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Yellow mealworm as an alternative to conventional plant- and animal-based protein sources in feedlot lambs' diets: Implications on blood parameters, growth and slaughter performance, carcass traits, and meat quality

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ABSTRACT

This study evaluates the effects of *Tenebrio molitor* meal (TMM) as an alternative to conventional plant-based (soybean meal, SBM) and animal-based (fishmeal, FM) protein sources on blood parameters, growth and slaughter performance, carcass traits, and meat quality of feedlot lambs. A total of 24 Suffolk lambs [3 months of age, 21.4 ± 1.08 kg body weight (BW)] were allocated for 60 days to three treatments (8 lambs per treatment) in a completely randomized design, as follows: 1- SBM at 150 g/kg dry matter (DM)], 2- FM at 50 g/kg DM, and 3- TMM at 60 g/kg DM. Feed intake, BW, and blood samples were collected on days 0, 20, 40, and 60. At the end of the trial, the lambs were slaughtered for carcass evaluation and meat quality assessment. The average daily gain was higher for SBM-fed than for TMM-fed lambs (0.25 and 0.21 kg/d; $P < 0.001$). Plasma levels of creatinine were higher in FM-fed when compared to SBM-fed lambs, while TMM-fed lambs showed intermediate values (88.72, 85.69 and 87.57 μmol/L, respectively; $P < 0.05$). The hot and cold carcass weights (15.99 and 15.52 kg *vs* 18.49 and 18.03 kg) and yields (44.31 and 43.02 % *vs* 46.03 and 44.87 %) were lower in the TMM group compared with the SBM group ($P < 0.05$). The dietary treatment had no effect ($P > 0.05$) on meat pH, color, texture, water loss and contents of fat, protein, and moisture. Further research should evaluate optimal inclusion levels of TMM in combination with conventional protein sources to avoid negative economic implications.

1. Introduction

Globally, climate change and population growth are causing food insecurity issues (Abbas et al., 2025). Therefore, alternative, innovative, and sustainable ingredients, characterized by high nutrient contents and health-promoting properties, are currently being evaluated as feed ingredients for farmed animals (Castillo & Hernández, 2023; Elahi et al., 2020).

Edible insects contain valuable quantities of protein [up to 80 % on a dry matter (DM) basis], lipids (up to 43 % DM), vitamins (thiamine, riboflavin, niacin), and minerals (iron, zinc, potassium, magnesium, calcium) (Zhou et al., 2022), therefore being considered as promising

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ingredients for animal feeding purposes (Gasco et al., 2023; Renna et al., 2023a). Insects also contribute to sustainability by promoting higher resource-use efficiency and by reducing greenhouse gas emissions compared to conventional protein sources such as soybean meal and fishmeal (Gasco et al., 2023). This environmental advantage makes them key contributors to several Sustainable Development Goals (Dicke, 2018; Gao et al., 2024).

Insect species, such as the yellow mealworm (*Tenebrio molitor* L.; TM), the black soldier fly (*Hermetia illucens* L.), the housefly (*Musca domestica* L.), and the silkworm (*Bombyx mori* L.) have been widely investigated as substitutes for soybean meal (SBM) and fishmeal (FM) in monogastric (*i.e.*, poultry, swine, and fish) diets, with maximum recommended inclusion levels set at 25–30 % for fish and 10 % for poultry and pigs (Gasco et al., 2023). For ruminants, preliminary studies suggest that insect-based diets could reduce enteric methane emissions, potentially offering a dual benefit of nutritional adequacy and environmental impact reduction (Gonzalez-Ronquillo et al., 2025). However, their evaluation and use in ruminant nutrition is limited, mainly due to the potential risks associated with the Bovine Spongiform Encephalopathy (BSE) zoonosis, commonly known as the mad cow disease (Renna, Rastello and Gasco, 2022a).

Since 2017 the European Union (EU) has allowed the use of insects as ingredients in aquafeeds (Commission Regulation (EU) 2017/893), with a further extension of the approval to feed poultry and swine since 2021 (Commission Regulation (EU) 2021/1372). Several developed countries, including EU ones, USA, Canada, China, and Japan, prohibit the use of insects as ruminant feed, with a certain variability when considering insect meals or insect oils. In contrast, in many developing countries there is no specific legislation for using insects as ruminant feed (Renna et al., 2023a).

On a DM basis, crude protein (CP) and lipid contents in TM larvae range from 40 % to 63 % and from 22 % to 40 %, respectively (Syahrulawal, Torske, Sapkota, Næss, & Khanal, 2023). The TM lipids comprise unsaturated fatty acids, such as oleic (C18:1 *c*9) and linoleic (C18:2 *c*9c12) acids (Renna et al., 2022b), which can reach relative concentrations up to 55 % and 38 % of total fatty acids, respectively, depending on the substrate used for larvae rearing (Rossi et al., 2022; Syahrulawal et al., 2023).

Ayaz et al. (2023) found that replacing 25 % of commercial concentrate feed with mealworm frass (the leftover larvae rearing substrate, which comprises spent feedstock, insect feces, and cuticles) in sheep diets resulted in a weight gain increase of about 0.9 kg when compared to a control group. Khanal et al. (2023) showed that, for ruminants, the *in vitro* DM digestibility of TM larvae and pupae were comparable to that of SBM, being in all cases greater than 90 %. On the contrary, the same authors reported lower *in vitro* CP digestibility of TM larvae and pupae (slightly lower than 90 %) compared to values obtained for SBM (98 %). Again *in vitro*, Samadi, Wajizah, Pratama, and Jayanegara (2023) highlighted the potential enteric methane emission reduction of several non-conventional protein sources, including insect meals (TMM 0.42 mL/g DM; grasshopper meal 0.21 mL/g DM), when compared to SBM (0.50 mL/g DM). Finally, Toral et al. (2022) compared the ruminal nitrogen degradation and intestinal digestibility of four insects, namely TM, *Acheta domesticus*, *Alphitobius diaperinus,* and *Zophobas morio*. These authors found that TM showed the lowest values of ruminal N degradation (46 %, 72 %, 72 %, and 74 % for the four species, respectively) and the greatest intestinal digestibility of the non-degraded nitrogen in the rumen (78 %, 73 %, 64 %, and 70 %), and concluded that TM would probably be the best option, among the four insect species studied, to replace dietary SBM and increase the sustainability of ruminant production.

With this trial, we aimed at investigating the effects of the dietary inclusion of full-fat TMM on the blood parameters, growth and slaughter performance, carcass traits, and meat quality of feedlot lambs. We hypothesized that TMM can fully replace conventional protein sources (SBM and FM) without compromising growth performance, carcass traits and meat quality, thereby offering a viable alternative to conventional protein sources in lamb finishing diets.

2. Materials and methods

2.1. Ethical statement

This trial was reviewed by the Professional Committee on Standardization of Experimental Animals of the Universidad Autónoma del Estado de México (project code CAT2021–0070) and was carried out according to the guidelines and regulations established by ARRIVE (Percie du Sert et al., 2020).

2.2. Experimental design, animals, and diets

Twenty-four male Suffolk lambs of 3 months of age, with an initial body weight (BW) of 21.4 ± 1.08 kg (mean ± SD), were randomly allocated to three total mixed ration (TMR) diets (eight lambs per diet) in a completely randomized design.

