrates: estimated using the Hall equation (2000); Starch and Pectin: Estimated by average obtained in CQBAL 4.0 (Valadares Filho and Lopes, 2018).
[3]Soluble carbohydrates = non-fiber carbohydrates—starch—pectin.

Table 2. Proximate composition of the fatty acid profile in experimental diets containing or not SM

Item, %	Diets[1]	
	CON	SM20
C4:0	0.22	0.39
C6:0	0.07	0.10
C8:0	0.01	0.01
C10:0	0.01	—
C12:0	0.17	0.13
C13:0 anteiso	0.03	0.02
C14:0	0.20	0.20
C15:0	0.04	0.07
C16:0	17.99	21.18
C16:1c9	0.18	0.13
C17:0	0.14	0.14
C17:1	0.03	—
C18:0	2.83	3.30
C18:1 c9	29.54	20.15
C18:1 c11	1.48	1.48
C18:1 c12	0.44	0.07
C18:1 c13	—	0.02
C18:1 t16	—	0.02
C18:2 c9c12	42.98	47.71
C20:0	0.44	0.23
C18:3 ω3	2.34	3.49
C20:1	0.22	0.75
C20:3 ω6	0.19	0.13
C20:4 ω6	<0.01	—
C23:0	0.09	0.06
C24:0	0.34	0.20
C24:1	0.01	—
SFA	22.24	25.52
MUFA	31.90	22.63
PUFA	45.52	51.33
ω6	0.20	0.13
ω3	2.34	3.49
Outros	0.31	0.51

[1]CON: control diet without the addition of SM; SM20: diet with the addition of 200 g/kg of SM on the DM basis.

calculated by weighing the offered ration and leftovers, expressed in kilogram and body weight percentage.

Sampling of leftovers was conducted on alternate days, ensuring a representative quantity of residues. These samples were grouped and stored as composite samples per animal, with each sample being taken every 2 wk. Similarly, feed samples were taken from each diet every 2 wk. Samples were stored at −20 °C until analysis.

Individual performance (average daily gain, **ADG**) was determined by the ratio of body weight obtained after a 16-h solid fasting performed at the beginning and at the end of the experimental period (64 d). Feed efficiency (**FE**) was calculated as the ratio of performance to feed intake

$$\text{ADG} = \frac{(\text{BWf} - \text{BWi})}{\text{feedlot days}} \quad (1)$$

where BWf = final body weight (kg); BWi = initial body weight (kg)

Chemical analysis

Samples of corn silage, feed, and leftovers were dried in a forced air oven at 55 °C for 72 h (method G-001/2), and ground in a Wiley mill with 1 mm sieves. Subsequently, they were placed in an oven at 105 °C for 16 h, along with the concentrate samples, to obtain DM (method G-003/1). Ash content was determined through total combustion in a muffle furnace at 600 °C for 4 h (MM, method M-001/2).

Total nitrogen was determined using the micro-Kjeldahl apparatus (method N-001/2), and CP levels were obtained by multiplying total *N* by 6.25. EE levels were obtained through petroleum ether extraction using a Soxhlet apparatus for a 4-h extraction period (EE, method G-004/1). However, the analysis of fresh SM was carried out as described by AOAC (method 920.39-C) for feed with a high content of soluble carbohydrates, utilizing low-quality hay as an adsorbent.

The contents of NDF and acid detergent fiber (**ADF**) were obtained using filtering crucibles (NDF, method F-013/1, and ADF, method F-015/1, respectively) according to the National Institute of Science and Technology in Animal Science (INCT-CA, Detmann et al., 2021), using thermostable α-amylase, without sodium sulfite. Nitrogen insoluble in neutral detergent was analyzed according to INCT-CA (method N-004/2, Detmann et al., 2021), and nitrogen insoluble in acid detergent by INCT-CA (method N-005/2, Detmann et al., 2021), as well as ash insoluble in neutral detergent (method M-002/2, Detmann et al., 2021) corrected for ash and protein according to the standard analytical procedures of INCT-CA (Detmann et al., 2021). NFC content was estimated using the equation of Hall (2000).

$$\text{NFC} = 1,000 - [(\text{CP} - \text{CPurea} + \text{urea}) + \text{aNDFom} + \text{EE} + \text{MM}] \quad (2)$$

where CP = crude protein; CPurea = crude protein in urea; aNDFom = insoluble fiber in neutral detergent corrected for ash and protein; EE = ether extract; MM = mineral matter.

Lignin analysis was conducted using the acid hydrolysis method (method F-005/2, INCT-CA, Detmann et al., 2021). The metabolizable energy of the diet was estimated according to the equation described by NRC (2001), using the composition of the feeds and ingredients obtained in the laboratory. The values of starch and pectin were estimated using the averages contained in the CQBAL 4.0 database (Valadares Filho e Lopes, 2018), while soluble carbohydrates were obtained by subtracting NFC values from the values of starch and pectin.

Blood parameters

Blood was collected at the beginning (day −6) and the end (day −75) of the experimental period. The first collection aimed to determine the biochemical status of the animals before the experiment, in the initial days of feedlot, while on the adaptation diet. On both occasions, blood samples were drawn from all lambs in the morning, before the first meal, through jugular venipuncture into tubes with clot activator. These samples were used to assess protein metabolism based on total protein and urea, as well as energy

metabolism based on total cholesterol, total triglycerides, and glucose.

After collection, samples were immediately processed in a refrigerated centrifuge at 4 °C, with a rotation of 2,000 rpm for 15 min. The serum obtained after centrifugation was stored at −20 °C until analysis. Analyses were performed using commercial kits provided by Quibasa-Bioclin, Belo Horizonte, MG, Brazil, covering total protein (K-031), urea (K-056), total cholesterol (K-083), total triglycerides (K-117), and glucose (K-082). Readings were performed on an automatic biochemistry analyzer SBA-200 (CELM, Barueri, SP, Brazil).

Slaughter, carcass traits and meat sampling

On the last day of the experiment, lambs were fasted from solids for 16 h, after which they were weighed to determine the final body weight (**BWf**). Subsequently, they were transported to the slaughterhouse, where they were rendered insensible by electronarcosis at two points, followed by the severing of the jugular veins and carotid arteries. After evisceration, the liver and still-warm carcasses were separated and weighed. Carcasses were refrigerated at approximately 4 °C for 24 h. After this period, carcasses were weighed again to determine the cold carcass weight. Carcass yield was calculated using the following equation:

$$\mathrm{CY} = \left(\frac{\text{Hot carcass weight}}{\text{Final body weight}}\right) * 100 \quad (3)$$

On carcass left side, a section was made to expose the longissimus thoracis muscle at the 12th rib for measuring subcutaneous fat thickness (**SFT**) with the aid of a digital caliper and assessment of the longissimus muscle area (**LMA**) using the equation by Silva Sobrinho (1999):

$$\mathrm{LMA} = \left(\frac{A}{2} * \frac{B}{2}\right) * \pi \quad (4)$$

where A is the maximum length of the muscle, and B is the minimum length of the muscle.

The longissimus thoracis muscle from both sides of the carcass was taken between the 6th and 13th ribs and portioned into four chops. The chops on the right side were used for chemical analyses, while the chops on the left side were used for physical analyses. The chops were vacuum-packed (Selovac 200S, São Paulo, Brazil) with 99% vacuum in polyamide/polyethylene bags with 120 µm, 1 $cm^3/m^2/24$ h O_2 permeability, and 3 $cm^3/m^2/24$ h CO_2 permeability measured at 5 °C and 75% relative humidity. The water vapor transmission rate was $3g/m^2/24$ h at 38 °C and 100% relative humidity. A vacuum value of 20 (50 Pa) was used for packing the samples, which were stored at −20 °C until analysis.

Physical analysis of meat

After 24 h of refrigeration, the final pH of the meat was measured directly in the longissimus thoracis muscle on the left side at the 12th rib using a digital H_2 potentiometer (Text 205 Sparta, NJ, USA). Three chops were separated for physical evaluations, which carried out 2 wk after slaughter. Water-holding capacity (**WHC**) was obtained by the difference in weight after 2 g of meat was subjected to a pressure of 10 kg for 5 min, where the weight difference in % represents WHC (Hamm, 1986). Cooking loss (**CKL**) was obtained by cooking approximately 130 g of chop from each animal on a preheated electric grill at 196 °C (George Foreman, Beachwood, OH, USA) until reaching an internal temperature of 75 °C, monitored by a probe thermometer (Incotermp, Porto Alegre, RS, Brazil).

Cooked chops were refrigerated at 4 °C overnight and weighed again the next morning. The difference in weight determined the CKL in %. From the same chop, six cylindrical cores with a diameter of 1.27 cm were taken parallel to the axis of the muscle fiber (AMSA, 1995) for shear force determination (Wheeler et al., 2002) using a texturometer device (CT3 25K Brookfield, Middleboro, MA, USA). Each cylinder was cut in half perpendicular to the axis of the muscle fiber using a Warner Bratzler blade, and the values obtained in kilogram-force (kgf) were converted to newton (N).

