Carcass trait, physiochemical, microstructure, and fatty acid profile of lamb meat fed different levels of tamanu kernel cake (Callophyllum inophyllum)

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Abstract

A possible substitute protein source for ruminants, tamanu kernel cake (TKC), a by-product of oil extraction from *Calophyllum inophyllum* kernel, is rich in protein and bioactive compounds. The purpose of this study is to examine the carcass characteristic, microstructure, physicochemical quality, and fatty acid (FA) profile lamb meat given a TKC. The dietary treatments consisted of forage and concentrate rations with TKC at 0% as a control (T0), 15% (T15), 30% (T30), and 30% with additional mineral premix supplementation (T30m). A total of 20 male thin-tailed lamb were used after three months of feeding. Each treatment group included five lamb as replicates. The results showed that in the yield grade evaluation, T30 produced the highest carcass percentage compared to other treatments. All TKC rations yielded a significantly higher Boneless Closely Trimmed Retail Cuts percentage (% BTCRC) than the control group. However, TKC had an insignificant effect on the overall yield grade. In terms of physicochemical quality, T30 and T30m produced lower cooking loss compared to T15 and the control groups. T30 also produced significantly higher intramuscular fat content than the control. In terms of microstructure, T30 and T30m yielded higher perimysium, endomysium, and muscle fiber area than the control group. FA profile analysis revealed that all TKC treatments increased unsaturated fatty acid (UFA) levels and decreased saturated fatty acid (SFA) levels. In conclusion, the T30 inclusion in the concentrate diet is recommended to improve carcass percentage, muscle microstructure quality, and UFA content in lamb meat.

INTRODUCTION

Lamb is a promising animal protein source because of its adaptability, relatively quick growth, and widespread cultural acceptance of the meat in many regions (Idayanti et al., 2024). The demand for lamb meat in Indonesia continues to rise, creating the market for a sustainable, high-quality supply. To address this need, efforts have been directed toward exploring potential local feed resources that can enhance both productivity and meat quality (Sujarwanta et al., 2024). The lamb meat quality, particularly the tenderness, fat content, and flavor, is crucial for consumer acceptance. Improving meat quality through nutritional management has become a key strategy to enhance the competitiveness of lamb farming (Prache et al., 2022).

Supplementation with plant secondary metabolites, unsaturated fatty acid (UFA), and essential microminerals is a promising approach to improve meat quality. Diets rich in plant secondary metabolites can influence meat characteristics through biological processes. For example, tannins and phenols have antioxidant properties, which help reduce spoilage and prolong the shelf life of the meat (Waqas et al., 2023; Tong et al., 2022). Lamb diets that include plant secondary metabolites like saponins, flavonoids, and phenols improve meat quality and nutritional value (Abdallah et al., 2020). Phenolic compounds from herbal sources in lamb diets (7.8–16 mg DM) containing flavonoids stimulate muscle protein synthesis (Qin et al., 2020). Tannins, in particular, inhibit the fatty acid (FA) biohydrogenation in the rumen, thereby increasing the UFA deposition in the meat (Al Rharad et al., 2025). By reducing ruminal biohydrogenation, dietary UFA can be more effectively deposited in muscle

tissues. In addition, premixes containing essential microminerals such as zinc (Zn), selenium (Se), copper (Cu), along with manganese (Mn) play important roles in muscle metabolism, antioxidant function, and maintaining the structural integrity of meat, particularly in lamb (Meng et al., 2024)

The growing demand for lamb meat in Indonesia has increased the need for sustainable and locally available feed resources, particularly protein sources. Conventional protein sources are often expensive and imported. Therefore, exploring alternative sources from local agro-industrial by-products is crucial, specifically for small ruminants such as lamb that are commonly raised in rural areas. Tamanu kernel cake (TKC), a by-product of the tamanu crude oil (TCO) business, is one such substitute (Paradhipta et al., 2023; 2025). Tamanu oil is a non-edible product, primarily used in biodiesel production and medicinal applications. The development of the TCO industry in Indonesia is expanding due to government support, as tamanu (*Calophyllum inophyllum*) is native and abundantly available in the country. This makes TKC a promising and sustainable feed ingredient for the livestock sector (Leksono et al., 2014). TKC, being locally available and underutilized, offers both economic and environmental advantages. Aside from the high crude protein content (19.9%–23.5%), TKC also contains significant levels of crude fat (13.9%–15.3%), making it a valuable energy and protein source for ruminants (Paradhipta et al., 2023; Umroni et al., 2024). It is rich in plant secondary metabolites, including 6.47% phenols, 1.7% flavonoids, 0.9% saponins, and 0.93% tannins, which contribute not only to the nutritional value but also environmental benefits such as methane reduction (Paradhipta et al., 2023; 2025)

To date, no research has examined how TKC as a dietary ingredient influences lambs' carcass traits and meat quality. Due to its high content of plant secondary metabolites and fat, TKC may affect the physicochemical properties and FA profile. Combining with essential micromineral supplementation could produce synergistic effects that enhance meat quality. This study's novelty lies in grounded in the integrated approach, using TKC as a local, underutilized protein source in combination with microminerals, to assess the combined effects on lamb meat quality. The primary hypothesis is that secondary metabolites in TKC could improve carcass and meat quality. This study not only explores the nutritional and functional value of TKC but also contributes to the development of sustainable feeding strategies for improving meat quality in tropical small ruminant production systems.

MATERIALS AND METHODS

The Ethical Clearance Commission, Integrated Laboratory for Research and Testing, Universitas Gadjah Mada, granted ethical clearance for this study under certificate number 00056/04/LPPT/XII/2023.

Animals and experimental diets

This study was conducted at Universitas Gadjah Mada in Yogyakarta, Indonesia (coordinates 7° 45' 57.276" S, 110° 22' 38.712" E, altitude 150 m). The region has a tropical climate with a distinct dry season, as classified by the Köppen system. A total of 20 male lambs, with no specific racial pattern, were assessed. They averaged 4 months old and weighed 16.9 ± 2.93 kg (BW). The animals were divided

based on TKC levels, with five lambs in each group. Prior to the experiment, lambs were identified, vaccinated against clostridiosis, and subjected to parasite control.

Over the course of a 74-day research (including a 14-day adaptation), lambs were randomly given one of four different dietary treatments (T0, T15, T30, and T30m). Each treatment group consisted of five subjects. The diets, composed of pollard, rice bran, corn, soybean meal, palm kernel cake, and TKC (Table 1), were formulated to meet nutritional needs and a 200 g target ADG NRC, 2007). The techniques employed to estimate the diets' chemical makeup and the resulting ingredient values are identical to those expressed by Paradhipta et al. (2025).

Table 1
Proportion of ingredients and chemical composition of experimental diets containing TKC.