The diets had a 30:70 forage to concentrate (F:C) ratio and were formulated to be isocaloric [12.0 MJ/kg metabolizable energy (ME)] and isonitrogenous (198 g/kg CP), and to meet the nutritional requirements of growing lambs, to achieve an average daily gain (ADG) of 300 g/d (NRC (National Research Council), 2007).

The diets were designed to evaluate the type of protein, as follows: 1- plant-based protein (SBM at 150 g/kg DM), 2- animal-based protein (FM at 50 g/kg DM), and 3- insect-based protein (TMM at 60 g/kg DM). The TMM was obtained from larvae reared in the insectary of the Institute of Biology of the Universidad Nacional Autónoma de México. Larvae were grown under controlled conditions at a temperature of 26–27 °C and a relative humidity of 60 %. They were fed with 90 % wheat bran (crude protein 160 g/kg, ether extract 106 g/kg, and carbohydrates 268 g/kg) and 10 % *Saccharomyces cerevisiae* yeast (nutritional content: thiamine hydrochloride 0.12 mg/g, riboflavin 0.04 mg/g, nicotinic acid 0.25 mg/g, CP 0.40 g/kg). For this purpose, 56 plastic trays (12 cm high, 22 cm wide and 29 cm long) were used, with a rearing period of 90 days. The larvae were then sorted by size using a Mont Inox sieve number 6, with an opening of 2 mm. They were cleaned manually to avoid contamination of the batches caused by excreta and bran residues. The larvae were sacrificed in a freezer at −18 °C (Amendt et al., 2007). They were dehydrated at 60 °C for 24 h in a Riossa model H3A oven and then ground using a cutting mill to pass a 2-mm screen sieve (Arthur Hill Thomas Corporation, Philadelphia, Pennsylvania, USA) (Kröncke et al., 2019). The TMM was finally placed in a Nieto model CHV 153 freezer at −4 °C for storage, before being incorporated into the TMR.

The lambs were housed in metabolic cages (1.20 m × 1.20 m) in an enclosed barn for 60 days, preceded by two weeks of diet adaptation. The average internal temperature in the barn varied from 16 to 18 °C; the barn is equipped with windows that open and close manually, therefore providing adequate ventilation. During the trial, the diets were individually administered twice a day (at 08:00 h and 15:00 h) for *ad libitum* consumption, to ensure a daily refusal margin of 10 %. The lambs had free access to clean and fresh water. The offered feeds and refusals were recorded daily to determine the dry matter intake (DMI). Individual BW was recorded on days 0, 20, 40, and 60, and used to determine the ADG and the feed conversion ratio (FCR).

The chemical composition of the ingredients used to formulate the experimental diets are shown in Table 1, while the ingredient and chemical compositions of the experimental diets are shown in Table 2.

2.3. Laboratory analysis of feeds

Samples of individual feedstuffs, TMRs, and orts were dried in a forced air oven (at 60 °C for 48 h), separately pooled and ground in a cutting mill to pass a 1-mm screen sieve (Arthur Hill Thomas Corporation, Philadelphia, Pennsylvania, USA). They were analyzed in triplicate

Table 1
Chemical composition (g/kg DM, unless otherwise stated) of the ingredients used in the feedlot lamb diets with the inclusion of different protein sources.

Item	SBM	FM	TMM	Wheat bran	Corn grain	Barley hay	Triticale whole grain	Soybean oil	Fish oil	Urea
DM, g/kg	892.4	895.0	900.4	880.0	946.0	905.1	884.8	995.0	995.0	990.0
OM	925.0	844.0	967.4	941.0	986.0	906.0	930.0	995.0	999.5	999.0
CP	536.8	595.7	567.0	160.0	80.0	120.0	120.0	0.0	0.0	2810.0
EE	69.9	153.0	189.0	106.0	102.0	95.9	93.1	990.0	990.0	0.0
NFC[a]	146.6	95.3	211.4	267.9	575.8	96.2	362	0.0	0.0	0.0
NDF	171.7	423.8	248.1	407.1	228.2	593.9	354.9	0.0	0.0	0.0
Hemicellulose[b]	108.1	270.7	86.3	289.8	181.7	217.4	294.4	0.0	0.0	0.0
ADF	63.6	153.1	161.8	117.3	46.5	376.5	60.5	0.0	0.0	0.0
Cellulose[c]	48.6	106.1	122.9	74.3	31.0	287.9	36.3	0.0	0.0	0.0
ADL	15.0	47.0	38.9	43.0	15.5	88.6	24.2	0.0	0.0	0.0
ME[d], MJ/kg DM	12.9	11.1	12.0	13.5	14.0	8.5	12.9	36.7	36.7	0.0

Abbreviations: SBM, soybean meal; FM, fish meal; TMM, *Tenebrio molitor* meal; DM; dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFC, non-fiber carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; ME, metabolizable energy.

[a] NFC = 100 – (NDF + CP + EE + ash).

[b] Estimated as NDF – ADF.

[c] Estimated as ADF – ADL.

[d] ME (MJ/kg DM) = 0.172 DMD (%) − 1.707 Freer, Dove, and Noan (2007). For soya oil and FM oil, ME data was taken from INRA (2018).

Table 2
Ingredients and chemical composition (g/kg DM, unless otherwise stated) of the experimental diets.

Item	Diet		
	SBM	FM	TMM
Ingredients			
SBM	150.0	0.0	0.0
FM	0.0	50.0	0.0
TMM	0.0	0.0	60.0
Wheat bran	78.0	68.0	69.0
Corn grain	250.0	300.0	300.0
Barley hay	250.0	300.0	300.0
Triticale whole grain	250.0	250.0	250.0
Oil[a]	12.0	12.0	0.0
Urea	0.0	10.0	11.0
Vit-Min mix[b]	10.0	10.0	10.0
Chemical composition			
DM, g/kg	903.6	892.0	886.2
OM	949.7	943.8	957.8
CP	197.2	200.4	197.2
EE	88.6	104.2	86.8
NFC[c]	319.8	344.8	252.0
NDF	360.1	394.4	326.8
Hemicellulose[d]	189.8	229.5	180.4
ADF	170.3	164.9	146.4
Cellulose[e]	140.5	138.0	122.7
ADL	29.8	26.9	23.7
ME[f], MJ/kg DM	12.57	12.17	12.74

Abbreviations: SBM, soybean meal; FM, fish meal; TMM, *Tenebrio molitor* meal; DM; dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFC, non-fiber carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; ME, metabolizable energy.

[a] Soybean oil and fish oil used in SBM and FM diets, respectively.

[b] Multitec de Malta (1.0 kg DM) containing antioxidant 25 mg, calcium carbonate 4.5 g, salt 6 g, ionophore 30 g, zinc oxide 50 g, sodium bicarbonate 6 g, copper sulphate 6 g, ferrous sulphate 20 g, sodium sulphate 125 g, vitamins E18 000 IU, A 3000000 UI, D 3750000 IU, potassium chloride 140 g, E.D.D. I ethylene-dynamine 0.500 g, cobalt carbonate 0.090 g, magnesium oxide 500 mg, manganese oxide 36 g, and selenium 0.090 g.

[c] NFC = 100 – (NDF + CP + EE + Ash).

[d] Estimated as NDF – ADF.

[e] Estimated as ADF – ADL.

