

Article

Effects of Untreated or NaOH-Treated Carob (*Ceratonia siliqua*) Leaves and Twigs as Partial Wheat Straw Replacements on Growth Performance, Carcass Traits, and Meat Quality of Growing–Finishing Assaf Lambs

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Abstract

This study evaluated the effects of replacing 25% of wheat straw with dried carob (*Ceratonia siliqua*) leaves and twigs, either untreated or treated with 5% sodium hydroxide (NaOH), on growth performance, nutrient digestibility, carcass traits, meat quality, blood metabolites, and rumen microbial populations in Assaf lambs. Twenty-four male lambs (2.5 months old; 29 ± 0.5 kg) were randomly assigned to three dietary treatments ($n = 8$): a control diet containing wheat straw as the sole roughage source, supplemented with a concentrate feed, a diet with 25% untreated carob leaves and twigs (UCL), and a diet with 25% NaOH-treated carob leaves and twigs (TCL). Following a 14-day adaptation period, lambs were fed the corresponding experimental diet for 14 weeks. Carob inclusion improved growth performance, with UCL lambs showing the highest average daily gain (214 g/d) compared with TCL (201 g/d) and control (160 g/d), resulting in improved feed conversion ratio (9.02 vs. 5.68 and 5.63, respectively) ($p < 0.001$). Blood urea nitrogen was reduced ($p < 0.001$) in UCL lambs (26.8 vs. 38.5 mg/dL in control), suggesting improved nitrogen retention. Digestibility responses differed between treatments ($p < 0.001$), as TCL increased dry matter digestibility to 72.6% compared with 65.4% (UCL) and 63.6% (control), indicating enhanced nutrient utilization following NaOH treatment. Both UCL and TCL increased ($p < 0.001$) carcass weights (up to 24.7 vs. 21.0 kg in control), while TCL achieved the highest dressing percentage (46.6% vs. 43.4%). Meat quality traits were generally unaffected in terms of color (lightness, redness, and yellowness) and water-holding capacity; however, shear force decreased from 33.6 N (control) to 30.0 N (TCL), indicating improved tenderness. Carob inclusion modified meat composition by increasing ($p < 0.001$) lipid content (12.0–12.2 vs. 9.6%) and improving fatty acid profile, with reduced saturated fatty acids (53.4–56.5 vs. 61.4%) and increased α -linolenic acid (2.04 vs. 1.58%), leading to a



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lower n-6/n-3 ratio (5.54–5.61 vs. 6.45). Rumen fermentation was also affected ($p < 0.001$), as carob diets increased total bacterial populations and reduced protozoal counts, suggesting shifts toward more efficient microbial activity. In conclusion, replacing 25% of wheat straw with carob leaves improved growth performance and feed efficiency, with untreated carob primarily enhancing nitrogen utilization and treated carob improving fiber digestibility and carcass yield. These findings support the use of carob by-products as a viable alternative feed resource, although responses depend on processing method and targeted production outcomes.

Keywords: roughage; alkali treatment; feed efficiency; lignocellulosic by-products; silvopastoral forage; tree fodder

1. Introduction

Sheep production is a fundamental component of agricultural systems across the Mediterranean region, contributing substantially to food security, rural livelihoods, and the preservation of traditional farming practices [1,2]. The predominance of semi-arid conditions and heterogeneous agroecological zones has favored the development of resilient sheep production systems that efficiently utilize marginal lands unsuitable for crop cultivation, while supporting sustainable land management through controlled grazing [3]. Within this context, the Assaf sheep breed represents a successful dual-purpose genotype, developed through crossbreeding programs combining the environmental adaptability of Awassi sheep with the high productive potential of East Friesian sheep [4,5]. This genetic background allows Assaf sheep to achieve annual milk yields of approximately 450 L while maintaining satisfactory growth performance and carcass quality, making them well suited to intensive and semi-intensive Mediterranean production systems.

Despite these advantages, sheep production in the Mediterranean basin faces increasing sustainability challenges driven by climate change, rising feed costs, and growing competition for arable land between livestock and crop production [6]. These pressures have intensified the search for alternative feeding strategies based on locally available and underutilized resources capable of improving production efficiency while reducing environmental impact [7,8].

