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Purified lignin in the diet of feedlot lambs and the effects on performance, carcass and meat quality

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ABSTRACT - This study aimed to establish the optimal concentration of purified Kraft lignin (PKL) in the diet of finishing lambs. The experiment was arranged in a randomized block design, with four diets and LKR concentrations of 0, 6, 12, and 18 g/kg DM, with eight Poll Dorset × Texel crossbred lambs per treatment. The experiment was conducted with newly weaned lambs, aged 60 ± 10 days, with an average initial body weight of 23 ± 5 kg. The animals were housed in individual stalls for 69 days. Their performance was measured during the experiment, and the carcass, meat quality, and viscera were evaluated after slaughter. An orthogonal polynomial regression study was performed with a 5% significance level. Including PKL did not alter the performance parameters body weight, average daily weight gain, feed efficiency, and dry matter intake. The inclusion of lignin increased the amount of superoxide dismutase and glutathione peroxidase in the blood ($P<0.05$). In addition, there were no changes in carcass quality parameters hot and cold carcass weight, hot and cold carcass yield, rib eye area, pH, and temperature. The L* content was lower in the treatment with 18 g of lignin ($P = 0.0135$). Fecal microbiology had a cubic effect ($P = 0.0509$) with lower values (154.81 CFU/mL) at 18 g/kg DM. The treatment with 12 g of lignin had zero rumenitis index, and the highest occurrence was with 6 g ($P<0.0001$). There was no difference among treatments for commercial meat cuts and viscera. At the concentrations used, PKL does not alter performance characteristics, carcass and meat quality parameters, but it has an antimicrobial and antioxidant effect.

Keywords: antimicrobial, antioxidant, GPX, Kraft, sheep

1. Introduction

The contemporary consumer increasingly demands food quality, leading to alterations in the meat production chain. Livestock farmers and ruminant nutritionists are exploring the potential of alternative feeds and additives to replace or reduce the use of ionophores in animal diets (Polizel et al., 2021). However, these feeds and additives must neither compromise the health or performance of animals nor the quality of carcasses or meat products. In consideration of the market context, alternative feeds may be derived from byproducts of the food or wood industry (Bezerra et al., 2020).

Lignin is a cross-linked aromatic polymer, a process that occurs when linear or branched polymer chains are linked by covalent bonds. It is found in plant cell wall, along with cellulose and hemicellulose, and contributes structurally and protects against pathogens (Baurhoo et al., 2008; Boarino and Klok, 2023). Cellulose is the most abundant element in plant cell walls, accounting for up to a third of the total (Pan et al., 2006). Lignin is the second most common compound in plant cell walls, with the two elements often occurring together. Lignin can be industrially isolated from various natural sources, including agricultural waste, energy crops, and woody biomass (Yu and Kim, 2020). In the papermaking industry, purified lignin is a byproduct of pulp production during the chemical pulping process of wood (Zemek et al., 1979). Kraft lignin is obtained by extracting the lignin from the cellulose in the wood fibers using sodium hydroxide and sodium sulfide, which is a more efficient and stronger fiber-producing process (Baurhoo et al., 2008).

Purified Kraft lignin (PKL) is distinguished by its phenolic fragments, which exhibit antioxidant effects and inhibit low-density lipoproteins (Catignani and Carter, 1982). The utilization of lignin in ruminant nutrition at a dosage of 12 g/kg of dry matter (DM) has been demonstrated to enhance antioxidant activity, thereby extending the shelf life of meat (Bezerra et al., 2020). At a dosage of 30 g/kg DM, the compound reduced dry matter intake, while improving feed efficiency and reducing methane production. Kraft lignin has antimicrobial action on Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, as well as Gram-negative bacteria such as *Escherichia coli* and *Salmonella enteritidis* (Lourençon et al., 2021).

The main objective of this study was to evaluate PKL doses in the nutrition of lambs in the finishing phase and its effects on performance, metabolism, antioxidant and microbial action, and carcass and meat quality.

2. Material and methods

2.1. Experimental treatment

The experiment was conducted at the Departamento de Zootecnia of the Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Universidade de São Paulo, in Pirassununga, SP, Brazil (21°59' south latitude, 47°26' west longitude (W. Gr), and an average altitude of 635 m). According to the Koppen classification, the climate in the region is classified as Cwa, mesothermal with predominantly summer rains, presenting dry winter and hot summers, with an average annual temperature of 22 °C and average annual rainfall close to 1363 mm. Research on animals was conducted according to the ethics committee on animal use of the FZEA, Protocol 5602281122.