[f] ME (MJ/kg DM) = 0.172 DMD (%) − 1.707 Freer, Dove, and Noan (2007). For soybean oil and FM oil, ME data was taken from INRA (2018).

for DM (method no. 930.15), organic matter (OM, method no. 942.05), CP [Kjeldahl N × 6.25 (method no. 990.03) and, in the case of TMM, Kjeldahl N × 4.75 (Janssen, Vincken, van den Broek, Fogliano, & Lakemond, 2017)], ether extract (EE, method no. 920.39), and ash (method no. 967.05), following Association of Official Analytical Chemists (AOAC) (1990) procedures.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using an ANKOM fiber system (Ankom 200 Fiber Analyzer; Ankom Technology, Macedon, NY, USA), with the addition of sodium sulfite and alpha amylase (Van Soest, Robertson, & Lewis, 1991). Non-fiber carbohydrates (NFC) were calculated as 100 – (NDF + CP + EE + ash). Hemicellulose and cellulose were calculated as NDF – ADF, and ADF – ADL, respectively.

To determine the ME, *in vitro* gas production was performed using a batch technique, incubating the ingredients and the diets with sheep rumen fluid and buffer, as described by Theodorou, Williams, Dhanoa, McAllan, and France (1994) up to 96 h of incubation, in three incubation runs. The rumen fluid was collected from three Suffolk lambs (35 ± 3.5 kg BW) fed with a 40:60 F:C TMR. After the incubations, the dry matter disappearance (DMD, g/100 g) was determined. Then the ME was calculated according to Freer, Dove, and Noan (2007), using the following formula:

$$\text{ME (MJ/kg DM)} = 0.172\ \text{DMD (g/100 g)} - 1.707$$

For soybean oil and FM oil, ME data were retrieved from INRA (2018).

2.4. Hematological analysis

Individual blood samples (10 mL) were collected before the morning feeding through the jugular vein using anticoagulant-free collection tubes (RotexMedica, Trittau, Germany) on days 0, 20, 40, and 60, according to Rivero et al. (2012). Serum samples were obtained (in duplicate within 2.0 mL vials) after centrifugation of the blood tubes for 10 min at 3500 ×*g*; they were then frozen at −20 °C until analysis. Serum samples were analyzed for their concentrations of glucose, urea, and creatinine by an auto-chemistry analyzer (DIRUI Model: CS-T240, constant speed 240 T/H; DIRUI, Güngören/İstanbul, Türkiye; CS-Anti-Bacterial Phosphor-Free Detergent, and CS-Alkaline Detergent), following the clinical chemistry parameters according to the manufacturer's instructions. In addition, minerals (ionized Ca^{++}, P, Mg, Na, K, and Cl) concentrations were determined using an Electrolyte Analyzer EasyLyte PLUS (Medica Corporation's EasyLyte®; Bedford, MA, USA). The specific ion-selective electrodes were employed to measure all minerals. Calibration of the instruments was performed automatically, utilizing standardized calibration solutions that contain two primary calibrants (Calibrant A and Calibrant B) and a wash solution. Calibrant A contained 800 mL at concentrations of 140 mmol/L for Na^{+}, 4.00 mmol/L for K^{+}, 125 mmol/L for Cl^{-}, 1.25 mmol/L for Ca^{++}, and 1.0 mmol/L for

Li^+. Calibrant B consisted of 180 mL with concentrations of 35.0 mmol/L for Na^+, 16.00 mmol/L for K^+, 41.0 mmol/L for Cl^-, 2.50 mmol/L for Ca^{++}, and 0.4 mmol/L for Li^+. The wash solution comprised 80 mL with a concentration of 0.1 mmol/L for ammonium bifluoride.

2.5. Slaughter performance and carcass traits

On day 60 (end of the trial), the lambs were weighed after 12 h of feed deprivation. The animals were then transported to the abattoir, located 53 km away from the experimental site. They were weighted and then slaughtered according to the Mexican Official Standard NOM-033-SAG/ZOO-2014 for the humane slaughter of animals. After slaughter, the hot carcass weights (HCW) and yields were recorded (Coyne, Evans, & Berry, 2019).

The pH was determined at slaughter and 24 h post-mortem, using a portable pH-meter specifically designed for meat (Hanna Instruments; HI-99163, Woonsocket, RI, USA) and equipped with a penetration probe (automatic temperature compensation); the pH-meter was calibrated prior to use and at an average environmental temperature of 20 °C.

Then the carcass was chilled at 4 °C for 24 h to determine the cold carcass weights (CCW) and yields.

The carcasses were divided into two parts along the backbone. On the right half of the carcass, using a measuring tape, the following measurements were recorded: thoracic width (maximum width between the ribs at the level of the 6th thoracic vertebra), thoracic girth (circumference of the carcass over the ribs at the level of the 6th thoracic vertebra width of the rump), rump girth (at the level of the trochanters of both femurs), thoracic depth (considered between the 5th and 7th thoracic vertebra and longitudinally surrounding the ribs until ending at the sternum), thigh length (most caudal distance from the perineum and the most distal point of the medial border of the tarsometatarsal articular surface), and carcass length (calculated as the sum of the dorsal length and thigh length data), following the methodology described by Ruiz de Huidobro, Miguel, Velasco, and Cañeque (2005).

The area of the eye of the chop (in the cutting zone at the level of the 13th rib) and the thickness of the dorsal subcutaneous fat (also in the cutting zone above the 13th rib), variables that are related to carcass quality, were measured on acetate paper and using a vernier caliper, respectively (Ruiz de Huidobro et al., 2005).

The carcass compactness index (CCI in kg/internal carcass length in cm) was calculated and the final 24 h pH was measured at the 12th thoracic vertebra using the same pH-meter reported above (Carrasco, Ripoll, Panea, Álvarez-Rodríguez, & Joy, 2009; Partida de la Peña, Ríos Rincón, Cruz Colín, Domínguez Vara, & Buendía Rodríguez, 2017).

2.6. Meat quality

Meat color of the *Longissimus thoracis et lumborum* (LTL) muscle was measured at room temperature (20 °C). Three slices (thickness 2 cm) per animal were cut from a freshly cut surface of the loin, at the level of the 7th lumbar vertebra, on the left side. The slices were placed in polystyrene trays, wrapped with 3 layers of household plastic film and stored in the dark at 4 °C (Valenti et al., 2019). Color measurements were recorded on days 0 (45 min after slaughter), 7, 14, and 21 post-mortem using a portable colorimeter (Konica Minolta Chroma Meter CR-400, Minolta Sensing Inc., Osaka, Japan). Lightness (L^*), redness (a^*) and yellowness (b^*) were measured in terms of the CIELAB color space model (Commission Internationale de l'Éclairage, 1976); chroma (C^*) was calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$, while hue angle ($h^*$) as $h^* = \tan^{-1}(b^*/a^*)$. The values were recorded for CIE standard illuminant D65 and the CIE 2° standard observer. Three readings per sample were performed and the color values were obtained considering the average of such readings.

Meat pH of the LTL muscle (at the level of the 7th lumbar vertebra) was measured in duplicate on days 0, 7, 14, and 21 post-mortem, using a portable pH-meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation probe.

To determine the proximate composition of the meat, the 9th or 10th rib was removed from the loin, and Association of Official Analytical Chemists (AOAC) (2007) methods were used [moisture (method no. 2001.12), protein (method no. 968.06), intramuscular fat (method no. 920.39), and ash as indicator of mineral composition (method no. 935.12)].

Indirect water holding capacity (water loss) was determined in the LTL muscle by the compression method and expressed as percentage of released juice (Pla, 2005). Briefly, a 0.30 ± 0.05 g of loin sample, between the 6th–8th ribs, was placed on a previously weighed Whatman No. 4 filter paper. The glass-covered sample was pressed under a weight of 2.25 kg for 5 min, then the filter paper was reweighed and the percentage of juice loss from the sample was calculated based on the difference in weight (Pla, 2005).