In this regard, the carob tree (*Ceratonia siliqua* L.), a drought-tolerant species native to the Mediterranean region, has gained attention as a promising alternative feed resource due to its nutritional value and adaptability to harsh environments [9,10]. While extensive research has focused on carob pulp and seeds for human and animal nutrition, a substantial proportion of carob biomass generated through annual pruning—primarily leaves and twigs—remains largely unexploited and is often discarded as agricultural waste [11]. This underutilization represents a missed opportunity within circular bioeconomy frameworks aimed at enhancing resource efficiency and sustainability in Mediterranean agro-silvo-pastoral systems [12]. Previous studies have primarily evaluated carob pulp or low inclusion levels of carob by-products (typically 10–20%), reporting moderate improvements in growth performance (approximately 5–15%) and feed efficiency, but with inconsistent effects on carcass traits and meat quality [13,14]. However, systematic evaluation of carob leaves and twigs, particularly at moderate inclusion levels that balance nutrient supply and anti-nutritional constraints, remains limited.

Moreover, the utilization of carob leaves is constrained by their high fiber content and the presence of condensed tannins (CTs), which can limit nutrient availability. Condensed tannins can reduce ruminal protein degradation by forming reversible complexes with

dietary proteins, thereby increasing the flow of undegraded protein to the small intestine, but excessive levels may depress intake and fiber digestion [15]. However, chemical treatments such as sodium hydroxide (NaOH) application have been shown to improve fiber digestibility and modify tannin activity, thereby enhancing the nutritional value of lignocellulosic by-products for ruminants [10]. Several chemical treatments have been explored to improve the fermentation and degradability of agricultural byproducts, enhancing their nutritional value [16]. Alkali treatment is considered safe for animal health and effective in upgrading low-quality roughages [17,18]. Sodium hydroxide disrupts ester bonds linking lignin to compounds such as acetic acid and phenolic acids in the plant cell wall via saponification [10,17,18]. This process also breaks down the cell wall structure, making polysaccharides like cellulose and hemicellulose more accessible to microbial enzymes for digestion [18,19]. As a result, alkali treatment can increase fiber digestibility by 10–25%, improve energy availability, reduce to some extent the biological activity of tannins and alter nitrogen utilization efficiency [18,20,21]. Recent studies by Zoabi et al. [18] on almond hulls and Ghzayel et al. [10] on carob leaves demonstrated that NaOH treatment reduced crude protein (CP) as well as both structural and nonstructural carbohydrates.

In addition to growth performance and carcass characteristics, meat quality is an important criterion in evaluating alternative feed sources. Consumer acceptance and market value of meat are determined by color, tenderness, and nutritional composition [22,23]. The post-slaughter pH decrease is a key factor in determining color stability and the meat's ability to retain water. Abnormal pH values can lead to undesirable meat quality defects, such as dark, firm, dry or pale, soft, exudative [24]. The rate of pH decrease is influenced by muscle type, temperature, and processing conditions. It should be noted that genetic factors also affect the pH, color, and tenderness of lamb [25]. It should also be noted that dietary interventions provided to lambs, including tannin-rich feeds or roughage treated with alkali, also affect meat quality standards [26–28]. Dietary tannins may improve oxidative stability and fatty acid composition by modulating ruminal biohydrogenation, whereas improved fiber digestibility through alkali treatment can enhance energy supply for intramuscular fat deposition and muscle development, thereby influencing carcass yield and meat tenderness [15,29,30]. Carob leaves contain bioactive compounds, such as phenolic compounds and CTs, which may exert antioxidant effects and influence meat quality [13].

Despite these advances, a clear knowledge gap remains regarding the comparative effects of untreated versus chemically treated carob leaves on growth performance, nutrient utilization, carcass characteristics, and meat quality within a single experimental framework. To date, most studies have evaluated either carob pulp or individual by-products, or have focused on a single processing method, without directly comparing untreated and chemically treated carob leaves and twigs under identical dietary and management conditions. In addition, the optimal inclusion level that maximizes benefits while avoiding negative effects associated with tannins and high fiber content has not been clearly established. A 25% replacement level was selected in the present study as a moderate inclusion rate based on previous literature, aiming to provide sufficient bioactive compounds and fiber substitution while minimizing potential anti-nutritional effects.