The experimental design was a randomized block, with four treatments and eight replications. The treatments were: L0 - without addition of PKL, and treatments with inclusion of PKL, L6 - 6 g/kg DM, L12 - 12 g/kg DM, and L18 - 18 g/kg DM.

We used 32 male lambs, recently weaned, not castrated, from an industrial cross between Poll Dorset and Texel, aged 60 ± 10 days, and with an average body weight of 23 ± 5 kg. The animals were allocated to treatments according to their initial body weight; they were kept confined in a covered shed, in individual stalls (2 × 1.25 m) with slatted floors and *ad libitum* water, and were fed at 07:00 and 14:00 h.

Prior to the initiation of the experiment, the animals were vaccinated against clostridium (Excell 10®, Vencofarma, Brazil) and administered an anthelmintic containing 5% levamisole hydrochloride (Ripercol®, Zoetis).

The lambs were acclimated to the facilities and the daily handling procedures for five days. The evaluation period was 64 days. The feed intake of the lambs was measured on a daily basis by calculating the difference between the amount of feed offered and the amount of feed left uneaten. The amount of feed offered was calculated in a manner that did not restrict intake.

The diet offered to the animals was based on the nutritional requirement for the growth phase (NRC, 2007), using the ingredients in the proportion of 20% roughage (hay of *Cynodon dactylon* (L) Pers cultivar *Coast Cross*) and 80% concentrate based on ground corn, soybean meal, limestone, mineral supplement, and PKL only in the diets with inclusion (Table 1).

Samples of the diets were collected and packed in plastic bags for analysis of dry matter, crude protein, ether extract, mineral matter, calcium, potassium (AOAC, 2000), neutral detergent fiber, and acid detergent fiber (Mertens et al., 2002).

Table 1 - Description of the feed and chemical composition of the experimental diet with different amounts of purified Kraft lignin (PKL) for feedlot lambs

Item	PKL (g/kg DM)			
	0	6	12	18
Ingredient (%)				
Coast cross hay	20.00	19.40	18.80	18.20
Ground grain corn	65.00	65.00	65.00	65.00
Soybean meal	12.00	12.00	12.00	12.00
Calcitic limestone	1.70	1.70	1.70	1.70
Mineral supplement[1]	1.3	1.3	1.3	1.3
Kraft lignin[2]	-	0.6	1.2	1.8
Total	100.00	100.00	100.00	100.00
Chemical composition (% DM)				
Crude protein	13.60	13.57	13.51	13.46
Ether extract	3.80	3.77	3.76	3.76
Neutral detergent fiber	26.20	26.32	26.41	26.50
Acid detergent fiber	16.00	16.03	16.10	16.15
Metabolizable energy[3] (Mcal)	2.770	2.742	2.708	2.672
Ca	0.84	0.83	0.83	0.84
P	0.34	0.34	0.34	0.34

[1] Brazil Boi® Animal Nutrition - Mineral Sheep SP: calcium (min), 130 g/kg; calcium (max), 150 g/kg; phosphorus (min), 60 g/kg; sodium (min), 120 g/kg; magnesium (min), 20 g/kg; sulphur (min), 20 g/kg; zinc (min), 2720 mg/kg; fluoride (max), 600 mg/kg; manganese (min), 1360 mg/kg; cobalt (min), 68 mg/kg; organic chromium (min) 20 mg/kg; iodine (min) 50 mg/kg; selenium (min) 20 mg/kg.
[2] Kraft lignin - Klabin S. A®.
[3] Metabolizable energy estimated according to NRC (2007).

2.2. Animal performance

The animals were weighed every 16 days, without prior fasting, using an electronic scale (model KM3-N, Coimma®) with a division of 0.1 kg and a capacity of 300 kg. The average daily dry matter intake per kg of body weight (DMI/BW, %) was calculated by the dry matter intake (DMI) ratio to body weight in kg, multiplied by 100, individually and daily. The average daily weight gain (ADG) was calculated by the difference in weights and divided by the days of the evaluation period. The feed efficiency (AE) of the animals was obtained as the ratio between ADG and DMI.

2.3. Laboratory analysis

On the 59th day of the experimental period, blood and fecal samples were collected for analysis. To minimize stress and movement during handling, the animals were restrained while the blood samples were collected via intrajugular venipuncture.