Chemical composition of meat

Chemical analyses were carried out 3 wk after slaughter. Two chops were used for chemical analyses of the meat, initially thawed at 4 °C for 24 h and ground using a food processor (method 983.18, AOAC, 1995). Subsequently, they were frozen at −20 °C for lyophilization for 72 h to obtain homogeneous and moisture-free samples. For determination of meat chemical composition, analyses were conducted following the AOAC protocols (1995) for ash (method 920.153) and CP (method 928.08). Intramuscular fat content was measured as total lipids through cold extraction by Bligh and Dyer (1959).

Lipid oxidation analysis (thiobarbituric acid reactive species - TBARS) was assessed based on malondialdehyde (**MDA**) content using thiobarbituric acid, as described by Souza et al. (2011) and Vyncke (1970). In 10 mL of TCA solution (7.5% trichloroacetic acid - TCA, 0.1% edetate disodium salt dihydrate - EDTA), 5 g of lamb meat was added and homogenized with an Ultra Turrax before being centrifuged (15 min, 4,000 rpm, 4 °C). The supernatant was filtered and mixed with thiobarbituric acid (TBA) solution (1% TBA, 15% TCA, and 562.5 µM HCl) in a 1:1 v/v ratio. The mixture was heated in a water bath (100 °C) for 15 min, cooled in an ice bath (5 min), and absorbance was read at 540 nm. MDA concentrations were calculated using a standard curve of 1,3,3-tetramethoxypropane (0 to 40 µM), and the results were expressed in mg MDA/kg of lamb meat.

Instrumental meat color

The assessment of meat color over time began the day after slaughter in the laboratory. For this, one chop was used for instrumental color evaluation, simulating real conditions on supermarket shelves. Samples of longissimus thoracis muscle were packed in polyethylene trays and wrapped in oxygen-permeable film (Goodyear, Americana, São Paulo, Brazil, with oxygen permeability of 8.200 $cm^3/ m^2/d$, and rates (RH) of 262 $cm^3/m^2/d$) without direct contact with the sample. They were placed in a refrigerated display case (Klima Expositor Practice, 05B0500.1, Venâncio Aires, RS, Brazil) at 4 °C ± 1 under fluorescent lights (1,200 lx, 12 h).

Meat color was evaluated on 0, 1, 7, 10, and 14 d of exposure. Three measurements at different locations on the chop were taken to obtain the average, using a colorimeter (CR-300 Minolta Chroma Meter Osaka, Japan) with a large-area aperture of 5 cm in diameter, illuminant D65, and a standard observer angle of 10°, as described by Houben et al. (2000). Before readings, a calibration at room temperature

(24 °C) was performed using a pure white standard (100% reflectance) and a black box (zero reflectance). The evaluated parameters were lightness L^*, red intensity a^*, and yellow intensity b^*, assessed according to the CIE L^*, a^*, b^* color system (CIE Publication, 2004). Additionally, hue angle and chroma saturation index were calculated according to AMSA (2012):

$$\text{hue} = \text{arctang}\ \frac{b}{a}$$

$$\text{Chroma} = \left(a^2 + b^2\right)^{1/2} \quad (5)$$

Adipocyte cellularity

While the carcass was still warm, approximately 2 g samples of longissimus thoracis muscle and subcutaneous fat were collected from the left side, at the 12th rib, and immediately stored in liquid N_2. For determining the size and number of adipocytes, following the method proposed by Etherton et al. (1977) and modified by Prior (1983), samples were thinly sliced (1 mm thickness) while still frozen. These slices were transferred to scintillation vials and fixed with 1% osmium tetroxide (Sigma-Aldrich, St Louis, MO, USA).

Fixed samples were filtered through screens with 250 and 10 µm mesh sizes, aided by 0.01% Triton x-100 buffer (Sigma-Aldrich, St Louis, MO, USA). Tissues retained on the 250 µm mesh were discarded, while those on the 10 µm screen were collected and suspended in a 10-mL solution with 55.5% glycerol. Adipocyte size was determined using image analysis software (Image-Pro v 4.5, Media Cybernetics Inc., Silver Spring, MD, USA), while the number of adipocytes was determined by the equation:

$$\begin{aligned}&\text{Number of cells g}^{-1}\\&= \textit{number cells counted}/((\textit{grid area} * \textit{number grids})/\textit{slide area})\\&* \textit{dilution}/\textit{g tissue}\end{aligned} \quad (6)$$

Fatty acid profile

The fatty acid profile was assessed by gas chromatography in samples from the right side of the longissimus thoracis muscle and the experimental diets.

For meat samples, homogenization was carried out, and fat was extracted using hexane: isopropanol (Hara and Radin, 1978). Methylation was performed according to Christie (1982). The fatty acid profile was determined using a gas chromatograph (Thermo Finnigan, Trace 2000 Thermo Fisher Scientific, Waltham, MA, USA) with a CP-Sil 88 capillary silica column (100 mm × 0.25 mm × 0.2 mm; Agilent Technologies, Ankeny, IA, USA). Hydrogen served as the carrier gas with a flow rate of 1.8 mL/min, and the vaporizer and detector temperatures were set at 250 and 300 °C, respectively. The oven temperature program initiated at 70 °C for 4 min, then increased to 175 °C at a rate of 13 °C/min, holding for 27 min, followed by an increase to 215 °C at a rate of 4 °C/min, holding for 9 min, and finally, a rise to 230 °C at a rate of 7 °C/min, holding for 5 min, with a total ramp duration of 65 min. A 2-µL aliquot of the esterified extract was injected into the chromatograph, and fatty acids were identified by comparing the retention time and percentages of each fatty acid using Chromquest 4.1 software (Thermo Electron, Thermo Fisher Scientific, Waltham, MA, USA).

For experimental diets, a composite sample of each TMR diet, ground to 1 mm, was taken. In the laboratory, 2 mL of methanol/acetyl chloride (20:1) was added to 200 mg of the sample along with 1 mL of hexane and heated in a water bath at 90 °C for 10 min. After cooling to room temperature, 2 mL of distilled water was added, and the mixture was centrifuged at 3,200 rpm for 5 min, following an adaptation by Rodríguez-Ruiz et al. (1998). If necessary to eliminate potential pigments, the supernatant was treated with activated charcoal and silica before being transferred to the chromatographic vial. The standards used were Supelco Component 37 FAME Mix (CRM47885, Supelco, St. Louis MO—USA) and linoleic acid (methyl ester, O5632, Sigma-Aldrich, St. Louis, MO, USA).

$$\Delta 9 \text{ desaturase } 16 \text{ activity} = 100 * \left[\frac{(\text{C16 : 1cis9})}{(\text{C16 : 1cis9}+\text{C16 : 0})}\right] \quad (7)$$

$$\Delta 9 \text{ desaturase } 18 \text{ activity} = 100 * \left[\frac{(\text{C18 : 1cis9})}{(\text{C18 : 1}\textit{cis}9 + \text{C18 : 0})}\right] \quad (8)$$

$$\text{Elongase activity} = 100 * \left[\frac{(\text{C18 : 0} + \text{C18 : 1cis9})}{(\text{C16 : 0} + \text{C16 : 1cis9}) + (\text{C18 : 0} + \text{C18 : 1cis9})}\right] \quad (9)$$

$$\text{Atherogenicity index} = \frac{[\text{C12 : 0} + (4 * \text{C14 : 0}) + \text{C16 : 0}]}{(\Sigma\text{MUFA} + \Sigma n3 + \Sigma n6)} \quad (10)$$

RNA extraction, qualitative analysis, library and sequencing

Approximately 500 mg of longissimus thoracis samples of the left side, at the 12th rib, were collected with the aid of a scalpel blade immediately after evisceration and promptly placed in liquid N_2 before being properly stored in an ultra-freezer at −80 °C.

For RNA sequencing (RNA-Seq), four samples were randomly selected for each experimental diet. Total RNA was extracted from the muscle tissue using a commercial RNA extraction kit (RNeasy Mini Kit, Qiagen, Hilden, Germany), following the manufacturer's instructions. RNA purity, quantity, and integrity of the samples were assessed using a Nanodrop spectrophotometer (Mustiskan GO, Thermo Scientific, Waltham, MA, USA), Qubit 3.0 Fluorometer (Thermo Fisher Scientific), and Bioanalyzer RNA, respectively. All samples had an RNA Integrity Number greater than or equal to 7.5 (Supplementary Table S1). For each sample, 2 µg of RNA was used for library preparation according to the protocol described in the TruSeq RNA Sample Preparation kit v2 guide (Illumina, San Diego, CA, USA). The average library size was estimated using the Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA), and library quantification was performed using quantitative PCR with the KAPA Library

Quantification kit (KAPA Biosystems, Foster City, CA, USA). Quantified libraries were diluted, indexed using the TruSeq DNA CD Index Plate (96 indices, 96 samples, Illumina, San Diego, CA, USA) and pooled at equimolar concentration. All samples were sequenced in a single sequencing lane using the TruSeq PE Cluster v4-cBot-HS kit (Illumina, San Diego, CA, USA) and were clustered and sequenced using the HiSeq2500 equipment (Illumina, San Diego, CA, USA) with the TruSeq SBS Kit v4-HS (200 cycles), following the manufacturer's instructions. All sequencing analyses were performed at the Genomic Center at ESALQ, located in the Animal Biotechnology Laboratory at ESALQ—USP, Piracicaba, São Paulo, Brazil.