Therefore, the novelty of the present study lies in the direct comparison between untreated and NaOH-treated carob leaves and twigs at the same inclusion level within a controlled experimental design, allowing the differentiation of their respective effects on nutrient utilization, rumen-related parameters, growth performance, carcass traits, and meat quality. Based on these considerations, it was hypothesized that replacing 25% of wheat straw with untreated or NaOH-treated carob leaves and twigs in diets of Assaf lambs would differentially affect animal performance through distinct mechanisms, with untreated carob enhancing nitrogen utilization via tannin–protein interactions and NaOH-

treated carob improving fiber digestibility and energy availability. The primary objective was to generate evidence-based data supporting the use of carob pruning residues as a sustainable, cost-effective feed resource capable of enhancing sheep productivity while contributing to environmentally resilient livestock systems in Mediterranean and similar agroecological regions.

2. Materials and Methods

2.1. Animals, Experimental Design, and Diets

The experiment was conducted starting in April 2024 on a private sheep farm located in Jenin, Palestine, following the guidelines of the Palestinian Association of Agricultural Engineers—Jerusalem Branch (Ethical Approval No. 2/A.E.A/2026). Twenty-four weaned male Assaf lambs [2.5 months of age; initial body weight (BW) 29.0 ± 0.5 kg] were randomly allocated to three dietary treatments using a completely randomized design. Randomization was performed by assigning animals to treatments using a random number generator without prior stratification based on BW. Initial BW was subsequently accounted for as a covariate in the statistical analysis to correct for baseline differences among groups.

The feeding trial lasted 16 weeks (from 2.5 to 6.5 months of age), including a 14 d adaptation period. Three dietary treatments were evaluated. Lambs in the control group received wheat straw as the sole roughage source, supplemented with a fixed amount of concentrate feed. In the UCL group, 25% of the wheat straw [dry matter (DM) basis] was replaced with sun-dried, untreated carob leaves and twigs, while the concentrate level remained identical to that of the control. In the TCL group, 25% of the wheat straw (DM basis) was replaced with sun-dried carob leaves and twigs treated with NaOH, with the same concentrate inclusion as in the other treatments. Diet composition and the quantities of each feed were adjusted monthly to meet the nutritional requirements of the lambs, as shown in Table 1.

Table 1. Composition of the diets (amounts as fed with dry matter intake between brackets) fed to the lambs during the 16 weeks of the study.

Week of the Study		Control	UCL	TCL
Week 0 to week 4	Straw	400 (372)	300 (279)	300 (279)
	UL	0	100 (90)	0
	TL	0	0	100 (92)
	Concentrate	500 (450)	500 (450)	500 (450)
	Total	900 (822)	900 (819)	900 (821)
Week 4 to week 8	Straw	400 (372)	300 (279)	300 (279)
	UL	0	100 (90)	0
	TL	0	0	100 (92)
	Concentrate	700 (629)	700 (629)	700 (629)
	Total	1100 (1001)	1100 (998)	1100 (1000)
Week 8 to week 12	Straw	500 (465)	375 (348)	375 (348)
	UL	0	125 (112)	0
	TL	0	0	125 (115)
	Concentrate	800 (719)	800 (719)	800 (719)
	Total	1300 (1184)	1300 (1179)	1300 (1182)
Week 12 to week 16	Straw	700 (651)	525 (488)	525 (488)
	UL	0	175 (157)	0
	TL	0	0	175 (161)
	Concentrate	900 (809)	900 (809)	900 (809)
	Total	1600 (1460)	1600 (1454)	1600 (1458)

UCL: diet with untreated carob leaves; TCL: diet with NaOH-treated carob leaves; UL: untreated carob leaves; TL: NaOH-treated carob leaves.

The experimental diets were not formulated to be strictly iso-nitrogenous or iso-energetic, as the replacement of wheat straw with carob leaves and twigs inherently altered the chemical composition of the diets. However, the level of concentrate supplementation was kept constant across treatments to minimize variation in nutrient supply and to allow the effects of the roughage source to be evaluated under practical feeding conditions. Chemical composition of wheat straw, concentrate, and carob leaves and twigs, either untreated or treated with NaOH are shown in Table 2.

Table 2. Chemical composition of wheat straw and carob leaves and twigs untreated or treated with NaOH) (% unless otherwise stated).