The protein and energy parameters evaluated included total protein, globulin, albumin, glucose, and urea. Blood samples were obtained from the animals by puncturing the jugular vein using an 18G needle (1.2 mm). A 4-mL BD Vacutainer® Fluoride/EDTA vacuum blood collection tube was employed to measure plasma glucose, while a 10-mL vacuum tube with a plastic clot activator was utilized for the other analyses. The samples were then subjected to centrifugation using a centrifuge (model Daiki® 80-2B - Digital Display) for 20 min at 2500 rpm to obtain serum and plasma.

The determination of serum levels of each parameter was performed using the Mindray BS 120 - China, with commercial kits from Labtest, Brazil (urea Ref. 104-4; total proteins ref. 99-100; albumin ref. 19/250; glucose ref. 133-2/500; Calibra H Ref. 80-1; Quoitrel 1H Ref. 71-1). The methodology used for the parameters was: glucose by colorimetry, GOD – Trinder; Urea by UV enzymatic test; albumin by colorimetric test – Bromocresol Green; total proteins by the colorimetric – Biuret test. Globulin levels were obtained by subtracting total proteins from albumin.

The activity of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPX) was assessed using specific commercial kits (BioAssay Systems®). The readings were made on a spectrophotometer (Multiskan GO - Thermo Scientific®) with the following wavelengths: 450 nM for SOD; 540 nm for MDA; and 340 nm for GPX.

The fecal samples were collected for microbiological analysis. The analysis was conducted within 2 h after data collection to obtain a better result. To perform the total bacterial count (TBC), the following methodology was used by Molosse et al. (2021) with 1 g of each stool sample diluted in 9 mL of buffered peptone water in sterile test tubes, homogenized in a vortex shaker, resulting in a dilution of 10^{-1}. The remaining dilutions were carried out in Eppendorf from 2 mL until they reached 10^{-2} and 10^{-3}, always inoculating 100 µL of the previous dilution in 900 µL of buffered peptone water. Then, 100 µL of the 10^{-2} and 10^{-3} dilutions of each sample was inoculated in a Petri dish previously prepared with standard agar for counting (PCA) and incubated in a bacteriological oven at 25 °C for 24 h. Next, a colony counter was used, and the results were expressed in colony-forming units per mL (CFU/mL).

2.4. Carcass and meat quality parameters

At the end of the experiment (69 days), the animals were immediately slaughtered. They were fasted from solid food for 16 h before slaughter and were transported in a truck suitable for sheep for a distance of 200 m. The slaughter was carried out by the humanitarian procedures established by Brazilian law (Brasil, 2017). The animals were stunned with a penetrating captive bolt gun, and then bleeding was performed within a maximum time of 1 min, by cutting the jugular vein and carotid artery.

After slaughter, the carcasses were skinned, gutted, washed, identified, and weighed to determine the hot carcass weight (HCW). Immediately after evisceration, the heart, lungs, spleen, liver, kidneys, visceral fat, rumen (empty), and intestine (empty) were weighed on a counter scale (model BP-15, Filizola®). The fat that surrounds the digestive system was divided into omental fat (rumen, reticulum, abomasum, and omasum), mesenteric fat (small and large intestine), and perirenal fat (kidney) and weighed on a counter scale (model BP-15, Filizola®). The proportion of the weight of the viscera to the weight of the fasting animal was calculated.

The rumenitis index was taken after the rumen was washed under running water. Rumen papillae were visually classified according to the incidence of lesions on a score scale from 0 to 10 in which each score point represents 10% of the rumen compromised, considering the incidence of rumenitis any classification above zero (Bigham and McManus, 1975). The evaluation was visual and subjective, so they were performed by a trained person.

In the *Longissimus dorsi* muscle, pH and temperature were measured between the 12th and 13th ribs. The pH and temperature were measured 30 min and 24 h after slaughter, with the aid of a digital pH meter (pH IN model, AKSO®), penetration probe, calibrated with pH 7.0 and 4.0.

The carcasses were weighed before being placed in the cold room (HCW). The carcasses were kept in the cold room (0-2 °C) for 24 h. After this period, the carcasses were weighed again (CCW). The hot carcass yield (HCY) was calculated as the ratio of HCW to the weight of the animal at slaughter, in percentage. Cold carcass yield (CCY) was calculated using CCW by the weight of the animal at slaughter, in percentage. The difference between the weight of the cold carcass and the hot carcass was used to calculate the water loss by cooling.

The carcasses were divided in half and the left half-carcass was sectioned between the 12th and 13th ribs to measure the loin eye area (LEA) of the *Longissimus dorsi* muscle. The outline of the muscle was drawn on a transparent plastic sheet with a permanent marker. The geometric method was used, in which points A and B were determined and then the values of the following equation were applied: $(A/2 \times B/2) \times \pi$, in which A is the maximum length of the *Longissimus dorsi* muscle, and B is its maximum depth (both measurements were taken perpendicular to each other); and π is equal to 3.1416 (Cezar and Sousa, 2007). Subcutaneous fat thickness (SFT) was measured using a digital caliper (Luatek®) in the region between the 12th and 13th ribs.