Ingredients[b]	Inclusion of the TKC (% dry matter)			
	T0	T15	T30	T30m
Pollard	30	30	30	30
Rice bran	20	20	20	20
Corn	10	10	10	10
Soybean Meal	10	10	10	10
Palm kernel cake	30	15	0	0
Tamanu kernel cake	0	15	30	0
Total	100	100	100	100
Trace mineral	0	0	0	0,5
	Chemical composition			
Dry matter[a]	86.78	89.08	89.73	89.88
Organic matter[b]	86.86	86.7	88.42	87.26
Crude protein[b]	15.71	15.02	15.28	15.01
Ether extract[b]	2.63	2.49	2.24	2.36
Neutral detergent fiber[b,c]	45.29	46.53	44.65	39.11
Acid detergent fiber[b,c]	27.63	27.88	28.27	25.25
	Total FA[d]			
C6	0.23	0.04	0	0
C8	1.97	0.75	0.11	0.04
C10	1.99	0.83	0.07	0.02
C12	14.9	8.25	0.42	0.14
C14	7.81	3.63	0.21	0.12
C15	0.08	0.06	0.07	0.06
C16	11.5	10.9	12	11.9
C16:1	0.12	0.19	0.3	0.29
C17	0.1	0.11	0.15	0.15

Ingredients[b]	Inclusion of the TKC (% dry matter)			
	T0	T15	T30	T30m
C17:1	0.05	0.05	0.07	0.06
C18	3.59	7.33	9.74	9.93
C18:1 (n9)	22.85	28.11	32.17	32.5
C18:2 (n6)	30	35.1	39.6	39.8
C18:3 (n3)	3.08	2.9	3.1	2.97
C20	0.34	0.61	0.8	0.81
C20:1 (n9)	0.65	0.56	0.6	0.57
C20:2 (n6)	0.05	0.04	0.06	0.05
C21	0.02	0.02	0	0
C22	0.2	0.25	0.27	0.28
C22:1 (n9)	0.05	0.06	0	0.05
C23	0.07	0.06	0	0.05
C24	0.3	0.23	0.22	0.22
C24:1 (n9)	0.04	0	0	0
SFA[e]	43.2	33	24.1	23.7
MUFA[f]	23.7	28.9	33.2	33.5
PUFA[g]	33.1	38	42.8	42.8
UFA[h]	56.8	66.9	76	76.3
UFA/SFA Ratio	1.32	2.03	3.15	3.21
Total FA[d]	94.7	95.0	95.2	95.2

Ingredients[b]	Inclusion of the TKC (% dry matter)			
	T0	T15	T30	T30m
[a] g/kg as fed				
[b] g/kg dry matter				
[c] corrected for ash and protein				
[d] mg/g dry matter				
[e] Saturated FA				
[f] Monounsaturated FA				
[g] Polyunsaturated FA				
[h] Unsaturated FA				
T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).				

Intake

Each lamb was fed a total mixed ration ad libitum, offered twice daily, half at 8 a.m. and the other half at 4 p.m. Daily feed intake was recorded by subtracting refusals (orts) from the provided amount. The feed quantity was modified according to each lamb's voluntary intake, with an estimated surplus of about 12%. During the experiment, the feed ingredients and refusals samples were gathered and kept at -18°C for subsequent chemical analysis. These samples were first thawed at room temperature, then pre-dried in a forced-air oven at 55°C for 72 h. After the trial ended, they were crushed using a knife mill fitted with 1 and 2 mm sieves.

The following procedures were observed in the analysis of dry matter (DM; method 934.01), mineral matter (MM; AOAC 942.05), ether extract (EE; AOAC 920.39), and crude protein (CP; AOAC 954.01) (AOAC, 2019). The method was utilized to select the neutral detergent fiber (NDF) and acid detergent fiber (ADF), with adjustments made for ash as well as protein (NDFac and ADFac) in accordance with Licitra et al. (1996).

Slaughter and carcass yield

After a 12-hour solid fast, the lambs were weighed at the conclusion of the experiment to determine their slaughter body weight (SBW). The carotid and jugular arteries were sliced in order to exsanguinate the lamb. The kidneys and pelvic-renal fat were retained after the head and feet were removed following

skinning and evisceration. A digital caliper was used to measure the thickness of the fat layer in the *Longissimus dorsi* muscle after a transverse cut was performed in the left half of the carcass between the 12th and 13th ribs to determine the subcutaneous fat thickness in the loin.

Chemical and instrumental analysis of meat

Each animal's left *Longissimus dorsi* (loin) muscle was collected, vacuum-sealed, and kept at -37°C for chemical and instrumental examinations. Before testing, it was thawed at 4°C. Following (AOAC, 2019) guidelines, the samples were lyophilized and subjected to analyses for moisture content (AOAC 950.46), crude protein (CP; AOAC 984.13), ether extract (EE; AOAC 920.39), and ash content (AOAC 920.153). The 9th rib was the starting point for sequential sampling. The shear force (SF), cooking loss (CL), and meat color (MC) was assessed between the 10th and 11th ribs, the 11th and 12th ribs, as well as the 9th and 10th ribs, respectively.

In order to calculate CL, 40 g steaks were prepared. After being weighed, each steak was cooked separately in a water bath until its internal temperature attained 80°C. Using Soeparno, (2015) method, CL was determined by comparing the weight of the sample before and after cooking. Six cylindrical cores were extracted from each cooked steak along the muscle fibers' longitudinal axis.

Meanwhile, in order to evaluate SF, at least four cylindrical samples (1×1×1 cm) were extracted along the orientation of the muscle fibers. The measurement was conducted utilizing a Warner-Bratzler shear blade at the 3 mm/sec speed, the 30 mm distance, 50% strain, and a preload of 5 g. SF values were recorded and averaged, with results expressed in Newtons, using a Warner-Bratzler device attached to a TA XT-2i Texture Analyzer (Stable Micro Systems Ltd., UK).

A Minolta CR300 colorimeter (d/8° geometry) was used to assess MC using the CIE color system, which provides values for L* (lightness), a* (redness), and b* (yellowness). A 10° standard observer and illuminant C were used to calibrate the instrument with a white reference tile. Samples were allowed to sit at room temperature for 30 min prior to assesment. Following the procedure described by Luzardo et al. (2019), color readings were taken at three distinct locations on the muscle surface, and the mean value for each coordinate (L*, a*, b*) was calculated per sample.

Microstructure analysis

Hematoxylin-Eosin (HE) Staining

Histological slides were prepared using Kiernan's (1990) method. This was carried out by performing an initial fixation with Bouin's solution for a day, followed by transfer to 70% alcohol. Samples were then trimmed to 2x2x1 mm, placed in cassettes, and dehydrated in increasing alcohol concentrations. After embedding, 4-micron sections were cut using a microtome, mounted on slides, and dried. The 24 h before staining with HE, the prepared slides were incubated at 37°C. The HE staining was conducted by

deparaffinization, rehydration, staining with Hematoxylin and Eosin, followed by dehydration. Quantitative data were collected from the images using ImageJ (NIH, United States), which included the perimysium length, endomysium length, muscle fiber area, muscle fiber diameter, and the total number of muscle fibers measurements.

Scanning Electron Microscopy (SEM)

The left *Longissimus dorsi* muscle microstructure was analyzed using SEM, following the procedure described by Estrada-Solís et al. (2016). Three tissue samples, each about 1×1×0.3 cm, were sliced perpendicular to the muscle fibers from the surface. Sample preparation followed the chemical fixation technique described by Sheldon and Sybers (1976), employing a critical point dryer (Tousimis Samdri 780a, Japan), a sputter coater (JEOL Fine Coat Ion Sputter JFC-1100, Japan), as well as SEM analysis (JEOL JSM-6390, Japan) operated at 5 kV. Quantitative analysis was performed on the SEM images using ImageJ software (NIH, USA). Each image was divided into grids of 100 × 100 μm squares, from which three squares were randomly selected and converted into binary images. For each chosen region, the black space region of interest (ROI) was measured, while the rest of the area was designated as the muscle fiber region. Images were taken at 300× magnification, with a 50-μm scale bar.

Fatty acid analysis of lamb feed

For FA analysis, 7 g of feed sample from each treatment group were measured, and the results were reported either as mg/g of total FAs or mg/100 g of feed. FA methyl esters (FAME) were generated and examined using gas chromatography with flame ionization detection (GC-FID; Agilent Technologies 7890B), featuring a capillary column (30 m in length, 250 μm internal diameter, 0.25 μm film thickness; Supelco Inc., Bellefonte, PA). The FAMEs were identified by comparing their retention times to those of a commercial standard mixture (38-Component FAME Mix, Supelco Inc.) as well as published chromatograms (Alves et al., 2021). Additionally, the identities of the FAME were further verified employing gas chromatography–mass spectrometry (GC-MS; Agilent Technologies 7890B), applying the same column and chromatographic conditions as in GC-FID.