Item	Concentrate Mixture ¹	Wheat Straw	UCL	TCL
Dry matter (% of fresh matter)	89.9	92.9	90.0	91.8
Organic matter	86.7	88.6	85.0	85.0
Crude protein	18.0	5.01	5.01	3.0
Crude fiber	29.6	27.5	33.4	34.6
Neutral detergent fiber	39.8	39.3	42.0	43.5
Acid detergent fiber	32.9	32.4	36.2	34.7
Acid detergent lignin	4.2	11.4	9.0	8.4
Tannins ²	NM	NM	6.74	4.84
Polyphenol ³	NM	NM	9.91	8.12

UCL: untreated carob leaves and twigs; TCL: NaOH-treated carob leaves and twigs. ¹ Concentrate feed mixture contained: 35% barley grain, 35% wheat bran, 10% yellow corn, 17% soybean meal, 2% limestone, 0.5% salt, and 0.5% mineral and vitamin mixture [composition (per kg premix): 250 g dicalcium phosphate, 60 g MgO, 10,000 mg Zn, 8000 mg Mn, 4000 mg Fe, 300 mg Cu, 100 mg I, 30 mg Co, 25 mg Se, 1,000,000 IU vitamin A, 150,000 IU vitamin D₃, and 2000 mg vitamin E]. ² Tannins contents: catechin equivalent/100 g DM, NM = not measured. ³ Polyphenol contents in mg equivalent gallic acid (GAE)/g DM.

Lambs were housed individually in shaded pens and had free access to clean drinking water throughout the experimental period. Feed was offered twice daily, and refusals were recorded daily to determine feed intake. Feed intake was monitored individually, and variability among animals was assessed by calculating the coefficient of variation within each treatment group. Animals remained clinically healthy throughout the trial, and no exclusions were required. Body weight was recorded at the start of the trial and every 10 d thereafter until the end of the experiment, as well as 24 h post-slaughter. Daily DM intake (DMI) was calculated, and feed conversion ratio (FCR) was expressed as the ratio of total DMI to total BW gain.

2.2. Preparation of Carob Leaves and Twigs and NaOH Treatment

Fresh carob leaves and twigs were collected during the annual pruning season, sun-dried for 10 d, and coarsely chopped. The carob tree biomass collected for use as a feed ingredient comprised entire compound leaves (consisting of leaflets, petioles, and rachides) along with a minor proportion of terminal thin twigs. Although the samples contained both foliar and woody fractions, the material is hereafter referred to as “carob leaves”, as the foliar portion constituted the predominant component of the total biomass. For the chemical treatment, a 5% (*w/v*) NaOH solution was prepared and applied at a rate of 50 g NaOH per kg of carob leaf DM. The solution was uniformly sprayed onto the chopped material and thoroughly mixed to ensure homogeneous distribution. The treated material was then sealed in plastic containers and stored at room temperature for 72 h to allow the chemical reaction to occur. No washing or chemical neutralization step was applied after NaOH treatment. Instead, the material was air-dried prior to incorporation into the experimental

diets, allowing residual moisture to evaporate and the reaction to stabilize. The pH of the treated material was not measured after the treatment period. However, air-drying is commonly used in alkali-treated roughages to reduce residual alkalinity, and the inclusion level of the treated material (25% of the roughage fraction) was moderate. No adverse effects on DMI, animal behavior, or health status were observed throughout the experimental period, suggesting that residual alkalinity did not negatively affect the animals.

2.3. Nutrient Digestibility

Two digestibility trials were conducted during the growth phase of the lambs to evaluate apparent nutrient digestibility at different physiological stages. The trials were conducted during the last week of the 3rd and 6th months of age, while animals continued to receive the same experimental dietary treatments assigned at the beginning of the feeding trial. Throughout the digestibility trials, lambs were housed individually and fed twice daily at 07:00 and 16:00 h, with free access to fresh drinking water and salt blocks. Feed allowances were adjusted according to age to meet the increasing nutrient requirements of growing lambs, while maintaining the relative proportions of roughage and concentrate within each dietary group. This design ensured that treatment effects were evaluated independently of age-related changes in DMI. At 2–3 months of age, lambs received 900 g feed/day per lamb. At 3–4 months of age, the daily feed allowance increased to 1100 g/day per lamb. Feed allowances were increased to 1300 g/day at 4–5 months and to 1600 g/day at 5–6 months of age. Across all stages, the relative inclusion levels of straw, carob leaves, and concentrate remained consistent within each treatment. Fecal samples were collected directly from the rectum of each lamb once daily immediately after the morning feeding for seven consecutive days during each digestibility trial. Collected samples were pooled by animal, oven-dried at 55 °C for 48 h, ground, and stored for subsequent chemical analyses. Feed and fecal samples were analyzed for DM and organic matter (OM). Acid-insoluble ash (AIA) was used as an internal marker [31]. The apparent digestibility of DM and OM was calculated according to Dhanoa et al. [32]:

$$\text{Apparent digestibility (\%)} = 100 - \left(\frac{\% \text{ AIA in feed DM}}{\% \text{ AIA in faeces DM}} \right) \times \left(\frac{\% \text{ nutrient in faeces DM}}{\% \text{ nutrient in feed DM}} \right) \times 100$$

2.4. Chemical Analysis of Feeds and Meat

Representative samples of wheat straw, carob by-products, and concentrate feeds were collected for chemical analysis. Dry matter (AOAC method 934.01), CP (AOAC method 984.13), ether extract (AOAC method 920.39), and ash (AOAC method 942.05) were determined according to AOAC official methods [33]. Crude fiber was analyzed following the method described by Thiex [34]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. [35] using an Ankom fiber analyzer (ANKOM Technology, Macedon, NY, USA).

2.5. Carcass Characteristics, Meat Quality, and Fatty Acid Analysis

At the end of the feeding trial, lambs were fasted for approximately 24 h with free access to water and slaughtered according to standard halal procedures. Hot carcass weight (HCW) was recorded immediately after slaughter, and cold carcass weight was measured after 24 h of chilling at 4 °C. Dressing percentage was calculated as (HCW/slaughter BW) × 100.

Meat quality traits were evaluated using samples from the *Longissimus dorsi* (LD) muscle. Meat tenderness was assessed by measuring shear force on cooked LD samples using a texture analyzer. For proximate composition, LD samples were homogenized and analyzed for moisture, CP, and intramuscular fat. Lipids were extracted following the chloroform–methanol method described by Folch et al. [36]. Fatty acid (FA) methyl

esters were prepared by acid-catalyzed transesterification and analyzed using gas chromatography equipped with a flame ionization detector and a capillary column, following the method of Pérez-Palacios et al. [37]. Meat color was measured using a colorimeter (Minolta Chroma Meter CR-400, Minolta, Osaka, Japan), and values for lightness (L^*), redness (a^*), and yellowness (b^*) were recorded according to Honikel [38]. Muscle pH was measured at 0 h, 6 h, and 24 h post-mortem using a portable pH meter (Hanna Instruments, model HI 99163, Italy) [38]. Early post-mortem measurements (0 h and 6 h) were included to characterize the rate and dynamics of pH decline during the glycolytic phase, in addition to the standard ultimate pH (24 h), which is commonly used for meat quality evaluation.

Water-holding capacity (WHC) was determined using the filter paper press method [38]. The amino acid profile was determined by high-performance liquid chromatography (HPLC) after acid hydrolysis of freeze-dried meat samples according to the AOAC method 982.30 [33]. The concentration of essential amino acids, including leucine, methionine, phenylalanine, and valine, was quantified. Collagen content was determined by measuring hydroxyproline concentration according to Woessner [39]. Soluble and insoluble collagen fractions were separated based on solubility in buffer solutions. Total collagen content was calculated from hydroxyproline content using a conversion factor.

2.6. Blood Sampling and Biochemical Analysis

Jugular blood samples were collected monthly using plain vacuum tubes without anti-coagulant. The samples were allowed to clot at room temperature for approximately 30 min and were subsequently centrifuged at $4000 \times g$ for 20 min at 4°C to minimize protein degradation. The obtained serum was stored at -24°C until biochemical analysis. All serum samples were analyzed within 1–2 weeks after collection to preserve sample integrity. Serum concentrations of total protein, albumin, blood urea nitrogen (BUN), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glutamic-oxaloacetic transaminase (GOT) were determined using commercial enzymatic kits (Spinreact, Girona, Spain) in conjunction with an automated Mindray BS-240 Vet biochemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), according to the manufacturers' instructions. All biochemical measurements were performed in triplicate, and the mean value was used for statistical analysis.