The loin, shoulder, shank, ribs, and neck were cut from the left half-carcass. The commercial cuts were weighed on a counter scale (model BP-15, Filizola®). The yield of the commercial cuts was calculated using the ratio of the weight of the cut to the weight of the half-carcass.

Samples of steak (2.5 cm each) were collected from the *Longissimus dorsi* muscle. The samples were then identified, wrapped in aluminum foil, placed in polyethylene bags, and vacuum-packed. The samples were kept frozen at a temperature of −20 °C. Before starting the analyses, the steaks were thawed at 4 °C for 12 h. After this period, the samples were placed under a counter at room temperature and exposed to oxygen for 30 min. Color determination was performed with a portable spectrophotometer (MiniScan EZ 4500 model, Hunterlab®), calibrated with black and white standards, operating in the CIE L* a* b* system (AMSA, 2012) considering the parameters of L*, a*, and b* (luminosity, red, and yellow content, respectively).

The pH of the meat was determined by a pH meter (Model HI 99163, HANNA) equipped with a combined electrode for reading by perforating three points of each sample in each replication. To calculate the water loss by cooking, the meat sample was weighed, cooked to an internal temperature of 72 °C, cooled to room temperature (22 °C) and weighed again to check the difference in sample weight before and after cooking.

Shear force analysis was performed with four to six cylinders (1.27 cm in diameter) that were removed from each sample, parallel to the orientation of the muscle fiber, and cut in a texture analyzer (TMS-PRO Food Technology Corporation, Sterling, USA). The shear force of each sample was obtained as the average of the replicates and expressed in Newton (N) (AMSA, 2015).

For the lipid oxidation evaluations by thiobarbituric acid reactive substances (TBARS) analysis, 5 g of *Longissimus dorsi* sample in triplicate were weighed, which were crushed and homogenized for analysis of 2-thiobarbituric acid reactive substances (Vyncke, 1970). Absorbance readings were performed at 532 and 600 nm in a spectrophotometer (Multiskan GO – Thermo Scientific®). To obtain the concentration of MDA in the samples, the equation provided by the curve was used. The results were expressed in µg MDA/kg meat.

2.5. Statistical analysis

A randomized block design with a treatment frame and eight replications was used. The treatments correspond to four PKL concentrations (0, 6, 12, and 18 g/kg DM). Data were checked for normality (Shapiro-Wilk and Levene's tests), and the data were subjected to analysis of variance (ANOVA) using the Proc GLM of the SAS program (Statistical Analysis System, 2013). An orthogonal polynomial regression study was performed with a significance level of 5%. The following model was used:

$$Y_{ij} = \mu + T_i + B_k + e_{ij},$$

in which Y_{ij} is the dependent variable (i = treatment and j = repetition), µ is the overall mean, T_i is the fixed effect of the treatment (i = 1-4), B is the block (initial body weight), and e_{ij} is the residual error.

3. Results

3.1. Performance characteristics

The inclusion of Kraft lignin in the diet did not alter the performance of lambs (Table 2, P>0.05). The mean final body weight was 44.53 kg. The treatments had averages for ADG of 0.333 kg, FE of 0.260 kg/kg, DMI/BW of 2.86%, and DMI of 1.28 kg.

Table 2 - Performance of feedlot lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

Performance	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Initial weight (kg)	23.87	23.77	24.25	23.75	5.50	23.91	0.972
Final weight (kg)	46.02	44.89	43.37	43.84	10.35	44.53	0.668
ADG (kg)	0.36	0.34	0.31	0.32	19.27	0.33	0.489
FE (kg/kg)	0.27	0.27	0.25	0.26	12.28	0.26	0.364
DMI/BW (%)	2.86	2.80	2.88	2.90	8.99	2.86	0.875
DMI (kg)	1.32	1.25	1.27	1.27	16.64	1.28	0.914

ADG - average daily gain; FE - feed efficiency; DMI/BW - average daily dry matter intake per kg body weight; CV - coefficient of variation.

3.2. Serum biochemistry

There was no influence (P>0.05) of the inclusion of PKL for the parameters of urea, total protein, albumin, globulin, and glucose with mean values of 43.28 mg/dL, 6.00 g/dL, 2.93 g/dL, 3.07 g/dL, and 84.70 mg/dL, respectively (Table 3).