Sequencing adapters and low-complexity reads were removed in an initial filtering step using the Trim Galore 0.6.5 software. Only reads with a length greater than 70 nucleotides and a Phred score greater than 33 were retained after trimming. Quality control and read statistics were estimated using FASTQC software version 0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) accessed on June 15, 2021. The reference genome adopted was Ovis_aries_Oar_rambouillet_V1.0104. The abundance (read count) of mRNAs for all annotated genes was calculated using featureCounts from the Rsubread package (Liao et al., 2019).

Identification of differentially expressed genes and transcription factors

Differentially expressed genes (**DEG**) were analyzed through contrasts between treatments using DESeq2, available in the Bioconductor package of the R software, employing a multifactor design (Love et al., 2014). Before statistical analysis, read counts were filtered as follows: (i) genes with zero counts for all samples were considered non-expressed genes; (ii) genes with fewer than one read per sample on average were removed (very low expression); (iii) genes that were not present in at least 50% of the samples were excluded (rarely expressed). The cutoff approach used to identify DEGs aimed to control the Benjamini-Hochberg adjusted *P* value (FDR) at 10%, and the log2 FC values were required to be greater in magnitude than 0.60 (0.60 > log2 FC < -0.60), following previous studies and DESeq2 recommendations (Love et al., 2014; Cesar et al., 2016).

Additionally, genes identified as hub genes in the CeTF analysis (Biagi Junior et al., 2021) were presented, employing regulatory impact factors and partial correlation and information theory analyses described by Reverter et al. (2010) and Reverter and Chan (2008). The sequencing data were submitted to NCBI Gene Expression Omnibus—GEO and are available under the GSE266406 accession number.

Statistical analysis

The experimental design adopted was a randomized complete block design, where the initial body weight was used as a criterion to divide the animals into five homogeneous blocks. Lambs were considered as experimental units ($n = 10$), and each treatment consisted of two replicates within each block (block 1: 15.91 ± 0.75 kg; block 2: 18.01 ± 0.40 kg; block 3: 19.63 ± 0.22 kg; block 4: 21.35 ± 0.64 kg; block 5: 25.39 ± 0.32 kg). According to the statistical model:

$$Yij = \mu + ti + bj + eij \quad (11)$$

where Yij = dependent variable in treatment i and block j; μ = overall mean effect; ti = treatment effect (i = CON and SM20); bj = block effect (j = 1 to 5); eij = random error of the plot that received treatment i in block j.

Instrumental meat color and blood parameters were assessed as repeated measures over time, where diet, evaluation day, and their interaction were considered as fixed effects, and the block was considered as a random effect. In this case, the best covariance structure was selected and adjusted based on the lowest AICC value. The variables of the fatty acid profile C18:2 cis9, cis12; C22:0; C20:5 ω3; C22:6 ω3; C20:2 ω6; and total PUFA did not have a normal distribution; therefore, they were analyzed using the Wilcoxon test for non-parametric data. All other variables were analyzed using the PROC MIXED procedure of SAS software (Inst., Cary, NC), with results considered significant when the *P* value ≤ 0.05 and a trend for *P* value < 0.10 for mean *t* test.

Results

Feed intake and performance

The inclusion of SM in the diet did not influence feed intake, final body weight, and daily gain ($P > 0.05$) of the animals, except for a significant trend for FE ($P = 0.07$; Table 3), where castrated lambs in the SM20 treatment tended to be less efficient compared to the CON treatment.

Blood parameters

In this study, blood parameters showed better results when presented as a repeated measure over time, even though no interaction effect for diet × day was observed ($P > 0.05$; Table 4). The inclusion of SM increased serum urea levels ($P = 0.03$) and decreased blood glucose levels ($P = 0.04$).

Carcass traits and meat quality

There was no effect of diet for most carcass traits ($P > 0.05$), except for subcutaneous fat ($P = 0.04$; Table 5). The inclusion of SM increased subcutaneous fat deposition by 29% (3.09 mm—CON vs. 4.38 mm—SM20).

Levels of MDA in the meat of castrated lambs fed SM (SM20) decreased compared to the control (CON), reducing lipid oxidation in the longissimus thoracis muscle by 49% ($P < 0.01$; Table 6). For other variables such as pH, LMA, WHC, CKL, and WBSF, there was no significant effect ($P > 0.05$).

Table 3. Feed intake and performance of castrated male lambs finished in feedlot fed or not SM

Item	Diets[1]		SEM[2]	*P* value
	CON	SM20		
Dry matter intake, g	1,152.72	1,139.46	47.52	0.87
Dry matter intake, %BW	4.27	4.24	0.11	0.90
Crude protein intake, g	184.95	182.22	7.43	0.84
Feed efficiency	0.20	0.17	0.01	0.07
Initial body weight, kg	20.87	21.30	0.66	0.29
Final body weight, kg	35.21	34.41	1.00	0.61
Average daily gain, g	227.70	208.10	11.59	0.38

[1]CON: control diet without the addition of SM, $n = 10$; SM20: diet with the addition of 200 g/kg of SM on the DM basis, $n = 10$ per diet.
[2]Standard error of the mean.

Table 4. Blood parameters of castrated male lambs finished in feedlot fed or not SM, evaluated at the beginning and end of the experimental period

Item	Diets[1]				SEM[2]	*P* value	
	CON		SM20				
	Initial	Final	Initial	Final		Diet	Diet × period
Protein total, g/dL	4.41	4.32	4.26	4.32	0.13	0.63	0.63
Urea, mg/dL	25.60	27.47	29.49	31.09	1.30	0.03	0.95
Cholesterol, mg/dL	55.19	58.44	55.63	59.15	3.22	0.74	0.85
Triglycerides, mg/dL	32.51	32.99	31.59	32.60	2.36	0.66	0.96
Glucose, mg/dL	93.13	91.92	85.69	87.28	2.06	0.04	0.62

[1]CON: control diet without the addition of SM, $n = 10$; SM20: diet with the addition of 200 g/kg of SM on the DM basis, $n = 10$ per diet.
[2]Standard error of the mean.

Table 5. Carcass traits of castrated male lambs finished in feedlot fed or not SM

Item	Diets[1]		SEM[2]	*P* value
	CON	SM20		
Hot carcass weight, kg	18.18	17.84	0.61	0.69
Cold carcass weight, kg	17.79	17.44	0.60	0.68
Carcass yield, %	51.49	51.76	0.56	0.77
Cooling loss, %	2.20	2.26	0.10	0.36
Liver, g	642.10	653.40	23.21	0.80
Subcutaneous fat thickness, mm	3.09	4.38	0.40	0.04

[1]CON: control diet without the addition of SM, $n = 10$; SM20: diet with the addition of 200 g/kg of SM on the DM basis, $n = 10$ per diet.
[2]Standard error of the mean.

Instrumental color of meat

The analysis of meat color on display aims to simulate supermarket shelves, demonstrating myoglobin oxidation over time. In the present study, there was no effect of diet and no interaction between diet and evaluation day on the assessed color parameters ($P > 0.05$; Table 7). However, the storage time significantly affected meat color parameters ($P < 0.05$; Supplementary Figure S1), regardless of the treatment. The L^* had little change throughout the 14-d display period. The b^* and Chroma reached higher value on day 1 of storage and showed a reduction over the storage period. In addition, a^* and a^*/b^* parameters decreased, while the hue parameter increased, over time.

Adipocyte cellularity

The results of adipocyte size exceeded the limits established by the mesh size of screens used in this study (10 and 250 µm) due to cellular clustering, which, combined with osmium staining, made it difficult to delineate adipose cells. Adipocyte cellularity was analyzed to better understand the effect of SM on adipocyte development and differentiation. As a result, SM increased the average diameter of subcutaneous adipose cells ($P < 0.01$; Table 8), as well as the proportion of adipocytes with average areas between 100 and 300 µm² ($P < 0.01$), consequently reducing the proportion of cells smaller than 100 µm² ($P < 0.01$). The SM effect on intramuscular adipocytes within the 100 to 300 µm² range appears to be more attributed to data dispersion than a genuine effect of the diet.