Samples for shear force (N/cm^2) were cooked in plastic bags in six batches of 24 samples for 60 min at 80 °C in a water bath. From each LTL sample, six 1 cm^2 subsamples were cut. After the cooking period was completed, the fluids in the bag were drained and the sample was cooled at 4 °C. The shear force was determined using a Lloyd texture analyzer (Model LRX, Lloyd Instruments, Hampshire, UK) fitted with a Warner–Bratzler shear blade (50 kg compression load cell), as described by Honikel (1998).

2.7. Statistical analysis

Data were analyzed as a completely randomized design using the SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA), with three treatments (diets) and eight replicates (lambs) per treatment.

Blood parameters, growth performance, meat pH, and color indices data were analyzed using the MIXED procedure for repeated measures over time, as follows:

$$y_{ijkl} = \mu + D_i + A_{j(i)} + T_k + D_i \times T_k + \varepsilon_{ijkl}$$

where y_{ijkl} is the dependent variable, μ is the overall mean, D_i is the fixed effect of diet, $A_{j(i)}$ is the random effect of the *j*th lamb within the *i*th diet, T_k is the fixed effect of time (repeated measure), $D_i \times T_k$ is the interaction effect between diet and time, and ε_{ijkl} is the residual error. When a significant D × T interaction was found, data were furtherly subjected to one-way ANOVA to explore the differences among the means for all the considered diet by time combinations.

Slaughter performance, carcass traits, as well as meat chemical composition and texture data were analyzed using a General Lineal Model (GLM). One-way analysis of covariance (ANCOVA) was performed for carcass measurements considering CCW as a covariate. The cooking batch was included as a random term in the analysis model for cooking loss and texture data.

Least square means were computed and tested for differences using the Tukey's test. Significance was declared at $P \leq 0.05$, while *P*-values >0.05 and ≤ 0.10 were considered as a trend towards statistical significance.

3. Results

3.1. Growth performance

The TMM-fed lambs showed lower ADG when compared to both the SBM-fed and the FM-fed lambs (−2.0 and − 3.1 g/kg $BW^{0.75}$, respectively; $P < 0.001$; Table 3). The feed conversion ratio (FCR) was 9 % higher for the SBM-fed lambs when compared to the FM-fed lambs, while intermediate values were recorded for the lambs belonging to the TMM group ($P < 0.05$).

Time significantly affected all the evaluated growth performance parameters ($P < 0.001$; Table 3). In particular, the DMI and ADG, when

Table 3
Growth performance of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Item	Diet (D)			Time (T), day of trial				SEM	*P*-values		
	SBM	FM	TMM	0	20	40	60		D	T	D × T
DMI, kg/d	1.17^{a}	1.06^{ab}	0.95^{b}	0.67^{w}	1.01^{z}	1.11^{yz}	1.26^{x}	0.052	<0.001	<0.001	0.290
ADG, kg/d	0.25^{a}	0.24^{ab}	0.21^{b}	0.15^{w}	0.23^{z}	0.25^{yz}	0.26^{x}	0.010	<0.001	<0.001	0.517
FCR	4.71^{a}	4.09^{b}	4.34^{ab}	4.52^{y}	4.03^{y}	4.40^{y}	6.14^{x}	0.338	0.040	<0.001	0.253
$BW^{0.75}$, kg	13.23	11.96	12.19	10.75^{w}	11.97^{z}	12.82^{y}	14.56^{x}	0.885	0.077	<0.001	0.224
ADG, g/kg $BW^{0.75}$	19.01^{a}	20.10^{a}	17.00^{b}	14.49^{y}	19.00^{x}	19.54^{x}	15.95^{y}	0.862	<0.001	<0.001	0.412

Abbreviations: SEM, standard error of the mean; DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; $BW^{0.75}$, metabolic body weight.
a,b Values within a row followed by different superscript letters are different for the diet effect ($P < 0.05$).
x,y,z,w Values within a row followed by different superscript letters are different for the time effect ($P < 0.05$).

expressed as kg/d, showed the following trend: d 60 > d 40 = d 20 > d 0, increasing by 0.59 kg/d and 0.11 kg/d from the beginning to the end of the trial, respectively. The FCR was significantly higher at d 60 when compared to the other considered times (on average, +1.82). The metabolic BW significantly increased at all considered measuring times, with values being 3.8 kg higher at the end when compared to the beginning of the trial.

In addition, a significant D × T interaction was found for both BW and DMI, the latter when expressed as g/kg $BW^{0.75}$ ($P < 0.001$; Table 4). In this regard, the highest and lowest BW values were found for the SBM at d 60 and for the FM at d 0, respectively. However, no clear trend for BW values across diet and time combinations was found. The DMI was the highest for the FM group at d 40 and the lowest for the TMM group at d 0. The DMI values for the SBM and FM groups at d 0, and for the TMM group at both d 0 and d 20, were significantly lower when compared to the value obtained for the FM group at d 40, while the other diet by time combinations showed intermediate values.

3.2. Blood parameters

The FM group showed higher concentrations of creatinine (+3.0 μmol/L) compared to the SBM group, while the TMM group showed intermediate values ($P < 0.05$; Table 5). The diet affected neither serum minerals (P, Mg, Na, K, and Cl) nor the Ca:P ratio ($P > 0.05$).

Regarding the effect of time, higher Mg levels were found at d 40 when compared to d 0 (+0.16 mmol/L), while intermediate values were found at d 20 and d 60 ($P < 0.01$). On the contrary, the levels of P, Na, K, and Cl did not change during the trial. The Ca:P ratio decreased as the feeding period progressed, with values ranging from 1.98 to 1.39 at d 0 and d 60, respectively ($P < 0.001$).

A significant D × T interaction effect was found for glucose, urea, and calcium contents ($P < 0.001$; Table 6). In particular, glucose levels reached the maximum concentration in the FM-fed lambs at d 20 (3.95 mmol/L), while the lowest levels were found in the SBM group at d 40, in the FM group at d 40 and d 60, and in the TMM group at d 60 (on average, 3.37 mmol/L); the other diet by time combinations showed intermediate values. The highest blood urea levels were found in the FM group at d 0 (6.25 mmol/L); such value did not differ from values found for the SBM group at d 0 and for the TMM group at both d 0 and d 40 (on average, 5.91 mmol/L). The absolute lowest urea level was found for the SBM group at d 20 (5.45 mmol/L), and the other diet by time combinations showed intermediate values (on average, 5.75 mmol/L). Finally, Ca levels were the highest for FM at d 60 and the lowest for FM at d 0, with a difference equal to 0.76 mmol/L. In both SBM and FM groups, Ca levels increased during the trial (total increase from d 0 to d 60 equal to 0.48 and 0.76 mmol/L, respectively), with different statistical trends (*i.e.*, trend for the SBM group: d 0 = d 20 < d 40 = d 60; trend for the FM group: d 0 < d 20 = d 40 = d 60), while no significant changes were found for Ca levels in the TMM group as the trial progressed.