The concentration of SOD (P = 0.043) and GPX (P = 0.032) in the blood increased with the inclusion of lignin, with a linear regression behavior, therefore with higher values in the treatment with 18 g of PKL (Table 3). The concentration of MDA (P = 0.539) was not modified by the inclusion of lignin in the diet.

Table 3 - Parameters of serum biochemistry analyses of lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Metabolism							
Urea (mg/dL)	48.45	40.44	42.24	42.01	21.86	43.28	0.360
Total proteins (g/dL)	5.92	6.09	6.03	5.98	6.00	6.00	0.816
Albumina (g/dL)	2.84	3.02	3.01	2.87	16.23	2.93	0.827
Globulins (g/dL)	3.08	3.07	3.02	3.12	19.46	3.07	0.989
Glucose (mg/dL)	82.59	81.45	93.41	82.06	11.61	84.70	0.121
Antioxidant							
SOD (%)	30.12b	33.20ab	33.43ab	37.2a	13.86	33.49	0.043*
MDA (nmol/mL)	34.31	27.84	34.99	32.43	32.79	32.39	0.539
GPX (µmol/L)	206.73b	236.78ab	207.93b	293.27a	26.15	236.18	0.032*

SOD - superoxide dismutase; MDA - malondialdehyde; GPX - glutathione peroxidase; CV - coefficient of variation.
a-b - Means in the same row with different letters differ by test (P<0.05).
* Linear regression.
Linear equation for SOD: $y = 0.3578x + 30.267$, $R^2 = 0.9143$.
Linear equation for GPX: $y = 3.8462x + 201.56$, $R^2 = 0.5407$.

3.3. Carcass quality characteristics

The inclusion of lignin did not alter the carcass quality measurements (P>0.05, Table 4). The mean slaughter weight was 42.48 kg, HCW was 19.68 kg, and HCY was 19.17 kg. The CCW was 46% and the CCY was 46%, with LEA value of 14.56 cm^2. The average SFT was 1.56 mm, and the pH was measured 30 min and 24 h after slaughter, with an initial mean pH of 6.07 and a mean temperature of 32.93 °C, being reduced to a mean pH of 5.59 and a mean temperature of 6.61 °C.

The inclusion of lignin in the diet of lambs did not alter (P>0.05) the proportion of meat cuts and viscera weight (Table 5). For meat quality parameters, there was no difference (P>0.05) in shear force (47.62 N), pH (5.54), cooking loss (23.41%), color a* (13.40), color b* (12.12), and TBARS (0.14 µg MDA/kg meat) (Table 6). However, for the color L*, there was a statistical difference (P = 0.0135) with a cubic effect, and higher value with 6 g/kg PKL than other treatments.

Table 4 - Carcass characteristics of lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Slaughter weight (kg)	43.86	42.68	41.31	42.06	9.88	42.48	0.667
HCW (kg)	20.35	19.68	19.20	19.50	11.71	19.68	0.784
HCY (kg)	19.84	19.13	18.69	19.01	12.04	19.17	0.776
CCY (%)	47.00	46.00	46.00	46.00	4.39	0.46	0.993
CCW (%)	45.00	45.00	45.00	45.00	4.41	0.46	0.990
LEA (cm^2)	15.47	13.91	14.52	14.05	12.51	14.56	0.391
SFT (mm)	1.85	1.57	1.40	1.44	27.03	1.56	0.168
pH, 30 min	5.86	6.13	6.13	6.18	8.86	6.07	0.639
T °C, 30 min	32.99	32.64	33.36	32.75	5.16	32.93	0.836
pH, 24 h	5.58	5.54	5.61	5.63	2.40	5.59	0.527
T °C, 24 h	6.05	7.46	6.80	6.11	22.35	6.61	0.210

Slaughter weight - fasting animal (16 h); HCW - hot carcass weight; CCW - cold carcass weight; HCY - hot carcass yield; CCY - cold carcass yield; LEA - rib eye area; SFT - subcutaneous fat thickness; T °C - temperature in degrees Celsius; CV - coefficient of variation.