2.7. Rumen Fluid Sampling and Microbial Analysis

Rumen fluid samples were collected from each lamb before the fasting period, on the final day of the experiment, 3 h after the morning feeding, using a stomach tube connected to a vacuum pump. Sampling at a standardized postprandial time point (3 h after feeding) was selected to minimize temporal variation in rumen fermentation parameters; however, it represents a single time-point measurement and therefore reflects relative treatment differences rather than diurnal fermentation dynamics. The initial 100 mL of rumen fluid was discarded to minimize saliva contamination. Rumen pH was measured immediately using a portable pH meter. After sample collection, lambs were returned to their pens and subsequently fasted overnight before slaughter.

For microbial enumeration, rumen fluid samples were serially diluted under anaerobic conditions. Total anaerobic bacteria were cultured on Wilkins–Chalgren anaerobic agar and incubated anaerobically at 39°C for 48 h [40]. Starch-degrading bacteria were enumerated on starch agar, while lactic acid bacteria (LAB) were cultured on de Man, Rogosa, and Sharpe (MRS) agar. Protozoa were counted microscopically using a counting chamber according to the method described by Saro et al. [41].

2.8. Statistical Analysis

Data were analyzed using the General Linear Model procedure (PROC GLM) of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Assumptions for analysis of variance (ANOVA) (normality and homogeneity of variance) were evaluated using Shapiro–Wilk and Levene’s tests, respectively. One-way ANOVA was applied according to the model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is the observed value, μ is the overall mean, T_i is the fixed effect of treatment, and e_{ij} is the residual error. For growth performance variables, including final BW, average daily gain (ADG), FCR and carcass traits, an analysis of covariance (ANCOVA) was performed to account for baseline differences in BW among animals at the start of the trial. Initial BW was included as a covariate in the model as follows: $Y_{ij} = \mu + T_i + \beta(X_{ij} - \bar{X}) + e_{ij}$, where X_{ij} is the initial BW, $\bar{X}$ is the overall mean initial BW, and β is the regression coefficient associated with the covariate. The assumption of homogeneity of regression slopes was tested and satisfied prior to conducting the ANCOVA. Residual plots were visually inspected to confirm model adequacy. No data transformation was required. Differences among treatments were considered significant at ($p < 0.05$), and trends were noted at $0.05 \leq p < 0.10$. When a significant treatment effect was detected, multiple pairwise comparisons among all treatment means (Control vs. UCL, Control vs. TCL, and UCL vs. TCL) were performed using Tukey’s honestly significant difference (HSD) test to control for Type I error.

3. Results

3.1. Growth Performance

Growth performance of Assaf lambs was significantly affected by dietary treatment (Table 3). Initial BW differed among groups ($p = 0.027$), with lambs in the control group having a higher initial BW than those in the UCL group, while TCL lambs were intermediate. Because this baseline difference may influence growth-related outcomes, growth performance variables were reanalyzed using ANCOVA with initial BW as a covariate. After adjustment, treatment effects on final BW, BW gain, ADG, and FCR remained significant ($p < 0.05$).

Table 3. Growth performance of Assaf lambs fed diets containing untreated or NaOH-treated carob leaves and twigs.

	Treatments ¹			SEM	p-Value	Covariate p-Value
	Control	UCL	TCL			
Initial body weight (kg)	29.0 ^a	27.0 ^b	28.9 ^{ab}	0.54	0.027	
Final body weight (kg)	46.9 ^b	51.0 ^a	51.4 ^a	0.57	<0.001	
[LS means $\pm$ SEM]	46.3 ^b $\pm$ 0.40	52.0 ^a $\pm$ 0.44	50.9 ^a $\pm$ 0.40		<0.001	<0.001
Body weight gain (kg)	17.9 ^c	24.0 ^a	22.5 ^b	0.39		
[LS means $\pm$ SEM]	18.0 ^b $\pm$ 0.40	23.7 ^a $\pm$ 0.44	22.6 ^a $\pm$ 0.40		<0.001	0.208
Average daily gain (g/d)	160 ^c	214 ^a	201 ^b	3.52	<0.001	
[LS means $\pm$ SEM]	161 ^b $\pm$ 3.6	212 ^a $\pm$ 3.9	202 ^a $\pm$ 3.6		<0.001	0.207
Feed conversion ratio	9.02 ^a	5.68 ^b	5.63 ^b	0.191	<0.001	
[LS means $\pm$ SEM]	8.94 ^a $\pm$ 0.195	5.81 ^b $\pm$ 0.212	5.57 ^b $\pm$ 0.193		<0.001	0.199
Feed efficiency (gain:feed)	0.112 ^b	0.177 ^a	0.178 ^a	0.0033	<0.001	
[LS means $\pm$ SEM]	0.113 ^b $\pm$ 0.0033	0.174 ^a $\pm$ 0.0035	0.179 ^a $\pm$ 0.0032		<0.001	0.107