3.3. Slaughter performance and carcass traits

The TMM-fed lambs showed 13.5 % lower HCW, 13.9 % lower CCW, 3.74 % lower hot carcass yield, 4.12 % lower cold carcass yield, 4.34 % lower width of thorax and 5.12 % when compared to the SBM-fed lambs, while the FM-fed lambs showed intermediate values ($P < 0.05$; Table 7). The carcass depth was found to be 4.59 % and 7.87 % higher in the SBM group when compared to the FM and TMM groups, respectively ($P < 0.001$). For the other morphometric measures, no differences were found among the diets ($P > 0.05$).

3.4. Meat quality traits

The diet did not significantly affect pH as well as color traits such as L^*, a^* and C^*, in the raw LTL muscle (Table 8).

All the above-mentioned parameters, except for a^*, significantly changed over time ($P < 0.05$), but no clear trends were detected. In particular, L^* showed higher values at d 7 and d 14 when compared to d 0 and d 21 post-mortem (on average, 46.31 *vs.* 42.65, respectively), while C^* showed on average 10.6 % lower values at d 21 when compared to the other time points considered. Meat pH ranked during time in the following order: d 21 > d 0 > d 14 > d 7.

A significant D × T interaction effect was found for b^* and h ($P < 0.01$ in both cases; Table 9). In detail, b^* was higher in the LTL muscle of the TMM group at d 7 when compared to all other diet by time combinations, except for SBM and TMM at d 14 which showed intermediate values. When considering h, significantly higher values were found for the LTL of the TMM-fed lambs at d 7 when compared to all the other diet by time combinations, except for the LTL of the FM-fed lambs at both d 7 and d 21, for which intermediate values were found.

Table 4
Effect of diet × time (D × T) interaction on the body weight and dry matter intake (expressed on metabolic body weight) of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Diet (D)	SBM				FM				TMM				SEM	*P*-value
Time (T), day of trial	0	20	40	60	0	20	40	60	0	20	40	60		
BW, kg	21.68^{de}	25.75^{bcde}	30.07^{ab}	32.68^{a}	21.00^{e}	21.65^{de}	26.21^{abcde}	28.50^{abcd}	21.81^{de}	24.08^{cde}	27.48^{abcd}	29.10^{abc}	1.404	<0.001
DMI, g/kg $BW^{0.75}$	71.68^{b}	86.04^{ab}	86.05^{ab}	92.20^{ab}	72.91^{b}	75.54^{ab}	94.58^{a}	89.21^{ab}	46.05^{c}	72.77^{b}	77.34^{ab}	82.36^{ab}	4.356	<0.001

Abbreviations: SEM, standard error of the mean; BW, body weight; DMI, dry matter intake; $BW^{0.75}$, metabolic body weight.
a,b,c,d,e Values within a row followed by different superscript letters are different ($P < 0.05$).

Table 5
Blood parameters (mmol/L, unless otherwise stated) of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Item	Diet (D)			Time (T), day of trial				SEM	*P*-values		D × T
	SBM	FM	TMM	0	20	40	60		D	T	
Creatinine, μmol/L	85.69^{b}	88.72^{a}	87.57^{ab}	88.03	86.21	87.71	87.77	0.831	0.038	0.559	0.515
Phosphorus	1.91	1.88	1.81	1.90	1.89	1.85	1.83	0.033	0.081	0.492	0.894
Magnesium	1.14	1.19	1.13	1.06^{y}	1.15^{xy}	1.22^{x}	1.17^{xy}	0.028	0.271	0.004	0.472
Sodium	142.02	142.46	142.77	143.05	143.20	140.50	143.21	0.842	0.819	0.143	0.942
Potassium	4.88	4.92	4.94	4.77	4.80	4.96	5.11	0.088	0.884	0.058	0.054
Chlorine	108.57	108.34	106.53	107.45	104.10	108.49	111.19	1.932	0.719	0.155	0.759
Ca:P ratio	1.65	1.69	1.53	1.98^{x}	1.67^{y}	1.47^{yz}	1.39^{z}	0.069	0.262	<0.001	0.073
Na-K-Cl	38.33	39.04	41.19	40.37	43.91	36.97	37.13	1.826	0.522	0.062	0.708
Na-Cl	33.45	34.12	36.25	35.60	39.10	32.01	32.02	1.829	0.535	0.048	0.679

Abbreviations: SEM, standard error of the mean; Ca, calcium; P, phosphorus; Na, sodium; K, potassium; Cl, chlorine.
a,b Values within a row followed by different superscript letters are different for the diet effect ($P < 0.05$).
x,y,z Values within a row followed by different superscript letters are different for the time effect ($P < 0.05$).

Table 6
Effect of diet × time (D × T) interaction on the blood glucose, urea, and calcium levels (mmol/L) of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Diet (D)	SBM				FM				TMM				SEM	*P*-value
Time (T), day of trial	0	20	40	60	0	20	40	60	0	20	40	60		
Glucose	3.60^{bc}	3.50^{bc}	3.41^{c}	3.45^{bc}	3.75^{ab}	3.95^{a}	3.40^{c}	3.35^{c}	3.57^{bc}	3.50^{bc}	3.46^{bc}	3.32^{c}	0.070	0.001
Urea	5.86^{ab}	5.45^{c}	5.62^{bc}	5.80^{bc}	6.25^{a}	5.81^{bc}	5.75^{bc}	5.81^{bc}	5.92^{ab}	5.78^{bc}	5.96^{ab}	5.69^{bc}	0.086	0.001
Calcium	1.94^{cd}	2.07^{cd}	2.35^{ab}	2.42^{ab}	1.75^{d}	2.30^{abc}	2.33^{ab}	2.51^{a}	2.13^{bc}	2.22^{abc}	2.33^{ab}	2.51^{ab}	0.075	0.001

Abbreviations: SEM, standard error of the mean.
a,b,c,d Values within a row followed by different superscript letters are different ($P < 0.05$).

Table 7
Slaughter performance and carcass traits of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Item	Diet			SEM	*P*-value
	SBM	FM	TMM		
Final body weight (FBW), kg	40.76	37.14	36.43	1.352	0.076
Slaughter weight, kg	40.15	36.95	36.04	1.239	0.072
Hot carcass weight (HCW), kg	18.49^{a}	16.69^{ab}	15.99^{b}	0.674	0.044
Cold carcass weight (CCW), kg	18.03^{a}	16.17^{ab}	15.52^{b}	0.664	0.039
Hot carcass yield, %	46.03^{a}	45.22^{ab}	44.31^{b}	0.949	0.045
Cold carcass yield, %	44.87^{a}	43.83^{ab}	43.02^{b}	1.023	0.038
Thickness of the dorsal SF, cm subcutaneous fat (mm)	2.75	3.31	2.63	0.460	0.548
Area of the eye of the chop, cm^2	17.05	17.20	17.00	0.435	0.654
Carcass length, cm	79.37	87.75	83.79	5.189	0.532
Carcass depth, cm	79.00^{a}	75.37^{b}	72.78^{b}	0.822	<0.001
Thigh length, cm	31.25	31.87	27.92	1.427	0.138
Diameter length, cm	37.12	36.12	34.97	1.322	0.531
Rump girth, cm	58.75	55.50	55.27	1.956	0.392
Width of the rump, cm	20.60	19.58	19.37	0.639	0.372
Width of thorax, cm	23.48^{a}	22.14^{ab}	22.46^{b}	0.560	0.036
Thorax depth, cm	17.93	17.11	17.54	0.540	0.567
pH values at slaughter	7.92	8.17	8.07	0.179	0.618
pH values 24 h post slaughter	6.62	6.87	6.46	0.140	0.138
Temperature carcass at slaughter, °C	35.52	34.08	33.45	0.979	0.973
Temperature 24 h post slaughter, °C	11.87	11.81	11.91	0.308	0.973
Carcass compactness index	0.25	0.19	0.19	0.025	0.194

Abbreviations: SEM, standard error of the mean. SF, subcutaneous fat.
a,b Values within a row followed by different superscript letters are different ($P < 0.05$).