Fatty acid profile

The inclusion of SM influenced fatty acid profile in lamb meat. The SM20 diet increased the levels of saturated fatty acids (**SFA**) C8:0, C10:0, C16:0, C18:0, and total SFA ($P < 0.05$; Table 9). Conversely, it reduced the levels of C16:0, C17:0 iso, and total branched-chain fatty acids (**BCFA**) ($P < 0.10$). However, C10:1, C18:1 cis12, and C18:1 trans16 showed a significant trend with higher levels in animals fed SM20 ($P < 0.10$). For the remaining variables, monounsaturated fatty acids (**MUFA**), polyunsaturated fatty acids (**PUFA**), and saturation indices, there was no significant effect ($P > 0.05$).

Transcriptome profile

The entire transcriptome of eight samples from the longissimus thoracis muscles of feedlot-finished castrated lambs was sequenced using the RNA-Seq technology. The concentration and integrity of mRNA from all samples are listed in Supplementary Table S1. Sequencing data were evaluated by contrasting CON vs. SM20, resulting in 11,247 expressed genes used in the analysis of DEGs and CeTF.

The transcriptome analysis revealed 294 DEGs (FDR < 0.10; Figure 1 and Supplementary Table S2), of which 171 were upregulated and 123 were downregulated for SM20 compared to CON. Additionally, the gene *ACOX2* was identified as exclusive to CON, and *MFSD4B* was exclusive to SM20, with log2 fold changes (**FCs**) of 2.08 and 1.22, respectively (Supplementary Table S3).

The principal component analysis containing the DEGs and transcription factors accounted for 33.2% of variability in Principal Component 1 and 13.5% of variability in Principal Component 2 (Figure 2). The CON diet was distributed in the positive side of Principal Component 1 (Dim1), with major influences from genes such as *FH* (–6.61), *COQ7* (–6.59), *NDUFA12* (–7.05), *AIFM1* (–6.75), *COQ9* (–7.64), *NDUFAV1* (–7.67), *NDUFS8* (–8.00), *NDUFS6* (–7.87), *GUK1* (–7.93), and *SCUBE2* (–7.23). Meanwhile, the SM20 diet was distributed in the negative side of Principal Component 1 (Dim1), with major influences from genes such as *AP4E1* (7.32), *EEA1* (7.28), *UBR3* (7.60), *DDX6* (7.77), *CACNA2D1* (7.65), *UGGT1* (7.77), *TARBP2* (7.71), *SLF2* (7.56), *FASTK* (7.74), *PLCE1* (7.63).

Network analysis was employed to investigate differences in co-expressed genes and identify transcription factors. The CeTF analysis identified 172 transcription factors for

Table 6. Analysis of the longissimus thoracis muscle of castrated male lambs finished in feedlot fed or not SM

Item[1]	Diets[2]		SEM[3]	*P* value
	CON	SM20		
Physical analysis				
pH	5.59	5.56	0.01	0.30
LMA, cm^2	13.03	12.50	0.58	0.63
Water holding capacity, WHC %	81.16	82.08	0.61	0.13
Cooking loss, CKL %	26.38	28.60	1.87	0.22
WBSF, N	29.02	27.91	1.31	0.69
Chemical analysis				
Moisture, %	75.33	75.04	0.30	0.39
Ash, %	1.18	1.15	0.03	0.59
Crude protein, %	19.08	19.11	0.25	0.93
Total lipids, %	4.53	4.71	0.12	0.47
Malondialdehyde, MDA mg/kg	0.75	0.38	0.07	<0.01

[1]LMA: Longissimus muscle area; WBSF: Warner Bratzler—Shear Force.
[2]CON: control diet without the addition of soy molasses, $n = 10$; SM20: diet with the addition of 200 g/kg of soy molasses on the DM basis, $n = 10$ per diet.
[3]Standard error of the mean.

Table 7. Effect of aging on color of meat from castrated male lambs finished in feedlot fed or not SM

Item[1]	Diets[2]	Storage day					SEM[3]	*P* value		
		0	1	7	10	14		Diet	Day	Diet × day
*L**	CON	46.03	46.00	46.32	47.0	46.93	0.22	0.36	0.04	0.79
	SM20	46.71	46.55	47.92	48.44	47.38				
*a**	CON	13.56	14.43	10.11	9.77	9.37	0.24	0.99	<0.01	0.97
	SM20	13.27	14.46	10.03	9.79	9.55				
*b**	CON	5.68	7.91	6.07	6.05	6.35	0.13	0.87	<0.01	0.99
	SM20	5.61	8.10	6.11	6.29	6.44				
*a**/*b**	CON	2.42	1.84	1.68	1.62	1.53	0.04	0.45	<0.01	0.99
	SM20	2.43	1.79	1.67	1.60	1.52				
Chroma	CON	14.71	16.46	11.80	11.50	11.34	0.25	0.95	<0.01	0.98
	SM20	14.43	16.58	11.75	11.66	11.54				
Hue	CON	22.64	28.71	30.98	31.88	34.29	0.50	0.71	<0.01	0.97
	SM20	22.75	29.27	31.27	32.45	33.79				

[1]*L**: Lightness; *a**: red intensity; *b**: yellow intensity.
[2]CON: control diet without the addition of SM, $n = 10$; SM20: diet with the addition of 200 g/kg of SM on the DM basis, $n = 10$ per diet.
[3]Standard error of the mean.

the CON vs. SM20 comparison (Supplementary Table S4). Additionally, functional analysis (gene ontology (**GO**) and pathway enrichment) was conducted with all DEGs and transcription factors using the ClusterProfiler package in the R software, grouping biological processes into GO terms and Kyoto Encyclopedia of Genes Genomes (KEGG) signaling pathways.

Over-represented pathways are illustrated in Figures 3 and 4, highlighting pathways such as response to reactive oxygen species (GO:0302; $P < 0.01$; Supplementary Table S5), response to oxidative stress (GO:6979; $P < 0.01$), oxidative phosphorylation (Hsa0190; $P < 0.01$; Supplementary Table S6), insulin resistance (Hsa04910; $P = 0.09$), citrate cycle TCA (Hsa0020; $P < 0.01$), as well as starch and sucrose metabolism (Hsa0500; $P = 0.08$).

The calculation of the statistical power for non-significant variables, but with a direct relationship with the study hypothesis, indicated that the lack of effect may have been due to the sample size (intramuscular fat—power = 55%, intramuscular adipocyte area—power = 82%, intramuscular adipocyte cell number—power = 82%, WBSF—power = 22%).

Discussion

In the production system, lamb castration is adopted to enhance carcass and meat quality (Claffey et al., 2018; Torres et al., 2024). Its effects are related to early fat deposition in the carcass, sensory properties, as well as changes in the lipid profile of the meat, influencing meat shelf life (Gkarane et al.,

Table 8. Adipocyte cellularity of castrated male lambs finished in feedlot fed or not SM

Item	Diets[1]		SEM[2]	*P* value
	CON	SM20		
Subcutaneous tissue				
Diameter, μm	15.95	18.18	0.55	<0.01
Area, μm²	287.45	317.26	17.94	0.17
Number of cells × 10^4 g^{-1}	1.7	1.8	0.21	0.81
% < 100 μm²	29.48	13.28	3.64	<0.01
100 < % < 300 μm²	36.57	59.05	4.31	<0.01
300 < % < 500 μm²	17.53	18.85	1.85	0.47
% > 500 μm²	19.69	12.59	2.57	0.23
Intramuscular tissue				
Diameter, μm	16.61	16.74	0.64	0.93
Area, μm²	291.73	338.70	22.42	0.31
Number of cells × 10^3 g^{-1}	5.16	4.37	0.38	0.27
% < 100 μm²	31.16	40.37	3.40	0.19
100 < % < 300 μm²	39.20	29.25	2.26	0.02
300 < % < 500 μm²	12.30	10.98	1.37	0.96
% > 500 μm²	18.57	19.40	2.08	0.85

[1]CON: control diet without the addition of SM, n = 10; SM20: diet with the addition of 200 g/kg of SM on the DM basis, n = 10 per diet.
[2]Standard error of the mean.

2017; Gravador et al., 2018). Therefore, our initial hypothesis was that associating the inclusion of SM in the diet with lamb castration leads to increased fat deposition both in the carcass and meat. This hypothesis was partially accepted because the increased fat deposition was limited to the carcass in the form of subcutaneous fat.

The inclusion of SM in the diet did not influence animal feed intake and performance, which indicates the by-product is a viable feedstuff in ruminant nutrition that can be used as a macro-ingredient substitute for corn in feedlot diets without causing rejections and declines in animal productivity. Arruda et al. (2021) and Rodrigues et al. (2020) evaluated SM inclusions of up to 30% in the DM of the diet and observed no effects on lamb feed intake and performance. Previous reports indicate that SM-containing diets have a higher digestibility (Pereira Junior et al., 2018; Arruda et al., 2019), an effect that may be attributed to the carbohydrate solubility that SM adds to the diet (35.1—CON vs. 230.8 g kg^{-1} SM20 of soluble carbohydrates). However, it is worth noting that these studies were based on in vitro assays, which for diets with SM do not represent the most accurate method for estimating digestibility.