SEM: standard error of the mean. LS: ANCOVA least square means and SEM (with initial body weight as the covariate) ^{a–c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

Final BW was significantly increased ($p < 0.001$) in lambs fed carob-based diets compared with the control, reaching 51.0 kg in UCL and 51.4 kg in TCL versus 46.9 kg in the control. Body weight gain was also significantly higher ($p = 0.001$) in UCL (24.0 kg) and TCL (22.5 kg) compared with the control group (17.9 kg). In addition, relative growth rate was markedly improved ($p < 0.001$), with UCL lambs showing the highest value (0.89), followed by TCL (0.78) and the control (0.62), indicating a greater proportional increase in BW in carob-fed lambs.

Average daily gain was markedly improved by carob inclusion ($p < 0.001$). UCL lambs exhibited the highest ADG (214 g/d), followed by TCL lambs (201 g/d), both significantly higher than the control (160 g/d), with UCL exceeding TCL. Dry matter intake (on average 1.11–1.12 kg/d throughout the trial) was not affected by dietary treatment ($p = 0.650$). Consequently, FCR was significantly improved ($p < 0.001$) in both carob-fed groups, with values of 5.68 in UCL and 5.63 in TCL compared with 9.02 in the control. Feed efficiency (gain:feed) values were 0.112, 0.177, and 0.178 for control, UCL, and TCL, respectively.

3.2. Nutrient Digestibility

Nutrient digestibility was significantly influenced by dietary treatment (Table 4). At 3 months of age, DM and OM digestibility were higher in TCL lambs compared with both UCL and control groups ($p < 0.001$), while UCL did not differ from the control. At 6 months, DM and OM digestibility remained significantly higher in TCL lambs ($p < 0.001$), whereas UCL showed intermediate values that were higher than the control but lower than TCL.

Table 4. Digestibility (%) at the 3rd and 6th months of age in Assaf lambs fed diets containing untreated or NaOH-treated carob leaves and twigs.

Treatments ¹				SEM	<i>p</i> -Value
	Control	UCL	TCL		
Dry matter					
3 months	63.6 ^b	62.0 ^b	71.7 ^a	0.71	<0.001
6 months	63.6 ^c	65.4 ^b	72.6 ^a	0.65	<0.001
Organic matter					
3 months	64.9 ^b	63.3 ^b	73.2 ^a	0.73	<0.001
6 months	66.9 ^b	67.9 ^b	74.8 ^a	0.60	<0.001

SEM: standard error of the mean. ^{a–c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

3.3. Carcass Characteristics and Meat Quality

Carcass characteristics were significantly affected by dietary treatment (Table 5). Hot carcass weight was higher in lambs fed carob-based diets compared with the control ($p < 0.001$), with values of 23.7 kg in UCL and 24.7 kg in TCL versus 21.0 kg in the control. Cold carcass weight followed a similar pattern, with UCL (22.7 kg) and TCL (23.9 kg) exceeding the control (20.4 kg) ($p = 0.009$). Dressing percentage was also significantly improved ($p < 0.001$), with TCL showing the highest value (46.6%), followed by UCL (44.6%) and the control (43.4%). Carcass composition was affected by diet. Carcass muscle percentage was reduced in UCL compared with the control and TCL groups ($p = 0.001$), whereas carcass bone percentage increased in UCL ($p = 0.001$). Carcass fat percentage was not significantly affected by dietary treatment ($p = 0.499$).