The ash content of the LTL muscle of the TMM-fed lambs was 22.6 % lower when compared to that of the FM-fed lambs ($P < 0.05$, Table 10), while the SBM group showed intermediate values. The contents of moisture, protein, and fat, as well as the water loss and the shear force, were unaffected by diet ($P > 0.05$). The random effect of batch was not significant for both water loss and shear force ($P > 0.05$; Table 10).

4. Discussion

4.1. Growth performance

In the current study, the inclusion of full-fat TMM in a diet destined to feedlot lambs resulted in more than 10 % reductions of both DMI and FBW, when compared to SBM-fed animals. The lower weight gain is most probably the consequence of the lower observed DMI of the lambs fed TMM (Robles-Jimenez et al., 2025), leading to lower growth performance when compared to SBM- and FM-fed animals. Such results can be attributable to the presence of chitin (a major component of insect exoskeleton) and to the high fat content of full-fat insect meals.

As confirmation of the role of chitin, while evaluating the *in vitro* digestibility for ruminants of TM at different metamorphic stages, Khanal et al. (2023) observed lower digestibility of the adults (about 65 % and 55 % for DM and protein digestibility, respectively), compared to larvae or pupae; >85 % in both cases). Such result is most probably the consequence of increasing chitin contents (3–5 g/100 g DM for larvae and pupae *vs.* >20 g/100 g DM for beetles) during the insect life cycle (Muñoz-Seijas et al., 2024). Chitin is known to be degraded in the rumen of sheep very slowly, and with high (18 h) lag time for starting degradation (Fadel El-Seed, Kamel, Sekine, Hishinuma, & Hamana, 2003). Moreover, chitin encapsulates nutrients, acting like a physical barrier that limits nutrient digestibility (Gasco et al., 2023). Grundy et al. (2016) also showed in broiler chickens that chitin can increase the viscosity of intestinal digesta, hereby hastening the passage of digesta through the gut and augmenting the quantity of nutrients that evade digestion within the small intestine, which could also be the reason why the lambs fed TMM did not achieve the weight gains of the lambs fed with the other treatments. Similarly to what observed for chitin, also chitosan, a polysaccharide derived from chitin deacetylation, has been reported to exert negative effects on nutrients intake and digestibility in ruminants (Dias et al., 2017; Shah, Qazi, Matra, & Wanapat, 2022). The polycationic nature of chitosan, attributed to its protonated amino

Table 8
Color and pH of the *Longissimus thoracis et lumborum* (LTL) muscle from lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Item	Diet (D)			Time (T), day post-mortem				SEM	*P*-values		
	SBM	FM	TMM	0	7	14	21		D	T	D × T
L^*	43.84	44.85	44.86	43.78^{y}	46.34^{x}	46.27^{x}	41.51^{y}	0.699	0.246	<0.001	0.463
a^*	16.75	15.38	14.81	16.69	15.94	15.85	14.10	0.749	0.138	0.145	0.330
C^*	17.01	16.78	16.77	17.80^{x}	16.90^{x}	17.23^{x}	15.48^{y}	0.383	0.832	<0.001	0.755
pH	6.89	6.90	6.89	7.04^{y}	6.20^{w}	6.73^{z}	7.59^{x}	0.100	0.988	<0.001	0.357

Abbreviations: SEM, standard error of the mean; L^* = lightness (greater values show lighter color), a^* = redness (greater values show redder color), C^* = chroma or saturation (calculated as $(a^{*2} + b^{*2})^{1/2}$ (greater values display greater total color/more vivid color).
x,y,z,w Values within a row followed by different superscript letters are different for the time effect ($P < 0.05$).

Table 9
Effect of diet × time (D × T) interaction on yellowness and hue angle of the *Longissimus thoracis et lumborum* (LTL) muscle from lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Diet (D)	SBM				FM				TMM				SEM	*P*-value
Time (T), day post-mortem	0	7	14	21	0	7	14	21	0	7	14	21		
b^*	6.13^{b}	5.38^{b}	6.77^{ab}	6.02^{b}	5.80^{b}	6.59^{b}	6.27^{b}	6.45^{b}	5.13^{b}	9.87^{a}	6.86^{ab}	5.75^{b}	0.657	0.007
h	19.83^{b}	19.24^{b}	22.87^{b}	22.68^{b}	18.62^{b}	24.09^{ab}	22.08^{b}	27.39^{ab}	17.51^{b}	37.11^{a}	23.04^{b}	21.98^{b}	2.856	0.009

Abbreviations: SEM, standard error of the mean; b^* = yellowness (greater values show more yellow color); h = hue angle (calculated as $\tan^{-1}$ (b^*/a^*), greater values display more shift from red to yellow).
a,b Values within a row followed by different superscript letters are different ($P < 0.05$).

Table 10
Chemical composition, water loss and share force of the *Longissimus thoracis et lumborum* (LTL) muscle of lambs fed diets containing soybean meal (SBM), fish meal (FM) and *Tenebrio molitor* meal (TMM) as protein sources.

Item	Diet (D)			SEM	*P*-values	
	SBM	FM	TMM		D	B
Water loss, g/100 g	30.07	30.02	30.90	0.573	0.474	0.802
Shear force, N/cm^2	25.68	22.34	25.28	1.793	0.353	0.901
Moisture, g/100 g	71.87	71.92	72.45	1.387	0.977	–
Fat, g/100 g	3.68	3.67	2.56	0.773	0.505	–
Protein, g/100 g	18.11	17.86	19.25	0.719	0.192	–
Ash, g/100 g	2.03^{ab}	2.30^{a}	1.78^{b}	0.118	0.014	–

Abbreviations: SEM, standard error of the mean; B, random effect of batch.
a,b Values within a row followed by different superscript letters are different ($P < 0.05$).

groups, allows it to interact with the negative charge of the outer cell membrane of diverse microbiota, leading to cell death (Kirwan et al., 2021). By reducing feed intake and chewing efficiency, and thanks to its antimicrobial properties against rumen microbiota, chitosan can impair volatile fatty acid production (Shah et al., 2022). To overcome the negative effects that chitin exerts on digestibility, chitinases (enzymes able to cleave chitin into various chitin oligosaccharides) may be supplemented to ruminant diets containing TMM meals or frass; such a strategy proved to be successful in aquaculture nutrition (Hasan et al., 2023).

Full-fat insect meals exert negative effects on *in vitro* ruminal digestibility of DM and OM also because of their high fat contents (Renna et al., 2025; Renna, Rastello and Gasco, 2022a). Prachumchai, Suntara, Kanakai, and Cherdthong (2025) recently reported that the dietary inclusion of 0, 1, 2, and 4 % DM of *H. illucens* larvae oil respectively resulted in a linear and quadratic decrease of feed intake and digestibility coefficients in Thai-indigenous steers. Renna et al., 2022b observed that total gas production, volatile fatty acid production, and *in vitro* OM disappearance were respectively 69 %, 58 % and 59 % lower when incubating a full-fat TMM (392 g EE/kg DM) when compared to a SBM (6 g EE/kg DM). By mechanically or chemically extracting part of the oil from insect larvae, defatting processes allow the production of insect meals characterized by higher protein and lower EE contents, which may partially address the above-mentioned negative outcomes, enhancing insect meal digestibility for ruminants (Renna et al., 2023a; Renna et al., 2025; Renna et al., 2023b).