Table 5 - Measurements of meat cuts and viscera of feedlot lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Commercial cuts (%)							
Leg	31.08	30.91	31.34	31.90	5.81	31.31	0.297
Loin	9.06	9.48	9.04	9.37	19.29	9.24	0.982
Rib and neck	42.15	41.33	40.80	40.28	5.57	41.14	0.116
Shoulder	17.58	18.31	18.80	18.50	8.00	18.30	0.117
Viscera (kg)							
Liver	1.66	1.72	1.69	1.77	11.55	1.71	0.703
Kidneys	0.25	0.26	0.27	0.26	16.83	0.26	0.671
Renal fat	0.39	0.62	0.40	0.39	81.29	0.45	0.517
Respiratory tract	1.51	1.67	1.56	1.62	15.52	1.60	0.585
Spleen	0.13	0.15	0.16	0.17	25.41	0.15	0.364
Pancreas	0.08	0.11	0.09	0.08	43.27	0.09	0.556
Heart	0.41	0.42	0.42	0.42	12.80	0.42	0.953

CV - coefficient of variation.

Table 6 - Meat quality parameters of lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Shear force (N)	47.38	49.33	46.74	47.03	28.55	47.62	0.066
pH	5.60	5.53	5.52	5.54	1.92	5.54	0.086
Cooking loss (%)	23.33	26.44	21.81	22.08	18.12	23.41	0.246
L*	44.32A	45.51B	43.23A	40.96A	8.44	43.46	0.013**
a*	12.94	13.39	13.75	13.53	8.06	13.40	0.229
b*	11.26	13.22	11.69	12.20	15.00	12.12	0.192
TBARS	0.16	0.16	0.11	0.14	67.07	0.14	0.881

TBARS - thiobarbituric acid reactive substances (μg malondialdehyde/kg tissue); CV - coefficient of variation.
A-B - Means in the same row with different letters differ by test (P<0.05).
** Cubic equation for L*: $y = 0.0027x^3 - 0.0965x^2 + 0.6808x + 44.32$, $R^2 = 0.9461$.

3.4. Ruminal and intestinal features

The inclusion of Kraft lignin in the diet did not alter the weight of the rumen, small and large intestines, and visceral fat (P>0.05). The rumenitis index was influenced by lignin in the diet (P<0.001), being absent in the treatment with 12 g/kg DM of PKL, and with a higher incidence in the treatment with 6 g/kg DM (Table 7).

Table 7 - Rumen weight, small and large intestine, mesenteric and omental fat, and incidence of rumenitis of lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
Rumen (kg)	1.01	0.97	0.84	0.94	14.71	0.94	0.095
Small and large intestine (kg)	1.37	1.37	1.22	1.28	16.70	1.31	0.470
Mesenteric and omental fat (kg)	0.76	0.62	0.72	0.66	31.06	0.69	0.554
Rumenitis index (%)	12.5B	37.5C	0.00A	25.00B	0.02	18.7	<0.0001*

CV - coefficient of variation.
A-C - Means in the same row with different letters differ by test (P<0.05).
* Cubic equation for rumenitis index: $y = 0.0965x^3 - 2.6042x^2 + 16.319x + 12.5$, $R^2 = 0.8156$.

3.5. Stool microbiology analysis

There was a significant cubic ratio (P = 0.0509) for the CFU in lamb feces when the diet was supplemented with purified lignin (Table 8). The administration of 18 g/kg DM led to a decrease in fecal bacterial concentrations (154.81×10^{-3}). The 0, 6, and 12 g/kg lignin treatments did not differ significantly from each other.

Table 8 - Colony forming unit (CFU) in feces of lambs fed diet supplemented with purified Kraft lignin (PKL) in different concentrations

	PKL (g/kg DM)				CV	Mean	P-value
	0	6	12	18			
CFU × 10^{-3}	194.56B	178.56B	266.12B	154.81A	58.24	198.52	0.0509*

CV - coefficient of variation.
A-B - Means in the same row with different letters differ by test (P<0.05).
* Cubic equation for CFU: $y = -0.2334x^3 + 5.6387x^2 - 28.098x + 194.56$, $R^2 = 0.9830$.

4. Discussion

4.1. Performance characteristics

The intake and productive performance of ruminants depend mainly on the quantity and quality of the feed offered (Liu et al., 2005). In the present experiment, the performance of finishing lambs was not altered with the supplementation of PKL in the diets in the concentrations of 6, 12, and 18 g/kg DM. Dry matter intake remained within the range indicated by the NRC (2007), from 1.0 to 1.5 kg/day. For ADG, there was no difference among treatments, with an average of 0.333 kg/day. Although in forages the role of lignin is to limit digestion of structural polysaccharides such as cellulose and hemicellulose, lignin can also act as a regulator of feed intake (Moore and Jung, 2001).