High inclusions of SM (above 10% in DM) indicate a greater imbalance between energy and protein in the rumen. The high solubility of soluble carbohydrates in SM allows for easy escape of microbial fermentation, resulting in the conversion of protein and non-protein nitrogen into ammonia in the rumen, and this, in turn, into urea by the liver (Hailemariam et al., 2021). Pereira-Junior et al. (2019), when evaluating inclusions of up to 30% of SM in DM, found a reduction in ruminal NH_3, indicating greater epithelial absorption. In turn, our current study found higher levels of urea in the blood, indicating high activity in the urea cycle. Therefore, the imbalance between energy and protein in the rumen prevented the lambs which fed SM from having greater gains, justifying the absence of effects on performance in the present study. However, a comprehensive metabolism study is needed for a better understanding of the synchrony pattern between energy and protein in diets with high SM.

With respect to fat deposition, lambs in the present study were slaughtered close to the maturity weight of 32.5 kg reported for Dorper × Santa Inês crossbred lambs (Malhado et al., 2009). In other words, lambs were slaughtered near the point at which they reached the peak of their body growth, when fat deposition is expected to increase. The inclusion of SM in the lambs' diet possibly provided a greater energy supply in the gut, derived from ruminal escape, which was likely allocated in energy storage tissues, e.g., adipose tissue, following its dissociated from protein. Lambs which fed SM20 had increased SFT, as supported by adipocyte hypertrophy, but had no effect on intramuscular fat deposits.

Considering that fat is first deposited in visceral fat, followed by subcutaneous, intermuscular, and intramuscular tissues of ruminants (Pewan et al., 2020), it can be inferred that intramuscular fat might increase if lambs were kept for a longer time under the SM20 diet, since CON lambs seemed to have higher β-oxidation activity as indicated by increased expression of the *ACOX2* gene involved in the degradation of long-chain fatty acids (Ferdinandusse et al., 2018). Accordingly, SM inclusion significantly increased intramuscular fat content when lambs were fed for a longer period and with higher levels of SM (Rodrigues et al., 2020), compared to this study. In keeping with this, when evaluating the addition of 4.3% fructose-rich corn syrup diluted in water for beef cattle, Volpi-Lagreca and Duckett (2016) reported differences only in adipocyte size, justifying the lack of effect on subcutaneous and intramuscular fat content due to the low dosage adopted and the short experimental period.

The increased intestinal energy supply from soluble carbohydrates is supported by the transcriptome results. Pathways such as the monosaccharide metabolic process were highly represented in the CON vs. SM20 contrast, with the *HK2* gene upregulated for SM20 being particularly noteworthy, a finding that may support a higher circulation of soluble sugars in the muscle, possibly fructose, as HK2 phosphorylates fructose with higher affinity than glucose (Hapeta et al., 2021). Additionally, *MFSD4B*, reported as a glucose and fructose transporter dependent on Na^+ in rats (Horiba et al., 2003a, 2003b), was upregulated and exclusively expressed in lambs of the SM20 group. This potential increase of fructose in the muscle tissue may be responsible for the higher local lipogenic activity thus contributing to fat accumulation in carcasses and meat, as reported by Rodrigues et al. (2020).

Indeed, SM seems to be associated with modifications in the simple carbohydrate metabolism in the muscle tissue, as its inclusion led to changes in the insulin signaling pathway and resistance, with increased expression of *PPP1R3A*, *GYS1*, and *SLC2A4* in the SM20 diet, which are involved in glycogen synthesis and intracellular glucose transport (Grindflek et al., 2002; Zuo et al., 2005; Savage et al., 2008). Elsharkawy et al. (2021) demonstrated an association of low expression of *PPP1R3A* with higher pH values in chicken meat, reinforcing its role in muscle glycogen synthesis, which determines the drop in meat pH in the postmortem period. Thus, establishing a relationship between changes in muscle energy metabolism conferred by SM and meat quality.

The greater SFT is of utmost importance for meat quality; carcasses with low fat are more prone to muscle fiber

Table 9. Fatty acid profile in longissimus thoracis muscle of castrated lambs finished in feedlot fed or not SM

Variable, mg/100 g meat		Diets[1]		SEM[2]	*P* value
		CON	SM20		
Saturated fatty acids—SFA					
Caproic acid	C6:0	0.36	0.56	0.09	0.36
Caprylic acid	C8:0	0.49	0.88	0.09	0.03
Capric acid	C10:0	6.91	9.47	0.67	0.03
Undecanoic acid	C11:0	0.18	0.21	0.03	0.63
Lauric acid	C12:0	5.74	5.52	0.66	0.57
Tridecanoic acid	C13:0	0.41	0.32	0.05	0.25
Myristic acid	C14:0	109.00	118.30	7.21	0.13
Pentadecanoic acid	C15:0	14.77	12.19	1.20	0.52
Palmitic acid	C16:0	1,071.23	1,171.46	54.92	0.01
Margaric acid	C17:0	53.11	45.96	3.32	0.37
Stearic acid	C18:0	703.12	839.24	36.45	0.04
Arachidic acid	C20:0	2.65	2.71	0.16	0.87
Behenic acid	C22:0	0.52	0.44	0.08	0.94
Total		1,968.50	2,175.11	92.53	0.01
Branched-chain fatty acids—BCFA					
Isomyristic acid	C14:0 iso	0.57	0.44	0.05	0.13
Isopentadecanoic acid	C15:0 iso	2.66	2.13	0.17	0.19
Sarcinic acid	C15:0 anteiso	4.61	3.86	0.31	0.40
Isopalmitic acid	C16:0 iso	3.18	2.15	0.24	0.03
Isomargaric acid	C17:0 iso	9.93	7.69	0.60	0.06
Total		20.96	16.26	1.22	0.05
Monounsaturated fatty acids—MUFA					
Decenoic acid	C10:1	0.07	0.18	0.03	0.06
Dodecenoic acid	C12:1	0.08	0.07	0.01	0.68
Myristoleic acid	C14:1 c9	3.05	3.46	0.25	0.42
Palmitoleic acid	C16:1 c9	77.85	85.75	4.83	0.50
Heptadecenoic acid	C17:1	24.46	19.88	1.60	0.16
trans-Vaccenic acid	C18:1 trans	186.41	176.63	13.08	0.92
Oleic acid	C18:1 c9	1,684.50	1,958.96	84.75	0.11
cis11-Vaccenic acid	C18:1 c11	112.63	124.13	6.59	0.30
cis12-Vaccenic acid	C18:1 c12	23.42	30.35	1.79	0.07
cis13-Vaccenic acid	C18:1 c13	6.28	6.83	0.35	0.44
trans16-Vaccenic acid	C18:1 t16	1.85	2.71	0.29	0.08
cis15-Vaccenic acid	C15:1 c15	2.75	2.74	0.22	0.97
Eicosenoic acid	C20:1	3,39	3,58	0.21	0.67
Erucic acid	C22:1 ω9	4.22	3.97	0.35	0.96
Total		2,130.95	2,426.94	98.74	0.14
Polyunsaturated fatty acids—PUFA					
Linoleic acid	C18:2 c9,c12	179.23	167.66	7.46	0.40
γ-Linolenic acid	C18:3 ω6	1.45	1.42	0.11	0.88
α-Linolenic acid	C18:3 ω3	3.74	4.20	0.22	0.21
Conjugate linoleic acid, CLA	C18:2 c9,t11	18.89	19.29	1.51	0.90
Eicosadienoic acid, EDA	C20:2 ω6	0.39	0.43	0.09	0.91
Dihomo-γ-linolenic acid	C20:3 ω6	3.37	3.38	0.23	0.97
Arachidonic acid	C20:4 ω6	42.29	45.45	3.04	0.62
Eicosapentaenoic acid, EPA	C20:5 ω3	0.79	0.96	0.10	0.36
Docosapentaenoic acid, DPA	C22:5 ω3	3.51	4.27	0.28	0.19
Docosahexaenoic acid, DHA	C22:6 ω3	0.16	0.21	0.07	0.56
Total		253.82	247.53	11.07	0.53
ω-3		8.20	9.90	0.52	0.10

Table 9. Continued

Variable, mg/100 g meat	Diets[1]		SEM[2]	*P* value
	CON	SM20		
ω-6	66.39	69.96	3.89	0.66
Indices				
ω-6/ω-3	8.18	7.21	0.37	0.20
Desaturase 16	6.82	6.38	0.19	0.25
Desaturase 18	70.84	69.57	0.90	0.50
Elongase	68.00	67.89	0.43	0.60
Atherogenicity	0.69	0.69	0.02	0.61

[1]CON: control diet without the addition of SM, *n* = 10; SM20: diet with the addition of 200 g/kg of SM on the DM basis, *n* = 10 per diet.
[2]Standard error of the mean.