Besides the aforementioned considerations, it must be pointed out that current, albeit limited, findings on the effects of insect-derived products on the growth performance of ruminants are not univocal. For example, Astuti, Anggraeny, Khotijah, Suharti, and Jayanegara (2019) showed that the dietary inclusion of cricket meal (up to 30 % of concentrate in a 30:70 F:C diet) did not affect DM and nutrient intake, ADG, and feed efficiency of post-weaning goat kids. Also, Fukuda, Cox, Wickersham, and Drewery (2022) showed that the inclusion of a full-fat *H. illucens* larvae meal as the main protein source in diets destined to beef steers altered neither feed intake nor digestibility of nutrients in comparison to conventional protein sources, such as cottonseed meal and SBM. Qaisrani et al. (2014) found shorter intestinal villi and deeper crypts with increasing levels of indigestible protein in broiler chicken diets, which contributed to inadequate digestion and reduced nutrient assimilation, ultimately leading to suboptimal animal performance.

Finally, Nekrasov et al. (2022) showed that the dietary ingestion of fat extracted from *H. illucens* larvae (dietary inclusion up to 100 g/head per day) had no negative effects on feed digestion in dairy cows. Moreover, Phesatcha et al. (2022) demonstrated that the dietary F:C ratio can significantly impact the digestibility parameters when using an insect meal (*Gryllus bimaculatus*) in place of conventional protein sources, irrespective of the insect dietary inclusion or substitution level. Therefore, current available literature clearly shows that the substitution of SBM with insects can differently impact ruminant performance, depending on basal diet composition, insect species, insect-derived product and inclusion level used (Gao et al., 2024).

A point for farmers to consider before opting for the use of TMM for ruminant feeding could be the gains they will obtain (cost-benefit). In the present work, a lower weight gain was observed in lambs fed TMM compared to lambs fed SBM (0.21 *vs* 0.24 kg), which can have drawbacks for farmers. Although such difference seems small, in the long term, this reduction in weight gain could translate into lower efficiency for meat production, affecting profitability. Farmers could face additional costs to achieve the same growth and marketing targets for lambs, as they would need to feed the animals for longer periods. Furthermore, if the lambs do not reach optimal weight in the expected time, this could affect the quality of the final product and, consequently, the selling

prices. The choice of proper feed sources and quantities, together with their actual costs, is crucial to maximizing performance and profitability in lamb production (Montossi et al., 2013).

4.2. Blood parameters

Monitoring blood parameters over time can help to identify changes in blood chemistry and alterations of metabolites; this can help to diagnose any potential conditions derived by the administration of feed sources (*i.e.* insects or FM) that are not conventionally administered to ruminant animals. In the current study, all the evaluated serum parameters fell within the physiological range for feedlot lambs (Ma et al., 2022). Our results show that TMM feeding exerted only limited effects on blood parameters in fattening lambs.

Creatinine is a nitrogenous waste product which is transported *via* blood to the kidneys, being finally excreted with urine. In ruminants, creatinine is considered a specific biomarker of renal dysfunction, with decreased concentrations reflecting renal function improvement (Athanasiou, Katsogiannou, Spanou, Dedousi, & Katsoulos, 2021). In some monogastric studies, the use of insect meals or chitosan has been shown to significantly affect creatinine levels. For example, the dietary inclusion (up to 90 g/kg) of a partially defatted *H. illucens* meal in Muscovy duck (*Cairina moschata domestica*) decreased creatinine levels, suggesting improved renal function (Gariglio et al., 2019). Biomedical applications of chitosan have also determined decreased serum creatinine levels in rats and humans, resulting in renal benefits *via* mechanisms involving the high antioxidant activity and the renoprotective effects of chitosan (Anraku et al., 2020; Satitsri & Muanprasat, 2020). However, available published data is not consistent. For example, different levels (up to 150 g/kg) of TMM in broiler diets or dietary chitosan supplementation (5 g chitosan/kg diet) in Nile tilapia (*Oreochromis niloticus* L.) did not alter serum creatinine levels (Biasato et al., 2018; Fadl et al., 2020). Similarly, also in the current study, plasma creatinine levels did not significantly differ when comparing the TMM-fed with both the SBM-fed and the FM-fed lambs. The available published data is still scarce, and the inconsistency of the obtained results suggests key roles exerted by insect species and dietary inclusion levels of insect-derived products, as well as farmed animal species involved. Therefore, further research will be needed to clarify the effect of the dietary inclusion of insect meals on blood creatinine of both monogastric and ruminant animals.

The stability of other blood parameters, such as glucose and total protein, further supports the idea that TMM had a minimal impact on the overall metabolic status of the lambs. Odeon et al. (2024) recently showed that 10 % dietary inclusion of *H. illucens* larvae meal did not alter classical stress indicators, such as glucose, cortisol and plasmatic proteins, in Merino lambs. Similarly, Ayaz et al. (2023) reported no changes in serum glucose levels in sheep fed diets in which part of a commercial concentrate feed was replaced by frass derived from TM rearing. Such results are in agreement with our findings. Also, in monogastrics, Biasato et al. (2018) reported that serum levels of glucose, cholesterol, triglycerides, albumin, uric acid, and total protein did not change in broilers fed different levels of TMM. Similarly, Elahi et al. (2020) reported that the hematological characteristics of broiler chicks fed TMM-containing diets (up to 8 %) were not different if compared to a diet not containing TMM. In growing pigs fed diets containing 5 % and 10 % TMM, Meyer et al. (2020) concluded that TMM can be considered as a viable alternative protein-rich ingredient, with only negligible impact on the intermediary metabolism of pigs.

Overall, the lack of significant changes in the blood parameters in the current study is hypothesized to reflect the lambs' efficient adaptation to TMM-containing diets. This adaptation may involve readily adjusted metabolic pathways, allowing for TMM nutrient utilization without significant disruptions of glucose homeostasis and protein metabolism. However, further research should explore the specific metabolic pathways involved.

4.3. Slaughter performance and carcass traits

Most of the evaluated variables related to the slaughter performance and carcass traits remained unaffected by diet. However, hot and cold carcass weights and yields decreased in the TMM-fed lambs when compared to the SBM-fed lambs, while there were no differences with the FM-fed lambs. In line with our results, Lu et al. (2024) reported that dressing percentage and carcass weight of insect-fed goats (supplemented with 10 % *H. illucens*) were lower than those of a control group (supplemented with 10 % full-fat soybeans), indicating that *H. illucens* feeding might have a negative influence on carcass yield.

Conversely, Pereira et al. (2020) found that supplementing chitosan at 2 g/kg of diet DM did not alter HCW, CCW, dressing percentage, and liver weight of lambs. Other studies in monogastrics show lack of negative effects of TMM on carcass traits of different species. For example, dietary TMM did not affect carcass traits in growing pigs (Meyer et al., 2020; Zacharis et al., 2023). Also, in broilers, Sedgh-Gooya et al. (2020) showed that a dietary inclusion level up to 5 % of TMM did not modify carcass yield and organ weights, while Biasato et al. (2018) reported that experimental diets containing TMM at levels up to 15 % did not modify carcass weights and carcass traits.