The injector and detector temperatures for the GC-FID were set at 200°C and 250°C, respectively. Starting at 60°C (no hold), the oven program climbed at a rate of 30°C per min to 150°C (held for 1 minute), then by 2°C per min to 200°C, and lastly by 3°C per min to 225°C (held for 5 minutes). The carrier gas, helium, was employed at a rate of 1 mL/min. A 10:1 split ratio was used to inject a 1 μL sample. The ion source and interface were both set to 200°C and 250°C, respectively, for the GC-MS analysis.

Fatty acid of rumen fluids

The gas chromatography approach was employed to measure the FA concentrations in both the oil source and rumen buffer. To determine the total FA concentration, one milligram of internal standard (C19:0) was included to each sample before freeze drying. FAME was prepared via a two-step methylation process and assessed with gas chromatography (GC Agilent Technologies 7890B), utilizing helium as the carrier gas, an autosampler (Agilent DB-FastFAME), a flame ionization detector, and a Varian capillary column (HP-88, 100 m x 0.3 mm x 0.2 μm). The injector and detector temperature was kept at 240°C. After starting at 100°C for five minutes, the temperature was raised by 5°C per min to 240°C for 10 min, and it remained there for 50 min. After identifying the peak, the concentration was computed using a recognized standard's peak area and retention time (Amanullah et al., 2022)

Fatty acid of lamb meat

7 g of each animal's left *Longissimus dorsi* were used for the FA analysis, and the results were reported as mg/g of total FAs or mg/100g of meat. The same capillary column and gas chromatography with flame ionization detection were utilized to measure the FAME. By comparing retention periods with published chromatograms and the same commercial standard mixture, FAME was identified (Alves et al., 2021).

Additionally, GC-MS used an Agilent Technologies 7890B GC to achieve confirmation. The chromatographic conditions for GC-FID comprise an oven temperature of 60°C for 0 min, followed by an increase of 30°C/min to 150°C and maintenance for 1 min, as well as injector and detector temperatures of 200°C and 250°C, respectively. The temperature was raised by 2°C per min to 200°C, then by 3°C per min to 225°C, and it was kept there for 5 min. At a flow rate of 1 mL/min, helium was utilized as the carrier gas. The split ratio was 10:1, and approximately 1 μl of the material was injected. The capillary column and GC settings utilized in the GC-FID study were also used in the GC-MS. The MS condition was 200°C for the ion source and 250°C for the interface.

Statistical analysis

SAS software was used to analyze the data (SAS Institute Inc., Cary, NC, USA). The SAS mixed technique (PROC MIXED) was used to analyze the TKC impact on lamb carcass characteristics and meat quality. The PROC MIXED LSMEANS statement was utilized to get the mean of the treatments. The following describes the statistical model that was employed.

$Yij = \mu + Ti + eij$

Where Yij is the variable calculated, μ = the variable’s overall mean, Ti is the treatment i’s effect, and eij represented the residual error. Substantial differences were determined at $P < 0.05$, while a trend was noted for $0.05 \leq P < 0.10$.

RESULTS

Carcass yield

T30 feed treatment led to a higher carcass percentage (p = 0.040; 39.9% vs. 37.1%, 38.0%, and 38.0%) compared to the T0, T15, and T30m. Conversely, the non-carcass percentage in T30 feed treatment (p = 0.039; 60.1% vs. 62.9%, 62.0%, and 62.0%) was lower compared to the T0, T15, and T30m feed treatments with premix (Table 2). The BCTRC percentage value of T0 TKC meal treatment resulted in a lower BCTRC percentage (p = 0.011; 57.6% vs. 65.3%, 65.3%, and 68.2%) compared to T15, T30, and T30m (Table 3).

Table 2
Lamb Carcass Components Fed Rations with Different Levels of TKC *(Callophyllum inophylum)*

Variables	**Inclusion of TKC (% dry matter)**				**SEM**	**p value**
	T0	**T15**	**T30**	**T30m**		
Initial bodyweight (kg)	16,9	17,2	17,2	17,0	1,034	0,937
Slaughter weight (kg)	22,3	23,6	22,7	21,9	1,306	0,254
Carcass (kg)	8,24	8,92	9,16	8,44	0,564	0,138
Carcass (%)	37,1[b]	38,0[b]	39,9[a]	38,0[b]	1,207	0,040
Meat (kg)	5,34	5,81	5,85	5,37	0,420	0,196
Bone (kg)	2,98	3,11	3,19	3,08	0,216	0,480
Meat bone ratio	1,86	1,87	1,78	1,75	0,125	0,412

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

[a,b] = Different superscripts on the same line show significant differences (P < 0.05)

Table 3
Yield Grade Evaluation of Lamb Fed Rations with Different Levels of TKC (*Callophyllum inophylum*)

Variables	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
Fat thickness (mm)	1,37^{b}	1,60^{ab}	1,73^{ab}	1,97^{a}	0,248	0,089
Bodywall (mm)	4,34	4,60	4,92	4,50	0,580	0,472
Eye muscle area (cm^2)	3,89^{a}	2,95^{b}	3,54^{ab}	2,79^{b}	0,560	0,027
BCTRC^2 (%)	57,6^{b}	65,3^{a}	65,3^{a}	68,2^{a}	1,924	0,011
Yield grade	1,77^{b}	2,00^{ab}	2,13^{ab}	2,37^{a}	0,248	0,089

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

^{a,b,c} = Different superscripts on the same line show significant differences ($P < 0.05$)

Physicochemical analysis of meat

Inclusion of TKC treatment affected the lamb meat's physical and chemical characteristics. Its pH value in T30m ($p = 0.009$; 5.72 vs. 5.60, 5.52, and 5.41) was higher compared to the T30, T15, and T0 treatments, respectively. The findings demonstrated that there were differences in CL, where T0 and T15 TKC meal treatments ($p = 0.025$; 33.4% and 32.9% vs. 28.7% and 28.5%) resulted in lower CL compared to T30 and T30m. Hardness in the T0 TKC feed treatment produced a lower value compared to T15, T30, and T30m ($p = 0.002$; 21.8 N vs. 25.9, 27.0, and 28.4 N). The crude fat content showed a difference in T30 ($p = 0.021$; 2.55% vs. 1.65% and 1.31%), which was higher compared to T0, and T15 (Table 4).

Table 4
Physicochemical Characteristics of Lamb Meat Fed Rations with Different Levels of TKC (*Callophyllum inophylum*)

Variables	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
pH	5,52^{bc}	5,41^{c}	5,60^{ab}	5,72^{a}	0,119	0,009
CL (%)	33,4^{a}	32,9^{a}	28,7^{b}	28,5^{b}	2,054	0,025
SF (N/cm^2)	21,8^{b}	25,9^{a}	27,0^{a}	28,4^{a}	2,133	0,002
Water holding capacity (%)	43,7	43,2	45,3	40,0	6,163	0,594
L*	43,3	43,4	45,4	44,8	2,851	0,599
a*	18,4	17,6	18,5	18,1	2,581	0,940
b*	10,5	11,4	12,1	12,2	1,696	0,371
DeltaHue	29,8	33,0	33,4	34,0	4,039	0,384
Protein (%)	19,0^{ab}	18,1^{c}	19,3^{a}	18,5^{bc}	0,392	0,001
Fat (%)	1,65^{b}	1,31^{b}	2,55^{a}	2,11^{ab}	0,565	0,021
Moisture (%)	75,5	76,5	76,5	76,5	0,981	0,344
Ash (%)	1,42^{a}	1,23^{b}	1,42^{a}	1,25^{b}	0,058	0,000
T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).						
^{a,b,c} = Different superscripts on the same line show significant differences (P < 0.05)						

Microstructure analysis

Quantitative calculation results based on HE staining microstructure images in Fig. 1 show that the perimysium size in the T0 (p = 0.010; 31.2 µm vs. 43.6 and 43.3 µm) was lower compared to T30 and T30m. Meanwhile, the endomysium size in T0 and T15 (p = 0.004; 7.59 and 8.56 µm vs. 9.20 µm) was lower compared to T30 TKC with premix treatment. The myofibril area showed a difference in T30m and the T30 (p = < 0.001; 566.6 and 542.4 µm2 vs. 371.4 and 351.7 µm2) that was higher compared to the T0 and T15. Similarly, the myofibril diameter calculation also showed a difference in the T30m (p = < 0.001; 30.3 µm vs. 25.2, 24.1, and 24.6 µm) that was higher compared to T0, T15, and T30 (Table 5).