The portion not digested by forage, which is the case of lignin, passes slowly through the digestive system and contributes to the satiety effect, consequently decreasing the amount of energy available to the animal (Moore and Jung, 2001). It is noteworthy that the PKL of this study did not alter DMI and performance of lambs, contrary to what is expected in diets with lignin-rich forages.

4.2. Serum biochemistry

The parameters of total proteins, albumin, globulins, urea, and glucose found in feedlot lambs supplemented with lignin remained within the ranges considered for the species (Kaneko et al., 1997; Silva et al., 2020).

Protein metabolism is a process that is related to protein in the diet. In a healthy animal, there tends to be a balance between the ingestion and synthesis of amino acids, as well as the lysis and excretion of excessive nitrogenous material in the form of urea. Therefore, urea is a parameter used to evaluate feed and protein intake. The inclusion of lignin did not compromise the amount of protein ingested by the animal (Kaneko et al., 1997).

Lignin is a limiting factor in the utilization of digestible energy in the organic matter of grasses; therefore, the higher the lignin content in the organic matter, the lower the concentration of metabolizable energy in the organic matter (Tedeschi et al., 2023). However, ruminants in nutritional energy deficit can use protein as an energy source (Nelson and Cox, 2017). Albumin levels are a good indicator of whether the animal is deficient in protein (Díaz González and Silva, 2017). In this experiment, protein and energy metabolism was not altered by the inclusion of PKL, therefore indicating that even with the inclusion of lignin, there was no reduction in the concentration of metabolizable energy in the total diet.

Oxidative stress results from an imbalance between the generation of oxidizing compounds and the action of defense (antioxidant) systems. The generation of free radicals and non-radical reactive species results from oxygen metabolism (Vaz et al., 2025). Thus, antioxidant activity removes free radicals from the body and inhibits their action. This reduces oxidative stress and keeps cells healthy. Superoxide dismutase, MDA, and GPX are important antioxidants (Gallo et al., 2020). Superoxide dismutase plays a fundamental role in the body's defense against ROS by acting to remove O_2. Glutathione peroxidase prevents the accumulation of hydrogen peroxide; increasing its concentration helps to ensure the balance of these radicals and the health of the animal. Purified lignin is rich in guaicol, which has high antioxidant activity and reduces a variety of free radicals (Bezerra et al., 2020). In our study, we observed a positive linear effect of the inclusion of lignin in the diet and an increase in the concentration of SOD and GPX, confirming the action of lignin as an activator of antioxidant enzymes (Vaz et al., 2025).

4.3. Microbiology analysis

In this study, PKL supplementation of feedlot lambs at a concentration of 18 g/kg DM showed a reduction (P = 0.0509) for the CFU in their feces, indicating a lower concentration of bacteria. The action of lignin as an antibacterial is related to the phenolic hydroxyl group, capable of disrupting the cell membrane of the bacterium, after its lysis (Miklasińska-Majdanik et al., 2018; Bouarab-Chibane et al., 2019). This action of phenols and polyphenols is well recognized, but its mechanism is not yet clear. Antibacterial efficacy changes depending on the lignin variety and bacterial strain.

The presence of purified eucalyptus lignin has been demonstrated to reduce the abundance of various bacterial strains, including *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella enteritidis* (Lourençon et al., 2021). However, the use of up to 30 g/kg of purified lignin from non-woody annual plants in the diet of lambs in the finishing phase did not alter the amount of *E. coli* in the feces (Wang et al., 2009). Kraft lignin, a byproduct of the paper manufacturing process, may increase the bioavailability of antimicrobial compounds when included in animal diets (García-Fuentevilla et al., 2022). The chemical composition of lignin varies depending on the source material (Liu and Eudes, 2022). Furthermore, the processing methods used to obtain specific types of lignin, such as Kraft lignin, can alter the availability of certain compounds, leading to different analytical or functional outcomes (García-Fuentevilla et al., 2022). Therefore, it is essential to understand both the sources of lignin and the effects of processing on its properties (Tedeschi et al., 2023).

4.4. Carcass and meat quality characteristics

Purified Kraft lignin did not modify carcass measurements or meat quality, such as slaughter weight, HCW, HCY, CCW, CCY, LEA, SFT, pH and T °C at 30 minutes, and pH and T °C at 24 h. These results corroborate findings evaluating the inclusion of PKL in feedlot lamb diets (Wang et al., 2009; Bezerra et al., 2020). Nutrition can alter carcass and meat characteristics (Gallo et al., 2009); however, the inclusion of Kraft lignin did not have this effect.