Overall, the results obtained in our study suggest that TMM acts more like an animal-based protein (*i.e.*, FM) rather than a plant-based protein (*i.e.*, SBM). This was already pointed out in an *in vitro* study by Renna et al., 2022b when evaluating the fermentation features of full-fat insect meals from eight different insect species, as compared to plant-derived (soybean, rapeseed, and sunflower meals) and animal-derived (fishmeal) protein sources. Further research is needed to investigate the specific metabolic pathways influenced by TMM and how these pathways affect carcass composition in ruminants. Specifically, studies should examine amino acid utilization, energy partitioning, and the potential role of the gut microbiome in mediating the effects of TMM on carcass traits.

4.4. Meat quality traits

Meat color is recognized as a direct factor in the assessment of meat quality and the purchasing intentions of customers. The pH value of muscle is considered an important indicator that reflects the rate of post-slaughter muscle glycolysis in animals (Garmyn, 2020). Therefore, meat color measurement is of outmost importance when evaluating the effects of the inclusion of innovative ingredients in diets destined to farmed animals. In the current study, the inclusion of TMM in the diet of feedlot lambs affected neither color (lightness, redness, yellowness, chroma, and hue angle) nor pH values of LTL muscle. Also, the meat composition (in terms of its moisture, and protein contents), water loss and the shear force remained unaffected by treatment. Such results suggest that meat from TMM-fed lambs is characterized by normal appearance, which is fundamental for product acceptance by consumers (Garmyn, 2020). Our findings are consistent with the limited available data on insect meals in ruminant nutrition. Confirming our results, a recent study by Lu et al. (2024) showed that dietary supplementation with 10 % *H. illucens* did not affect shear force, water holding capacity, and cooking loss in meat from Qianbei goats. Similarly, chitosan-supplemented diets (2 g/kg of DM) for fattening lambs did not affect color, cooking loss, water holding capacity, tenderness, and composition of meat (Pereira et al., 2020). Overall, our findings also align with those of previous studies that examined the physicochemical properties of meat from insect-fed poultry, which revealed no alterations in pH, color, moisture content, and shear force (Dabbou et al., 2020; Elahi et al., 2020; Pietras, Orczewska-Dudek, Szczurek, & Pieszka, 2021). Also, the dietary inclusion of 4 % TMM in rabbits did not alter meat composition (Kowalska, Gugołek, & Strychalski, 2020). Similarly, in a study where pigs were fed diets with full-fat *H. illucens* larvae meal replacing fish meal and soybean meal (4 % and 8 % as-fed) for 16 weeks, no changes in the color and pH of longissimus thoracis muscle were observed; however, drip loss and

intramuscular fat content were improved in pork from pigs fed *H. illucens* meal (Zhu et al., 2022).

Even if not statistically significant, we found a numerical decrease in the fat content of meat in the TMM-fed lambs, when compared to the other experimental groups. Similarly, da Silva Magalhães et al. (2020) reported that a dietary supplementation with chitosan (at levels of 136 mg/kg BW) in feedlot lambs promoted a decrease in the fat content of the meat. This effect can be attributed to the ability of chitosan to selectively bind to specific organic compounds, including cholesterol, proteins, fats, and triglycerides, rendering them unavailable (Muzzarelli, 1999) and thereby influencing their absorption, ultimately leading to reduced body fat. Lu et al. (2024) also reported a significant decrease in the EE content of the meat from goats supplemented with 10 % heat-treated *H. illucens*, when compared to a control group supplemented with the same percentage of full-fat soybeans. These authors hypothesized a weaker lipid deposition capacity in the muscles of the goats supplemented with *H. illucens*, which seems also consistent with some studies conducted in poultry and mainly related to the high lauric acid content typically found in this insect species (Aprianto, Muhlisin Kurniawati, Hanim, Ariyadi, & Anas, 2023).

It should be noted that the pH values recorded 24-h postmortem in the current study were relatively high. It has been well established that high pH levels in meat can be attributed to several factors, including activities that cause fear and/or physical exhaustion, such as transportation, excessive activity, weather and temperature extremes, and any factor led to depletion of muscle glycogen (Fernández & Vieira, 2012; Ponnampalam et al., 2017; Prache, Schreurs, & Guillier, 2022). A fluctuation in mean environmental temperatures can cause physiological adjustments in animals susceptible to stress, for example, during transport, whose muscle consequently undergoes extremely rapid rates of postmortem change (Judge, 1969; McGeehin, Sheridan, & Butler, 2001). In our study, we followed the standard handling and transportation protocols to minimize potential impacts on pH; however, individual stress levels and/or human/equipment interference may have contributed to the observed high pH levels.

4.5. Economic implications

A document by FAO (2022) reports that the price of insect protein ranges from € 3500 to € 5500 per tonne, being higher than those of both FM (€ 1000 to € 1700 per tonne) and SBM (< € 550/t; Nunes, Dalen, Leonardi, & Burri, 2022). However, the insect sector has not reached maturity yet. The price of insect meals has been forecast to fall by € 2000/t at maturity, and the sustainability benefits of replacing conventional proteins with insect protein might be able to justify a slightly higher insect meal price (FAO, 2022), provided that no impairment of animal performance derives from the inclusion of insect-derived products in ruminant diets.

4.6. Limitations of the study

The calculation of effect size, using the partial eta statistic (η2) (Richardson, 2011), outlined small effects for sodium concentration, thickness of the dorsal subcutaneous fat, temperature 24 h post slaughter, and chroma. The number of replicates ($n = 8$) may have partially influenced the statistical outcomes, thus making further research on this topic recommended, especially for the above-mentioned parameters. In addition, our experiment used only a single inclusion level of TMM, limiting our ability to determine optimal inclusion rates or potential dose-dependent effects. The trial duration (60 days) also may not have been sufficient to evaluate long-term adaptations of the rumen microbiome to TMM, which could potentially improve utilization over extended feeding periods. Finally, we used full-fat TMM, while defatted TMM meals might yield more positive results by reducing negative impacts on digestibility.

5. Conclusion

Feeding TMM (insect protein) as an alternative to SBM (conventional vegetable protein) or FM (conventional animal protein) did not impair either the blood hematological profile of lambs or the physical (color, tenderness) and nutritional (fat, protein) characteristics of lamb meat. However, the TMM-containing diet was associated with lower DMI and ADG compared to the diet containing SBM. It is herein hypothesized that the chitin and high fat contents of full-fat TMM meal are responsible for these negative effects on lamb performance. To mitigate the negative effect of chitin on digestibility, dietary strategies such as enzyme (chitinases) supplementation of TMM could be explored. Defatting could be another solution to enhance the digestibility of TMM for ruminants. Lastly, dietary strategies should be explored to identify optimal inclusion levels of TMM in combination with conventional protein sources (*i.e.* SBM or FM) at varying ratios for fattening lambs.

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Author statement

No human subjects were involved in this trial.

Consent form

No consent form was used, as no human subjects were involved in this trial.

CRediT authorship contribution statement

Lizbeth Esmeralda Robles-Jimenez: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Eduardo Cardoso-Gutierrez:** Conceptualization, Visualization. **José Manuel Pino-Moreno:** Validation, Investigation. **Manuela Renna:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Laura Gasco:** Investigation, Writing – review & editing. **Navid Ghavipanje:** Formal analysis, Writing – original draft, Writing – review & editing. **Ignacio Arturo Dominguez-Vara:** Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing. **Manuel Gonzalez-Ronquillo:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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