Table 5
Microstructure of Lamb Meat Fed Rations with Different Levels of TKC (*Callophyllum inophylum*)

Variables	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
Perimysium (µm)	31,2[b]	39,4[ab]	43,6[a]	43,3[a]	12,46	0,010
Endomysium (µm)	7,59[b]	7,705[b]	8,56[ab]	9,20[a]	1,559	0,004
Muscle fiber area (µm^2)	371,4[b]	351,7[b]	5424[a]	566,6[a]	117,4	<,001
Muscle fiber diameter (µm)	25,2[b]	24,1[b]	24,6[b]	30,3[a]	3,421	<,001
Number of muscle fiber[2]	62[b]	99[a]	46[d]	55[c]	4,780	<,001
T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).						
[2]40 magnification						
[a,b,c] = Different superscripts on the same line show significant differences (P < 0.05)						

Fatty acid composition

Feed. Graded supplementation of TKC (T0, T15, T30, and T30m) altered the feed's FA profile. Overall, the SFA proportion decreased from 43.2% in T0 to 23.7% in the highest treatment. In contrast, monounsaturated (MUFA) and polyunsaturated FA (PUFA) increased, from 23.7% and 33.1% in T0 to 33.5% and 42.8%, respectively. Consequently, the UFA/SFA ratio improved significantly, rising from 1.32 to 3.21 (Table 1).

Rumen Fluids. Changes in FA profiles also occurred in the rumen due to feed composition. SFA significantly decreased from 13.58% to 4.82% (p = < 0.0001; 13.58% vs. 6.86, 4.71, and 4.82%). Lauric acid (C:12) also followed the same trend (p = < 0.0001; 9.02% vs. 3.28, 0.82, 0.57%). The T30m treatment showed the highest MUFA content (p = 0.0001; 94.95% vs 55.60, 52.44, and 52.03). T30 produced the highest PUFA content (p = 0.0001; 0% vs. 43.14, 37.41, and 34.36%), and the highest UFA/SFA ratio was found in T30 and T30m (p = < 0.0001; 6.42 and 13.61 vs. 20.29 and 19.88) (Table 6).

Table 6
Fatty Acid Composition of Lamb Rumen Fluids Fed Rations with Different Levels of TKC (*Callophyllum inophylum*)

Item	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
C12 (% total FA)	9.02^{a}	3.28^{b}	0.82^{c}	0.57^{c}	0.395	< .001
C14 (% total FA)	3.07^{a}	2.04^{b}	2.64^{ab}	2.10^{b}	0.339	0.017
C14:1 (% total FA)	7.24^{a}	4.89^{b}	2.46^{c}	1.35^{d}	0.137	< .001
C15 (% total FA)	1.36^{ab}	1.20^{b}	1.06^{b}	1.97^{a}	0.353	0.056
C15:1 (% total FA)	0.43^{b}	1.33^{a}	1.39^{a}	1.45^{a}	0.370	0.028
C16:1 (% total FA)	23.61^{b}	24.55^{a}	22.47^{c}	20.71^{d}	0.273	< .001
C18:1 (n9) (% total FA)	32.24	37.43	40.20	71.58	20.49	0.26
C18:2 (n6) (% total FA)	34.36^{c}	37.41^{b}	43.14^{a}	0.00^{d}	0.610	< .001
C21 (% total FA)	0.14	0.34	0.19	0.17	0.132	0.317
SFA[e] (% total FA)	13.58^{a}	6.86^{b}	4.71^{c}	4.82^{c}	0.750	< .001
MUFA[f] (% total FA)	52.44^{c}	55.60^{b}	52.03^{c}	94.95^{a}	0.844	< .001
PUFA[g] (% total FA)	34.36^{c}	37.41^{b}	43.14^{a}	0.00^{d}	0.610	< .001
UFA[h] (% total FA)	86.42^{c}	93.14^{b}	95.29^{a}	95.18^{a}	0.750	< .001
UFASFA Ratio	6.42^{c}	13.61^{b}	20.29^{a}	19.88^{a}	1.292	< .001
Total FA (mg/mL)	94.85^{d}	94.98^{c}	95.06^{b}	95.09^{a}	0.013	< .001

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

[a,b,c,d] = Different superscripts on the same line show significant differences (P < 0.05)

[e] Saturated FA

[f] Monounsaturated FA

[g] Polyunsaturated FA

[h] Unsaturated FA

Lamb meat. T0 had the highest SFA content (p = 0.000; 45.7% vs. 39.8,41.6, and 41.2%), with lauric, myristic, and palmitic acid as the dominant components. The PUFA content was significantly different in T30m (p = 0.004; 20.4% vs. 13.1 and 15.9%), containing linoleic (C18:2 n6), arachidonic (C20:4 n6) acid, as well as eicosatrienoic (C20:3 n6). T0 had the lowest UFA/SFA ratio (p = 0.001; 1.19 vs. 1.52, 1.41, 1.42) (Table 7).

Table 7
Fatty Acid Composition of Lamb Meat Fed Rations with Different Levels of TKC (*Callophyllum inophylum*)

Item	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
C8 (% total FA)	0.05^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.013	0.011
C10 (% total FA)	0.12^{a}	0.10^{ab}	0.10^{ab}	0.08^{b}	0.020	0.080
C12 (% total FA)	0.42^{a}	0.17^{b}	0.12^{b}	0.10^{b}	0.065	< .001
$C13^{ns}$ (% total FA)	0.00	0.00	0.00	0.00	0.005	0.879
C14 (% total FA)	2.92^{a}	1.72^{b}	1.46^{b}	1.27^{b}	0.430	0.000
C14:1 (% total FA)	0.07^{a}	0.05^{b}	0.03^{cb}	0.02^{c}	0.017	0.001
$C15^{ns}$ (% total FA)	0.39	0.39	0.37	0.36	0.080	0.897
C16 (% total FA)	20.3^{a}	17.1^{b}	17.8^{b}	17.0^{b}	0.998	0.001
C16:1 (% total FA)	2.05^{a}	1.98^{a}	1.39^{b}	1.37^{b}	0.208	0.000
C17 (% total FA)	0.86^{b}	1.04^{a}	1.12^{a}	1.13^{a}	0.099	0.003
C17:1 (% total FA)	0.64^{b}	0.89^{a}	0.72^{b}	0.68^{b}	0.087	0.004
C18 (% total FA)	20.7^{ab}	19.1^{b}	20.5^{ab}	21.2^{a}	1.188	0.088
C18:1 (n9) (% total FA)	38.4^{ab}	37.3^{b}	40.4^{a}	36.3^{b}	2.110	0.046
C18:2 (n6) (% total FA)	8.08^{c}	12.4^{ab}	11.0^{b}	13.6^{a}	1.716	0.001
C18:3 (n6) (% total FA)	0.01	0.03	0.01	0.04	0.030	0.299
C18:3 (n3) (% total FA)	0.50	0.75	0.74	0.76	0.205	0.203
$C20^{ns}$ (% total FA)	0.05	0.09	0.07	0.07	0.035	0.377
C20:1 (n9) (% total FA)	0.04	0.06	0.07	0.06	0.051	0.834
C20:3 (n6) (% total FA)	0.23^{b}	0.39^{a}	0.28^{b}	0.40^{a}	0.080	0.014
C20:4 (n6) (% total FA)	3.58^{bc}	5.43^{a}	3.38^{c}	5.02^{ab}	1.066	0.021