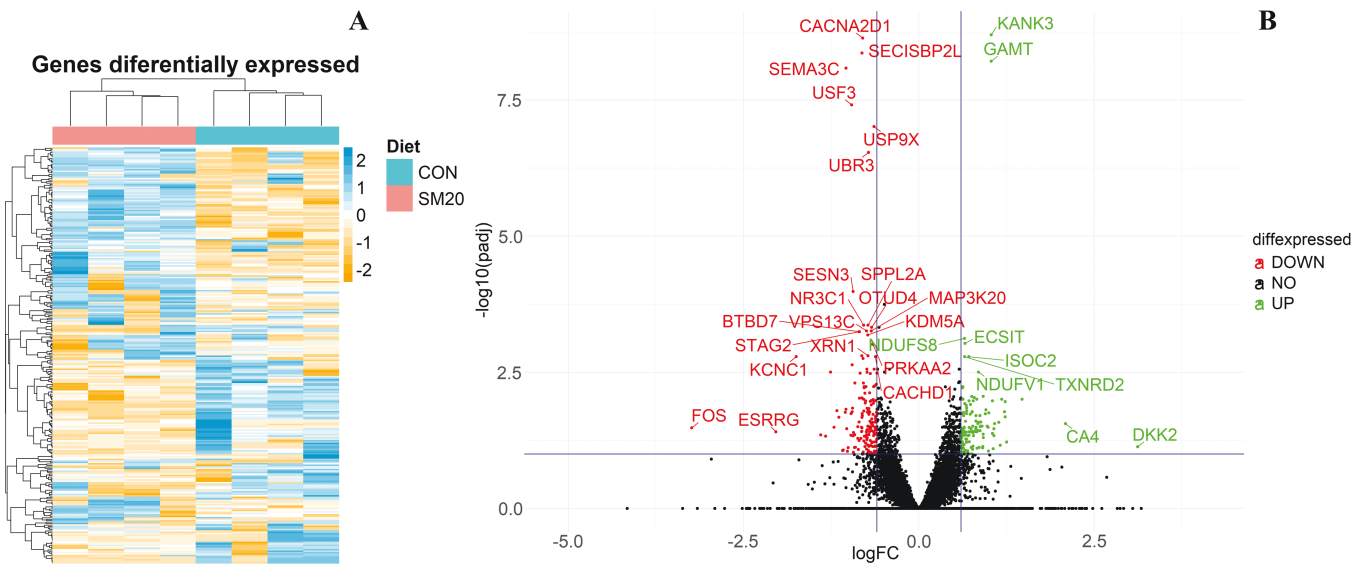

Figure 1. (A) Heatmap graph showing differentially expressed genes. (B) Volcano plot graph showing differentially expressed genes between animals on CON vs SM20 diets, *n* = 4 per diet.

shortening due to cold exposure during rigor mortis, contributing to tougher meat (Savell et al., 2005). In the present study, the effect of SM on carcass fat deposition was remarkable, with a 29% increase in subcutaneous fat, predominantly with larger adipocytes in animals feeding the by-product. However, in both treatments, carcasses were above the 2.5 mm minimum coverage to guarantee carcass protection (Savell et al., 2005). In subcutaneous tissue, the preference is for acetate utilization as a substrate for fat synthesis, rather than glucose (Smith et al., 2018). However, in the current study, this does not appear to be the reason for the greater fat deposition in this tissue. Previous studies have shown that replacing corn by SM does not seem to lead to modifications in the profile of short-chain fatty acids when considering inclusions of up to 20% (Almeida et al., 2018) and 30% SM in the diet (Pereira-Junior et al., 2019). The current study did not focus on analyzing in detail the lipid metabolism in subcutaneous tissue though, thus not allowing us to infer how SM affected fat deposition and highlighting the need for further research seeking to elucidate the underpinning mechanisms.

The greater fat deposition, in turn, explains the trend to lower FE observed in SM20 lambs. More efficient lambs have leaner meat (Zhang et al., 2023), due to the higher energy cost of fat deposition compared to protein deposition. The energy allocation of adipose tissue is 39.3 kJ/g compared to 23.1 kJ/g for other organic components, such as proteins (Liu et al., 2024). Consequently, the energy demand for fat deposition is substantially higher, agreeing with previous reports where inclusion of SM significantly decreased FE in lambs (van Cleef et al., 2018; Rodrigues et al., 2020).

In keeping with the lower FE of animals which fed SM, key pathways related to energetic metabolism were dysregulated in the muscle of SM20 lambs such. For instance, 32 genes belonging to oxidative phosphorylation and 6 genes (e.g., *IDH2, FH, MDH2, OGDH, PDHB,* and *SUCLG1*) belonging to the citric acid cycle were all upregulated in the CON group. This finding suggests that SM inclusion impaired mitochondrial function, corroborating previous reports that efficient animals have higher electron transport chain activity in the muscle compared to less efficient ones (Sharifabadi et al., 2012; Fernandez et al., 2020). Taking into account that SM20

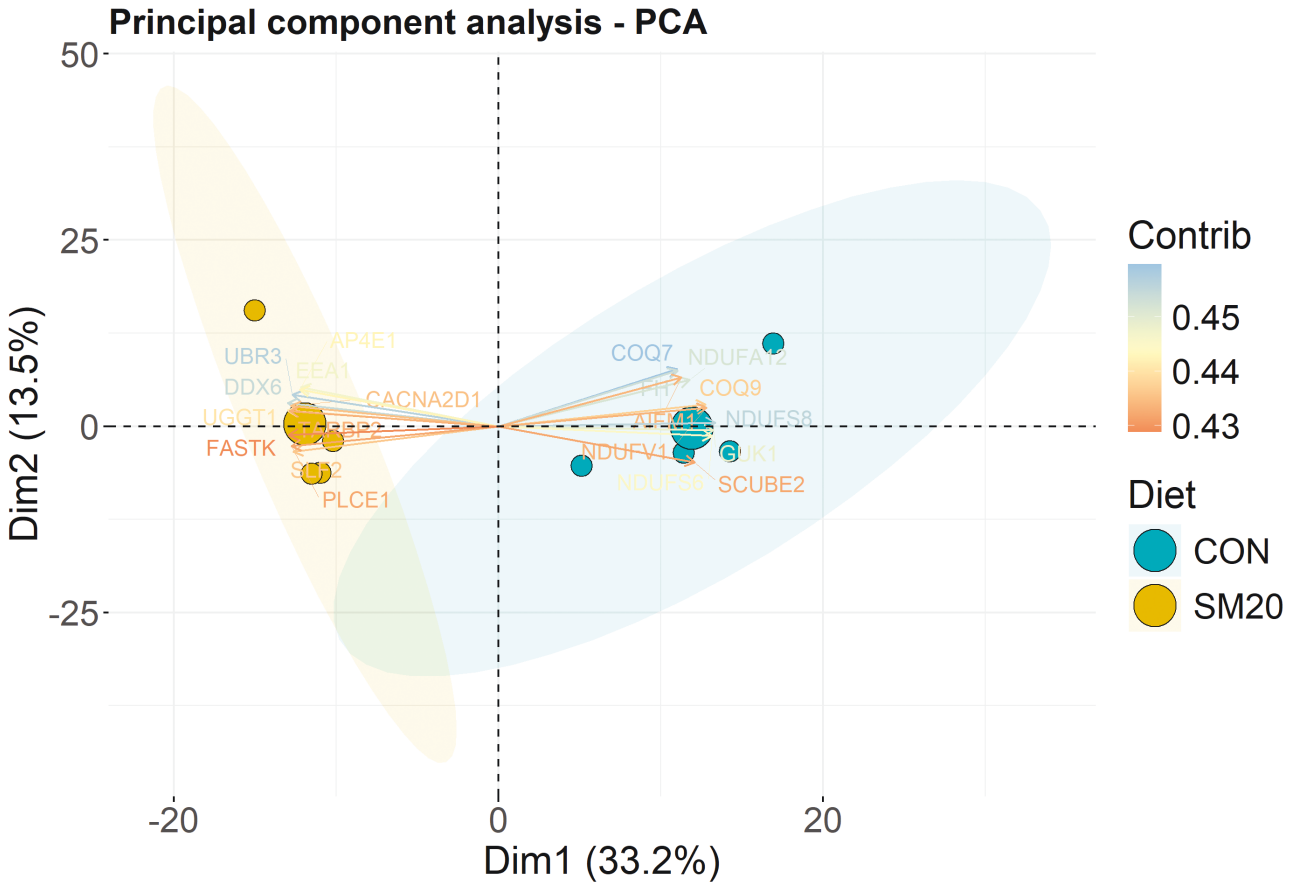

Figure 2. Principal component analysis scatter plot based on differentially expressed genes and transcription factors between animals on CON vs SM20 diets, *n* = 4 per diet.

contained 37.6% of starch against 52.3% in the CON diet, and that starch has a higher energy density than monosaccharides and disaccharides present in the SM20 diet (Broderick et al., 2008), the lower amount of glucose derived from ruminal escape of starch and propionate metabolism (gluconeogenesis) in the liver may explain the lower blood glucose in SM20 lambs. Accordingly, Membrive (2016) has shown a positive association between propionate and blood glucose indicative of increased gluconeogenic activity in the liver of CON lambs.