Purified Kraft lignin did not modify meat quality parameters such as pH, temperature, water loss by cooking, and shear force in the present study. Similar results were described by Bezerra et al. (2020), who provided 12.5 g/kg DM of PKL to feedlot lambs.

Muscle and adipose tissue development can alter pH and temperature values of meat, as well as the establishment of rigor mortis. These characteristics affect the loss of water from the muscle in cooking, which consequently alters the tenderness (Ponnampalam et al., 2016). The inclusion of PKL did not alter muscle or adipose tissue development, so it had little effect on meat quality characteristics.

In this study, the color parameter L* (lightness) was influenced by the treatment with a decreasing linear behavior ($P = 0.045$). Luminosity is associated with the type of muscle fiber, pH value, carcass fat thickness, and other post-mortem factors that determine the degree of hydration and condition of muscle proteins. However, in this experiment, none of these factors were influenced by the inclusion of lignin, only the luminosity content, as an isolated factor. However, in another experiment with the inclusion of purified lignin (Bezerra et al., 2020), a change in the L* content was also observed, and more studies are needed to fully understand this effect.

Lipid peroxidation is related to sensory characteristics such as color, taste, and odor. In this experiment, TBARS levels were not influenced by PKL with means of 0.14 µg malondialdehyde/kg.

4.5. Rumen and intestinal parameters

No effects of diets supplemented with PKL were observed on ruminal or intestinal parameters, including rumen weight, small and large intestine weights, and the weights of omental and mesenteric fat. However, for the diet with 12 g/kg DM of PKL, the rumenitis rate was zero ($P<0.001$) and in the diets with 6 and 18 g/kg DM, there was a frequency of rumen lesions of 61.54 and 30.77%, respectively. The composition of PKL is rich in guaiacol, and depending on the dose, it can present high toxicity, consequently being corrosive to cellular tissues (Anouar et al., 2009; Tisserand and Young, 2014). An experiment with 12.5 g/kg DM of PKL resulted in a rumenitis index above 3 in confined lambs (Bezerra et al., 2020). Therefore, it is necessary to carry out more research to find out what concentration and type of purified lignin can be harmful to the rumen.

5. Conclusions

Purified Kraft lignin has an effect on antioxidants, linearly increasing the activity of superoxide dismutase and glutathione peroxidase. In addition, the amount of 12 g/kg of DM has an effect on bacterial colonies in feces, with an antimicrobial effect. At this same concentration, the rate of ruminal papilla injury is zero. Furthermore, at this amount, the performance parameters, meat and carcass quality, metabolic parameters, and viscera weight are not altered, showing that it is a safe ingredient to provide in the diet of ruminants.

Data availability

The contents underlying the research text are included in the manuscript.

Author contributions

Conceptualization: Carvalho, A.; Oliveira, G. G. and Gallo, S. B. **Data curation:** Carvalho, A.; Silva, M. M.; Vedovate, A. V.; Sousa, R. L. M. and Gallo, S. B. **Formal analysis:** Carvalho, A.; Silva, M. M.; Oliveira, G. G.; Lima, C. G. and Gallo, S. B. **Funding acquisition:** Gallo, S. B. **Investigation:** Carvalho, A.; Almeida, D. L.; Silva, M. M.; Vedovate, A. V.; Oliveira, G. G.; Sousa, R. L. M.; Passarelli, D. and Gallo, S. B. **Methodology:** Carvalho, A.; Gonçalves, L. A.; Sousa, R. L. M.; Passarelli, D.; Furusho-Garcia, I. F.; Lima, C. G. and Gallo, S. B. **Project administration:** Carvalho, A.; Almeida, D. L.; Silva, M. M.; Vedovate, A. V.; Oliveira, G. G. and Gallo, S. B. **Resources:** Carvalho, A.; Almeida, D. L.; Silva, M. M.; Vedovate, A. V.; Oliveira, G. G.; Gonçalves, L. A.; Sousa, R. L. M.; Passarelli, D.; Furusho-Garcia, I. F.; Lima, C. G. and Gallo, S. B. **Software:** Lima, C. G. and Gallo, S. B. **Supervision:** Almeida, D. L. and Gallo, S. B. **Validation:** Carvalho, A.; Gonçalves, L. A.; Furusho-Garcia, I. F.; Lima, C. G. and Gallo, S. B. **Visualization:** Gallo, S. B. **Writing – original draft:** Carvalho, A.; Furusho-Garcia, I. F. Lima, C. G. and Gallo, S. B. **Writing – review & editing:** Carvalho, A.; Furusho-Garcia, I. F. and Gallo, S. B.

Conflict of interest

The authors declare no conflict of interest.

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