Item	Inclusion of TKC (% dry matter)				SEM	p value
	T0	T15	T30	T30m		
C20:5 (n3) (% total FA)	0.55[ab]	0.79[a]	0.39[b]	0.47[ab]	0.233	0.089
C22:6 (n3) (% total FA)	0.10	0.12	0.04	0.04	0.100	0.471
SFA[d] (% total FA)	45.7[a]	39.8[b]	41.6[b]	41.2[b]	1.545	0.000
MUFA[e] (% total FA)	41.2[ab]	40.3[ab]	42.6[a]	38.4[b]	2.289	0.076
PUFA[f] (% total FA)	13.1[b]	20.0[a]	15.9[b]	20.4[a]	2.838	0.004
UFA[g] (% total FA)	54.3[b]	60.2[a]	58.4[a]	58.8[a]	1.545	0.000
UFASFA Ratio	1.19[b]	1.52[a]	1.41[a]	1.43[a]	0.094	0.001
Total FA (mg/g)	95.1[b]	95.2[a]	95.2[a]	95.2[a]	0.011	< .0001
T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).						
[a,b,c] = Different superscripts on the same line show significant differences (P < 0.05)						
[d] Saturated FA						
[e] Monounsaturated FA						
[f] Polyunsaturated FA						
[g] Unsaturated FA						

DISCUSSION

Lamb meat, rich in protein and PUFA, as shown in Table 1, can enhance performance (Baptiste et al., 2018). The findings noted that T30 treatment increased carcass and %BCTRC. This can be attributed to the secondary metabolite content, which is high in phenols and flavonoids at 3.5 and 13.5 mg, respectively, as well as protein at 20.1% (Paradhipta et al., 2023). Phenols and flavonoids are strong antioxidants that protect muscle cells from oxidative stress during growth (Li et al., 2024) and in the mixed feed, can increase muscle protein synthesis (Poli et al., 2021). The percentage of BCTRC was quite high, reaching 68.2% (Table 2). Previous studies indicate that % BCTRC is an effective metric for describing a lean, sellable product. Most lamb carcasses (51.3%) had a BCTRC between 41% and 45% (Whaley et al., 2019). According to (O'rourke et al., 2005), the Grade 1 result in the T0 TKC treatment implies minimal fat, a high percentage of lean meat, while the Grade 2 in the T15, T30, and T30m

suggests slightly more fat. The higher the yield grade, the greater the carcass, which may reduce the efficiency of boneless meat but increase the tenderness and flavor. Therefore, the presence of phenols and flavonoids in TKC help to reduce oxidative stress and inflammation, which in turn enhances nutrient utilization efficiency, ultimately improving muscle growth, carcass percentage, % BCTRC, and yield grade.

Changes in the lamb meat's physical quality, specifically pH, CL, and tenderness (Table 3), may be attributed to secondary metabolites in TKC, which can influence intramuscular fat deposition. The meat's pH level increased notably but stayed within the normal range of 5.4 to 5.8 (Alice, 2010). Flavonoids in lamb feed could enhance muscle juiciness and tenderness by boosting the muscle's fat content (Li et al., 2024). Typically, higher intramuscular fat is associated with reduced meat tenderness, indicating that increased fat within the muscle tends to decrease its tenderness (Silva et al., 2024). Structurally, water holding capacity can be influenced by the myofibril shrinkage, the damage to cell membrane structure, the integrity of the intracellular cytoskeleton, and the development of intercellular spaces that allow fluid accumulation (Hughes et al., 2014). Nutritional management is often used to enhance the lamb meat's physical and chemical attributes. The feed in this study was isoprotein and isoenergetic, but the chemical quality analysis of the LD muscle showed an increase in fat content in T30 (Table 3). A prior study demonstrated that adding flavonoids to lamb feed resulted in increased fat content (Liu et al., 2019). A diet rich in flavonoids and phenolic compounds improves several meat quality aspects, such as lowering CL, boosting fat content, and enhancing FA profiles (Li et al., 2024).

The increase in the size of perimysium and endomysium (Table 5) surrounding the fasciculus shows a strengthening of the connective tissue that may occur due to the high flavonoid content of secondary metabolites in TKC. This is aligned with Ouyang et al. (2016), where flavonoids in mixed feed acted as mediators in fibroblast proliferation and collagen synthesis. Fibroblast cell metabolism stimulation produces more collagen fibers and extracellular matrix. This is also supported by a significant increase in SF and lower CL in the T30 and T30m, which shows the tissue ability to retain water better due to the increase in connective tissue. The higher UFA content in TKC also influences intramuscular fat deposition (Nuernberg et al., 2005). The simultaneous increase in muscle fasciculus diameter and perimysium diameter can be an indication of growth. This is because in the postnatal life of lamb, the growth of muscle cross-sectional area, the proportion of connective tissue, perimysium, and myofibril diameter are closely related. The number of muscle fibers per fasciculus, together with the muscle fiber area and perimysium thickness, describes the myofibril size and meat texture (Hena et al., 2017). Microstructure results of lamb meat by SEM (Fig. 1) showed that muscle fibers of T0 appeared smaller with relatively larger spaces between. Conversely, in T30 and T30m, muscle fibers were larger, denser, and the spaces between were narrower. The increased diameter and area of muscle fibers can be attributed to the bioactive effects of TKC, particularly phenols and flavonoids, known to support muscle cell growth and stability through antioxidant activity (Poli et al., 2021). This more compact muscle structure presumably led to an increase in meat SF, as reflected in the higher hardness values. Furthermore, the larger and more compact fiber structure may have helped enhance water retention within the tissue during cooking, consistent with the reduced loss observed in the TKC groups. Estrada-

Solís et al. (2016) reported that higher structural resistance of muscle tissue can retain more intramuscular fluid.

Regarding FA at the feed level, TKC contains a higher proportion of UFA (MUFA and PUFA) and a lower proportion of SFA. Notably, the PUFA content is especially high in linoleic acid (C18:2 n6) and α-linolenic acid (C18:3 n3). Bioactive compounds in TKC likely help protect UFA from oxidation both in the feed and during early digestion stages (Liu et al., 2022). In the rumen fluid, the FA profile exhibits a similar trend namely SFA levels decrease while MUFA and PUFA increase. Nowak et al. (2022), found that feeds high in phenolic compounds substantially influence the rumen FA profile, possibly enhancing milk and meat FA composition by altering VFAs during biohydrogenation. The inclusion of TKC may lead to a reduction in the biohydrogenation of UFA. Phenol and flavonoid compounds can reduce biohydrogenation by inhibiting the process at various stages of isomerization and saturation. This is achieved by modulating the rumen microbiota, the microbial community in the rumen that facilitates biohydrogenation. By changing the composition of these microorganisms, polyphenols decrease the efficiency of biohydrogenation, resulting in an increased amount of UFA in the rumen (Pratiwi et al., 2024).