Increased fat deposition in carcass and meat raises some concerns about meat quality, especially in ovine meat, which has a strong and distinctive flavor. Excessive subcutaneous fat depositions can hinder meat oxygenation, leading to changes on meat color due to low myoglobin oxygenation, which was not the case in our study (Vergara et al., 1999). Meanwhile, intramuscular fat is related to lipid oxidation, which can lead to meat rancidity. Lipid oxidation is closely associated with meat durability, being the primary non-microbial cause of quality deterioration, leading to the loss of essential fatty acids and vitamins. Lipid oxidation is defined as the reaction of PUFA with oxygen through free radical mechanisms, resulting in the formation of hydroperoxides as the initial reaction products. However, these hydroperoxides are highly unstable, and their decomposition is responsible for unpleasant flavors and odors in meat (Domínguez et al., 2019).

Therefore, the high concentration of PUFA is directly related to less durable products. However, in the present study, there were no differences in individual and total PUFA levels; thus, this is not the reason for the lower lipid oxidation observed in SM20-fed animals. Although lipid oxidation occurs naturally, it is not spontaneous; the oxygen state must be singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), or hydroxyl radical (OH) (Domínguez et al., 2019). As mentioned earlier, the gene expression profile supports a lower mitochondrial activity in of the SM20 group. If this is the case, one would expect a decreased release of reactive oxygen species, thus making SM20 meat less prove to oxidation. Additionally, Rakita et al. (2021) also reported the presence of isoflavones in SM, with antioxidant properties in poultry, which could represent an additional reason why lipid oxidation was decreased in SM20-fed lambs.

It is worth noting that SM inclusion enriched the meat with palmitic acid (C16:0), which is well associated with a positive energy status of the animal. Earlier reports have shown, for instance, that increased intake and deposition of C16:0 result in higher weight gains and increased body reserves, being the C16:0 the first product in the de novo fatty acid synthesis (Loften et al., 2014; Bai et al., 2023). On the other hand, SM reduced the content of BCFA in the muscle. This effect can likely be explained by lower level of fiber in the SM20 diet given that BCFA such as C15:0 iso are mainly derived from microbial synthesis (Fievez et al., 2012; Parente et al., 2020). On the hand, the lower fiber content in the SM20 diet did not seem to affect ruminal pH, as indicated by the higher muscle content of stearic acid (C18:0), the content of which is narrowly linked to biohydrogenation in the rumen (Santos-Silva et al., 2016). Agreeing with this, SM inclusion up to 30% in the diet of adult sheep directly increased ruminal

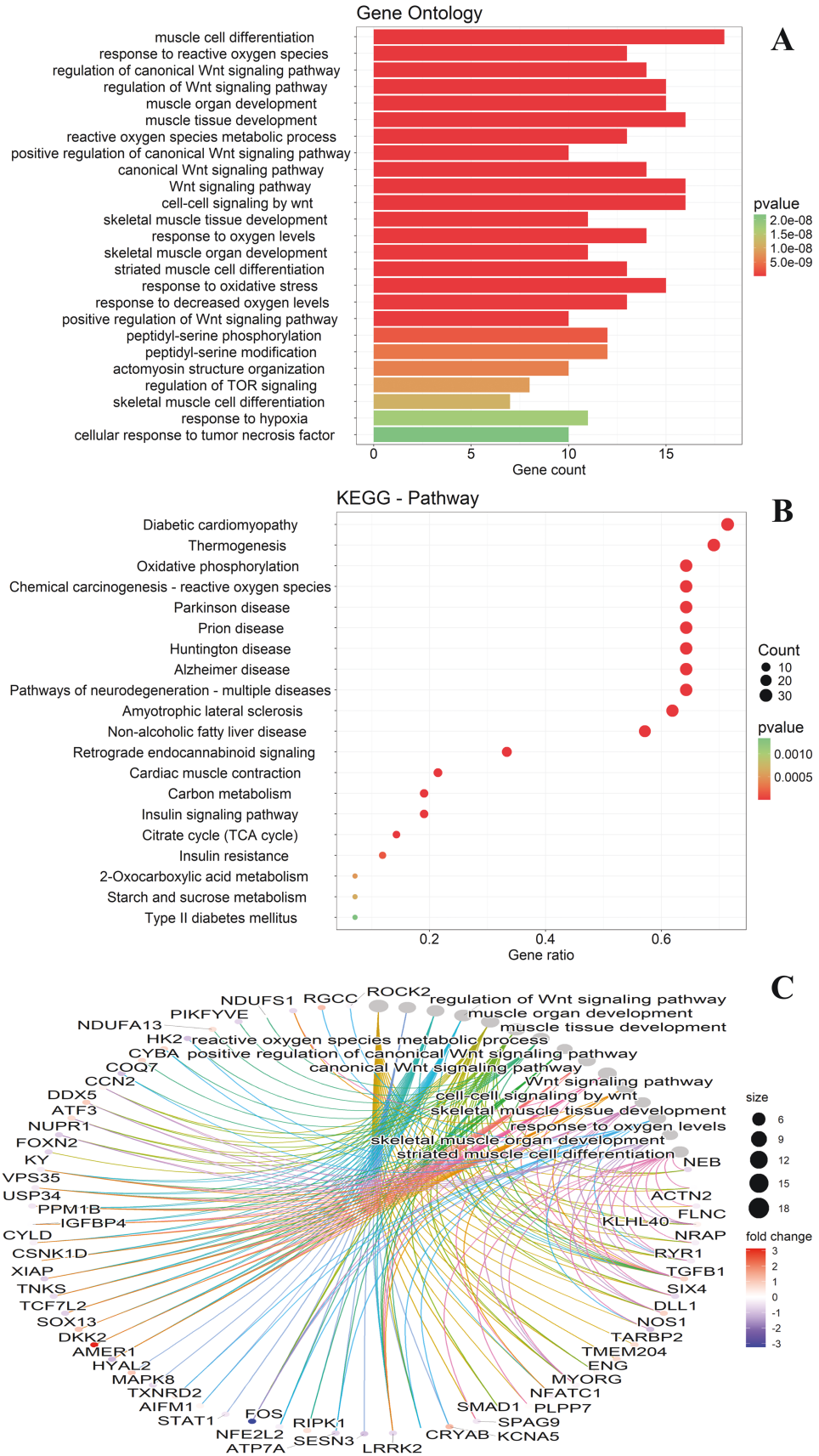

Figure 3. (A) Barplot graph with the main ontological terms over-represented in GO, (B) Dotplot graph with the main pathways over-represented in Kyoto Encyclopedia of Genes and Genomes (KEGG), (C) Cnetplot graph with the main biological processes and their respective genes, showing expression in the contrast between animals on CON vs SM20 diet, $n = 4$ per diet.