The reduction of biohydrogenation by phenol is possibly due to the ability to bind lipase enzymes and inhibit the lipolysis process (Morales and Ungerfeld, 2015). In the biohydrogenation process, C:18 undergoes isomerization from the cis-9, cis-12 form to cis-9, trans-11 (vaccenic acid), and then reduction at the cis-9 double bond to form trans-11-18:1. Biohydrogenation can also produce trans-18:1 and CLA (conjugated linoleic acid) from C:18, although CLA is not formed from linolenic acid (Demeyer and Doreau, 1999). Aside from changes in long-chain FA (LCFA), the inclusion of TKC also showed significant effects on medium-chain FA (MCFA), specifically C6 to C12. MCFA are FA with carbon chains that are generally 6 to 12 carbons long, and can be found in natural products such as coconut oil (comprising about 15%), palm kernel oil (around 7.9%) (Huang et al., 2021). Studies indicate that free medium-chain FA (C6:0–C12:0) are more effective at killing a wide range of gram-negative as well as gram-positive bacteria than free short-chain FA (Van Immerseel et al., 2004). In the context of biohydrogenation, the low levels of MCFA, particularly C12 in T30, may also contribute to the inhibition of UFA conversion to SFA, as shown by the increased MUFA and PUFA content in the rumen fluid. This phenomenon strengthens the hypothesis that phenols and flavonoids in TKC work synergistically with low MCFA to suppress biohydrogenation activity, either through microbial modulation or direct inhibition of enzymes converting UFA to SFA. Triglycerides in circulation are carried by chylomicrons and are broken down in muscle and fat tissue by lipoprotein lipase, releasing free FA. These FA are then taken up by muscle and fat, where they are utilized to form chylomicron remnants (Feingold, 2024).

This effect extended to the muscle tissue, where C12:0 decreased from T0 to T30m, and the overall proportion of total MCFA decreased, while PUFA increased (Table 7). Based on the results, substituting feed ingredients containing MCFA with TKC led to a healthier meat fat profile, characterized by a higher UFA/SFA ratio and more optimal PUFA levels. The FA content, especially the PUFA composition and levels, was greatly influenced by the TKC dietary inclusion. FA in meat are commonly used to evaluate nutritional and product quality, including effects on health and taste (Prache et al., 2022). Lamb meat

from the T30 group predominantly contained PUFA and MUFA, consistent with (Nuernberg et al., 2005) who noted that UFAs absorbed by the body tend to deposit in the intramuscular fat, whereas SFAs are more prevalent in subcutaneous and abdominal fat.. This result is also evident from the significant fat content (Table 4) as well as the size of the perimysium and endomysium observed through microstructure analysis (Table 5). Therefore, UFA levels in meat were higher in the T30 due to the inhibition of biohydrogenation in the rumen, increased UFA absorption, and selective UFA deposition into intramuscular tissue. According to Brunetto et al. (2024), a positive correlation exists between higher levels of UFA and health benefits. This is because while SFA consumption is often considered detrimental due to the connection to coronary disease, many UFAs are associated with reduced inflammation, stroke risk, oxidative stress, and liver problems.

CONCLUSION

Overall, this study demonstrated that adding T30 to the concentrate diet improved lamb carcass performance and meat quality. T30 increased the percentage of carcass and BTCRC, reduced CL, and improved fat content as well as muscle microstructure quality, including perimysium, endomysium, and muscle fiber area. Using TKC at all treatment levels increased the UFA content and decreased the SFA content in lamb meat than the control. However, adding mineral premix to TKC did not substantially impact carcass and meat quality. Including T30 TKC can be suggested as an subtitute protein source in lamb diets to enhance carcass and meat quality.

Declarations

ETHICS APPROVAL

The Ethical Clearance Commission, Integrated Laboratory for Research and Testing, Universitas Gadjah Mada, granted ethical clearance for this study under certificate number 00056/04/LPPT/XII/2023.

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Author Contribution

Conceptualization: DHVP, CH, ETData curation: ET, CHFormal analysis: HDA, DHVPMethodology: HDA, DHVPSoftware: HDA, DHVPValidation: IHAInvestigation: BLWriting - original draft: HDA, DHVP, EET, CHWriting - review & editing: HDA, DHVP, EET, CH, IHA, BL

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Figures

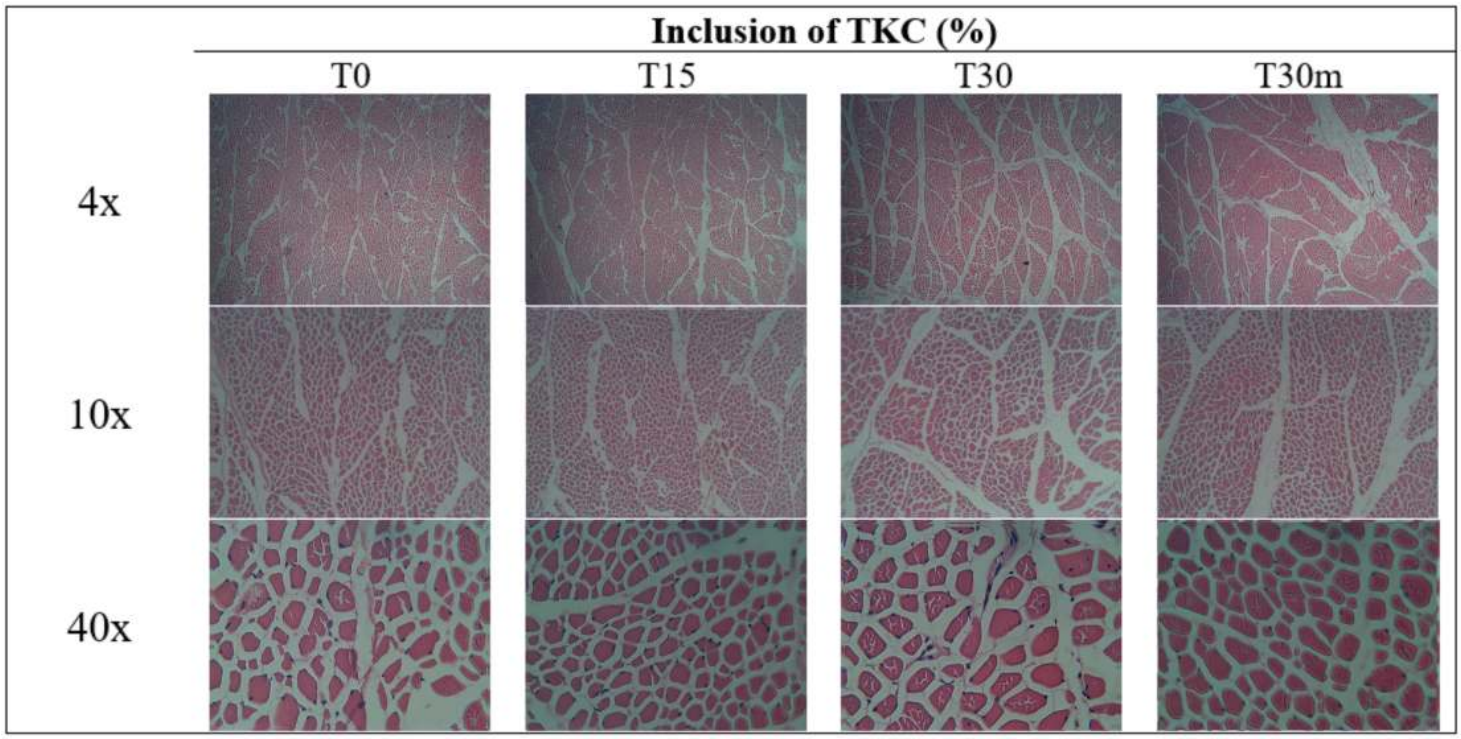

Figure 1

Microstructure of the *Longissimus dorsi* muscle cross-section of lamb with Hematoxylin-Eosin (HE) staining for various levels of Tamanu Kernel Cake (TKC) (*Callophyllum inophyllum*)

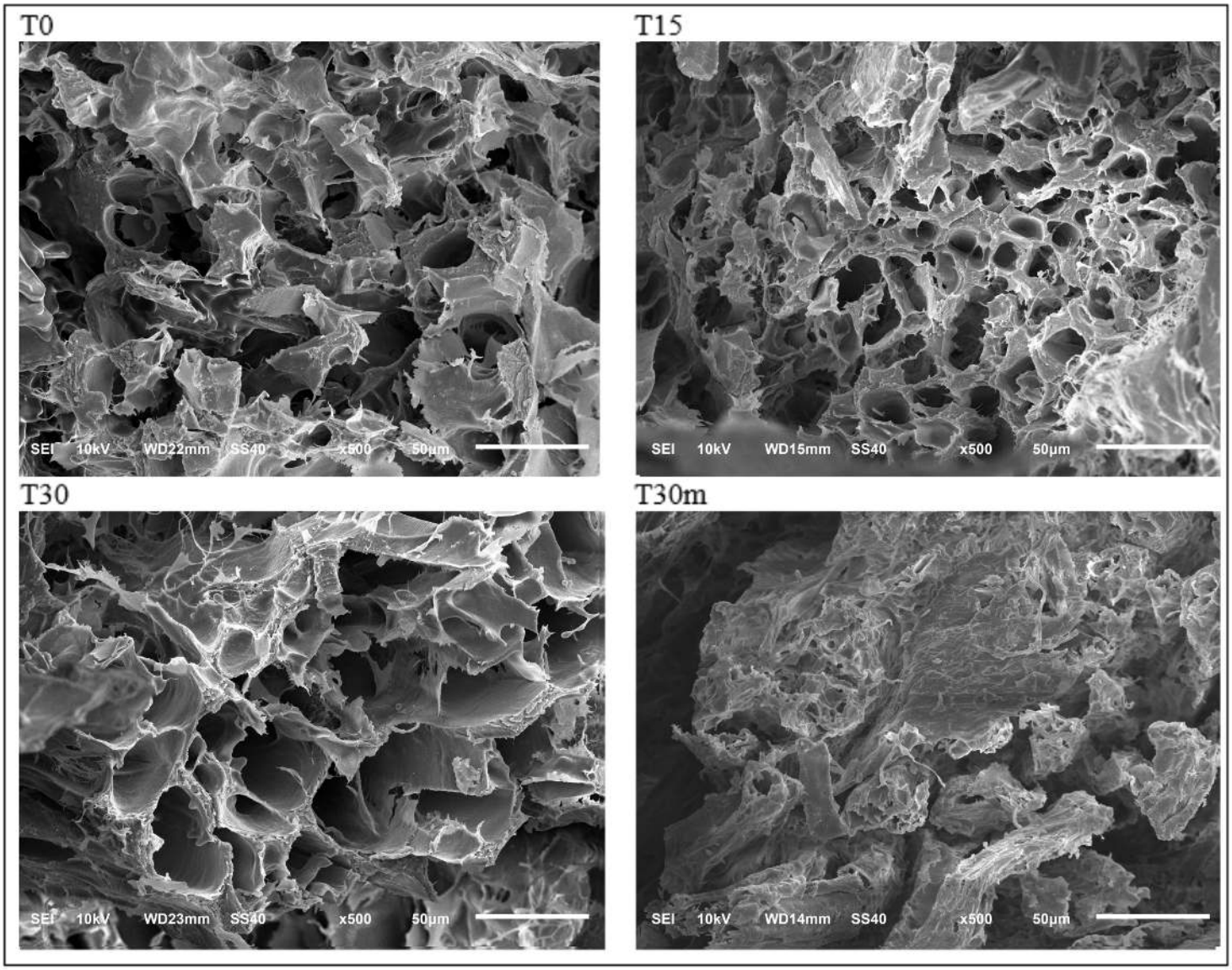

Figure 2

Microstructure of the *Longissimus dorsi* muscle cross-section of lamb with SEM at 500x magnification for various levels of TKC in the ration: T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

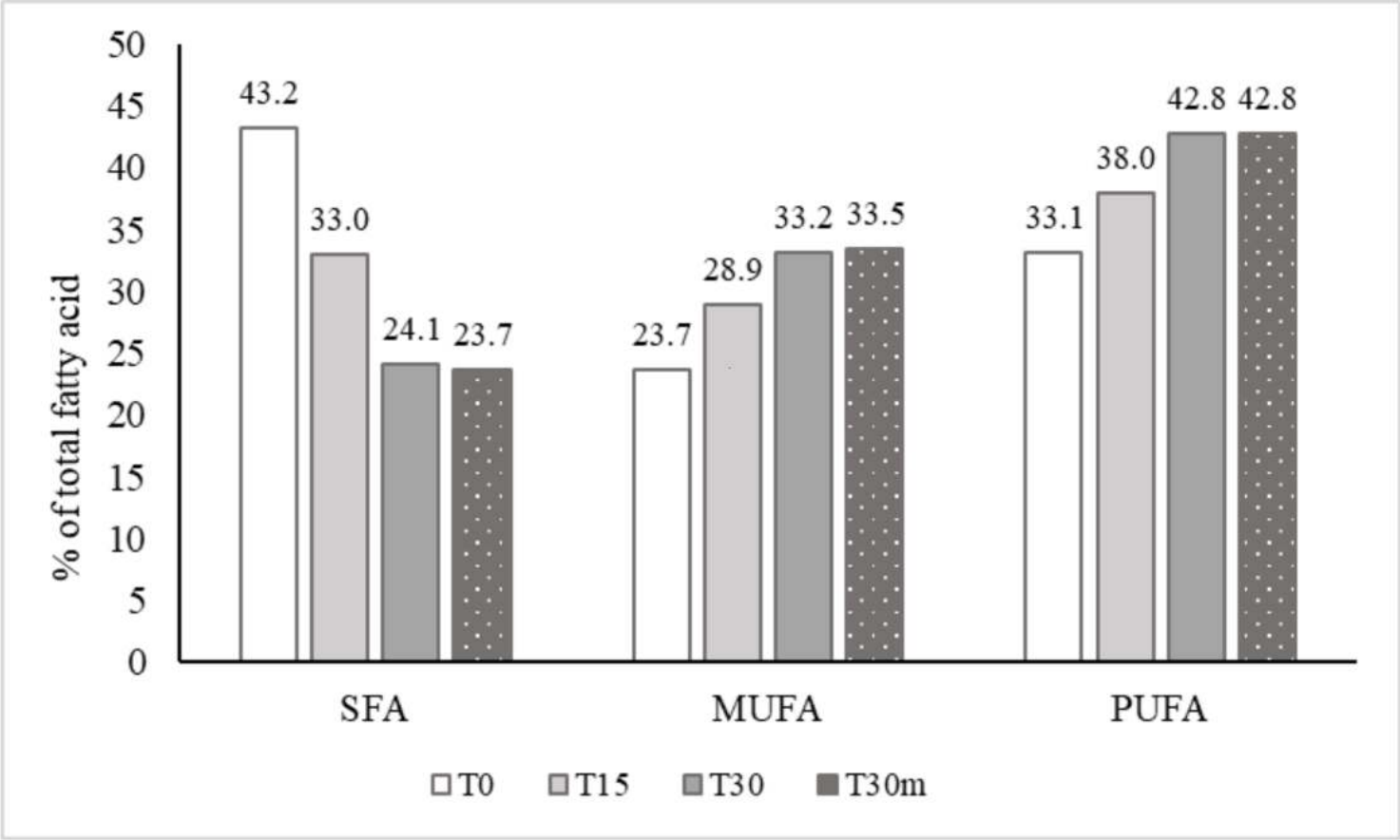

Figure 3

FA profiles on animal diet.