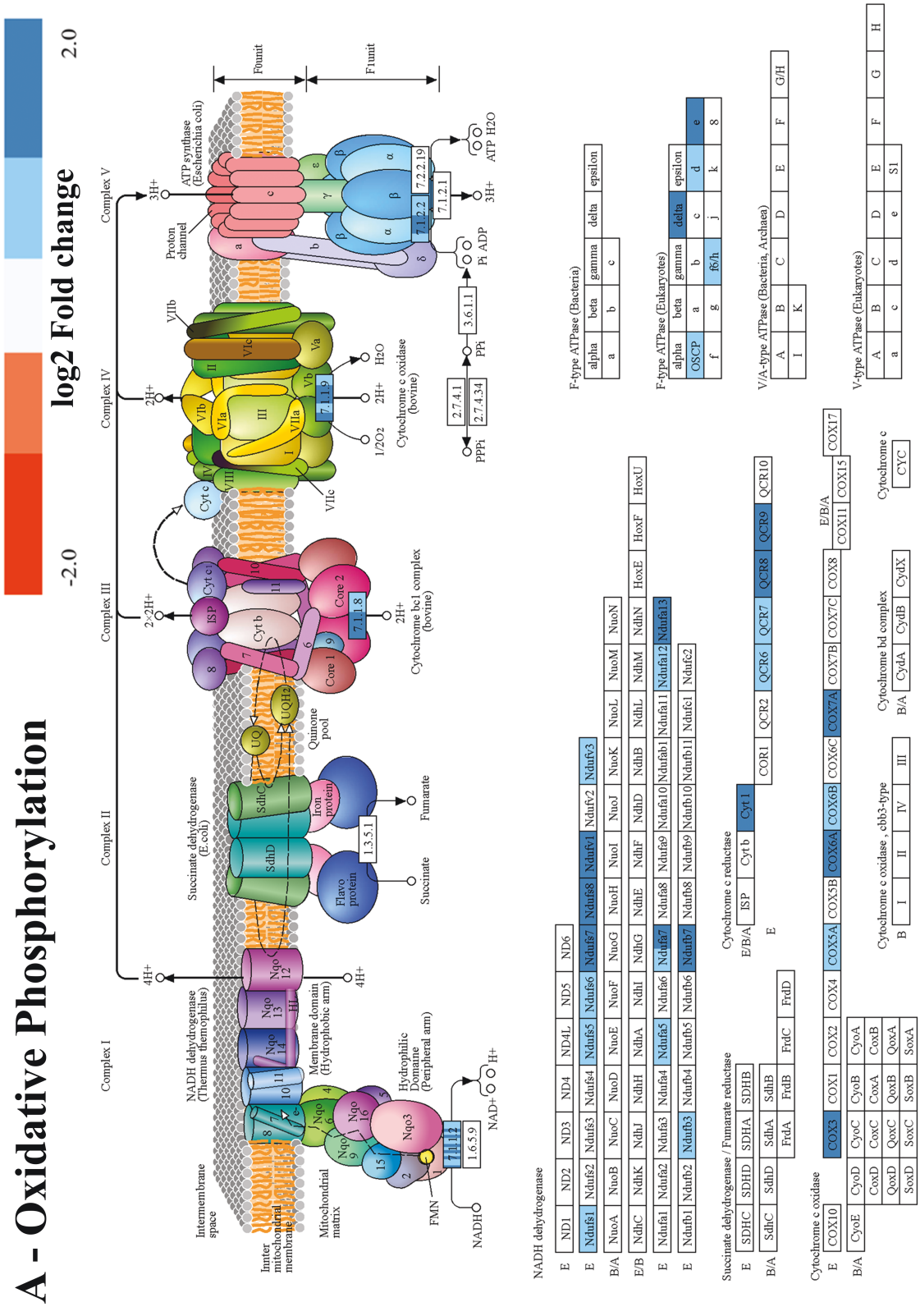

Figure 4. (A) Schematic pathway of oxidative phosphorylation by the Kyoto Encyclopedia of Genes and Genomes (KEGG), (B) Schematic pathway of the citric acid cycle by the Kyoto Encyclopedia of Genes and Genomes (KEGG), showing expression in the contrast between animals on CON vs SM20 diet, $n = 4$ per diet.

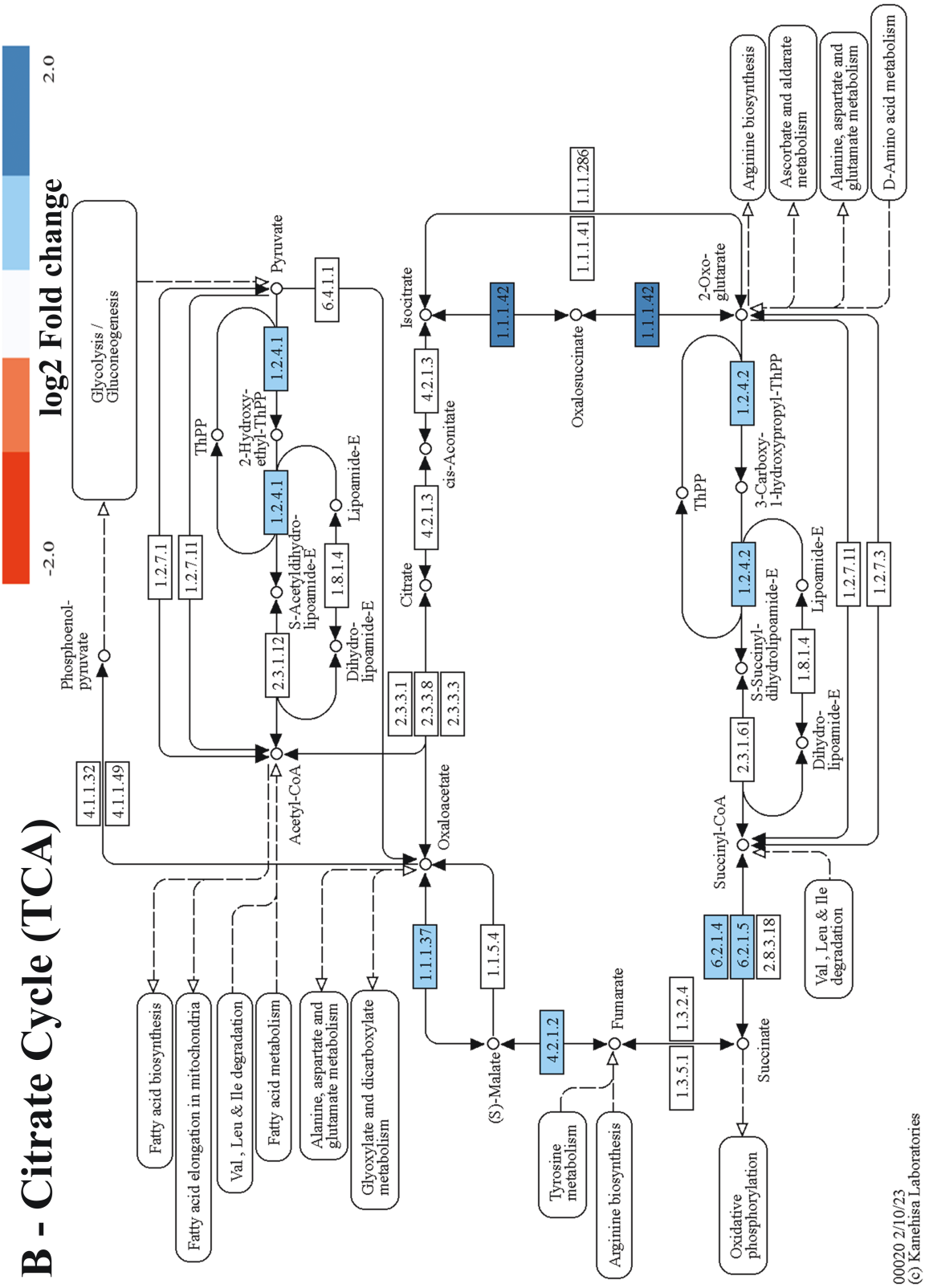

Figure 4. Continued

pH (Pereira-Junior et al., 2019). Another indicatives of good ruminal biohydrogenation capacity are the similar levels of PUFA and higher amounts of SFA and some MUFA in the muscle of SM20 lambs, despite the presence of 45% and 51% of PUFA in CON and SM20 diets, respectively.

In addition, the absence of SM20 effect on total PUFA may have contributed to no change in the meat color over time. The increase in meat deterioration and discoloration occurs due to the oxidation of lipids and myoglobin, especially the polyunsaturated fatty acid that can oxide quickly

(Wood et al., 2004). Exposure of the meat surface to oxygen causes an oxidative process of muscle pigment myoglobin leading the red myoglobin to brownness meat e.g., increased metmyoglobin (Jose et al., 2016). Overall, as expected, over storage time, the variables' behaviors were similar to previous studies regarding the evolution of color in lamb meat during retail storage, which is an oxidative natural process (Serrano et al., 2014; Guerra-Rivas et al., 2016).

The brightness of red meat is associated with freshness by consumers (Oliveira et al., 2013), and its reduction is related to metmyoglobin production (Fruet et al., 2018). Here, none of the color outcomes were affected by SM20, a finding that is supported by greater values of the a^* parameter at the end of the retail compared to other studies (Guerra-Rivas et al., 2016; Rubio et al., 2016). In addition, color stability over time as indicated by L^* showed little changes, while the hue angle, an indicator of discoloration, presented lower values than what is seen in the literature (Serrano et al., 2014; Guerra-Rivas et al., 2016).

Limitations

It is worth acknowledging some of the limitations of the present study such as analysis of gene expression but not protein abundance or activity. Coding genes identified as differentially expressed not necessarily will be translated to active proteins as posttranscriptional and posttranslation mechanism may apply. To minimize potential erroneous inferences, we focused on discussing gene expression changes that affected several genes in a pathway. Moreover, we restricted our discussion to transcriptomic differences that were supported by phenotypic data as the main reason why we included the RNA-Seq analysis was to confirm and provide a more in-depth explanation to the phenotypes found. However, further research should benefit from inclusion of addition analyses at the protein level in the muscle and additional tissues such as the adipose one. Furthermore, other fat deposition sites should be investigated, such as perirenal fat, for a better understanding of the effect of SM on fat deposition throughout the animals' body. In summary, we recognize that studies with SM are still in their early stages and still require additional research, for example, studies with comprehensive evaluations of digestibility and microbial synthesis. Additional studies with a larger number of experimental units are also needed to confirm some of the findings in this study.

Conclusion

Inclusion of SM in the diet of castrated male lambs as a partial substitute for corn can be an attractive option as it increases subcutaneous fat deposition in the carcass by adipocyte hypertrophy, while not impacting animal performance. Moreover, SM inclusion did not change the PUFA level in the meat, but reduced lipid oxidation.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflicts of interest statement

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Author statement

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