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

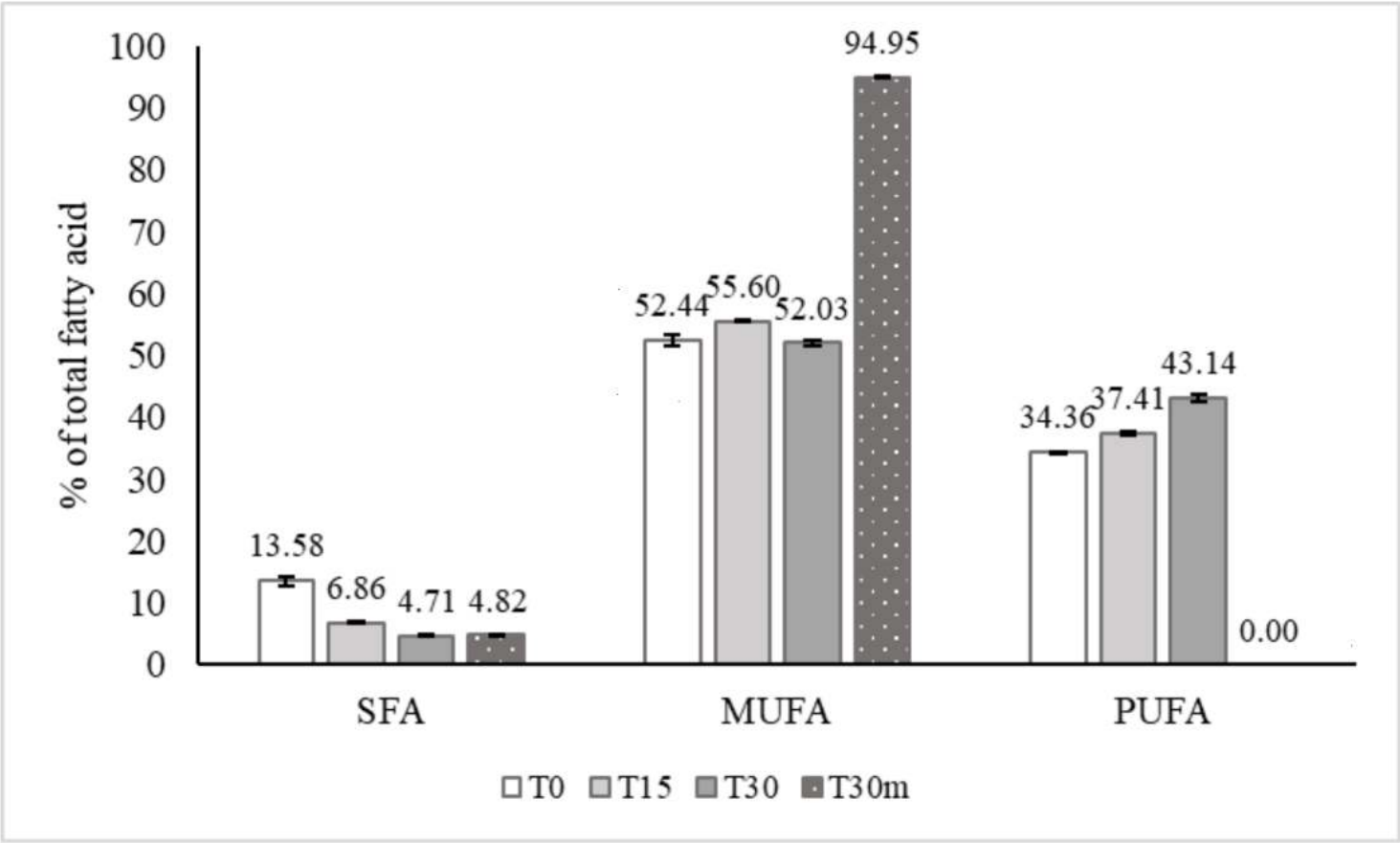

Figure 4

FA profiles on rumen fluid.

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).

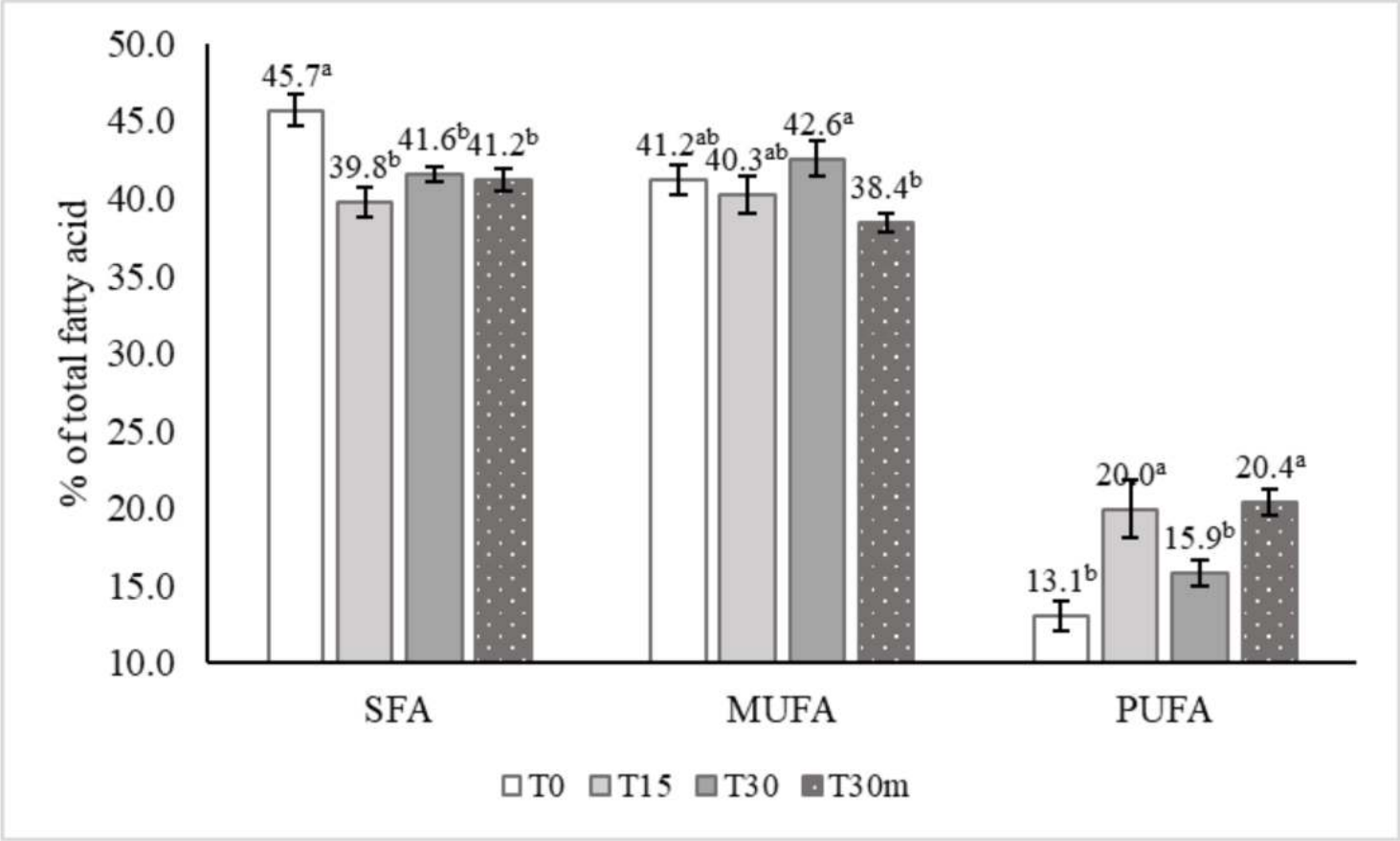

Figure 5

FA profiles on lamb meat

T0, inclusion of TKC 0%; T15, inclusion of TKC 15%; TKC 30, inclusion of TKC 30%; T30m, inclusion of TKC 30% added trace minerals including Fe, Zn, Mg, Se, and vitamin complex (A, D, E).