Table 5. Carcass traits and meat tenderness of Assaf lambs fed diets containing untreated or NaOH-treated carob leaves and twigs.

	Treatments ¹			SEM	<i>p</i> -Value	Covariate <i>p</i> -Value
	Control	UCL	TCL			
Hot carcass weight (kg)	21.0 ^b	23.7 ^a	24.7 ^a	0.49	<0.001	
[LS means ± SEM]	20.7 ^b ± 0.46	24.2 ^a ± 0.50	24.5 ^a ± 0.45		<0.001	0.021
Cold carcass weight (kg)	20.4 ^b	22.7 ^a	23.9 ^a	0.49	<0.001	
[LS means ± SEM]	20.1 ^b ± 0.46	23.3 ^a ± 0.50	23.7 ^a ± 0.45		<0.001	0.029
Dressing percentage (%)	43.4 ^b	44.6 ^{ab}	46.6 ^a	0.65	0.009	
[LS means ± SEM]	43.3 ^b ± 0.69	44.7 ^{ab} ± 0.75	46.5 ^a ± 0.68		0.011	0.639
Carcass bone (%)	18.4 ^b	19.5 ^a	18.6 ^b	0.19	0.001	
[LS means ± SEM]	18.3 ^b ± 0.20	19.6 ^a ± 0.21	18.6 ^b ± 0.19		0.002	0.412
Carcass muscle (%)	65.7 ^a	64.4 ^b	65.4 ^a	0.22	0.001	
[LS means ± SEM]	65.7 ^a ± 0.24	64.3 ^b ± 0.26	65.4 ^a ± 0.23		0.003	0.597
Carcass fat (%)	15.6	15.7	15.9	0.13	0.499	
[LS means ± SEM]	15.6 ± 0.14	15.7 ± 0.15	15.8 ± 0.14		0.515	0.985

SEM: standard error of the mean. LS: ANCOVA least square means and SEM (with initial body weight as the covariate) ^{a,b} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

Meat color parameters (L^* , a^* , and b^*) were not significantly influenced by diet ($p > 0.05$), indicating no effect of carob inclusion on visual appearance (Table 6). Although b^* values showed numerical differences, these were not statistically significant ($p = 0.057$). Muscle pH at 0 h and 6 h post-mortem was not affected by dietary treatment; however, pH at 24 h (pH24) was significantly reduced in UCL lambs compared with the control ($p = 0.031$), with TCL showing intermediate values. Water-holding capacity was not affected by treatment ($p = 0.342$). Meat tenderness, assessed by shear force, was significantly improved by carob inclusion ($p < 0.001$). Both UCL and TCL treatments reduced shear force values compared with the control, with the lowest values observed in TCL, followed by UCL and the control.

Table 6. Effect of dietary carob leaf inclusion on meat color, pH, water-holding capacity, and tenderness (shear force) of lamb meat.

	Treatments ¹			SEM	<i>p</i> -Value
	Control	UCL	TCL		
L^*	44.6	44.7	44.5	0.20	0.670
a^*	22.7	22.2	22.5	0.25	0.451
b^*	3.98	3.51	3.81	0.13	0.057
pH0	6.98	6.54	6.78	0.17	0.214
pH6	6.35	6.08	6.28	0.09	0.114
pH24	5.9 ^a	5.65 ^b	5.84 ^{ab}	0.07	0.031
WHC (%)	90.9	89.9	89.8	0.57	0.342
Tenderness (N)	33.6 ^a	32.0 ^b	30.0 ^c	0.23	<0.001

SEM: standard error of the mean. ^{a–c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

3.4. Meat Chemical Composition and Fatty Acid Profile

The chemical composition and fatty acid profile of LD muscle were significantly affected by dietary treatment (Table 7). Moisture content was not influenced by diet ($p = 0.362$). Crude protein content was significantly reduced in carob-fed lambs ($p < 0.001$), whereas intramuscular lipid content increased in UCL and TCL compared with the control ($p < 0.001$).

Table 7. Chemical composition and fatty acid (FA) profile (% of total fatty acids) of *Longissimus dorsi* muscle from Assaf lambs fed different diets.

	Treatments ¹			SEM	<i>p</i> -Value
	Control	UCL	TCL		
Chemical composition (%)					
Moisture	67.9	69.4	69.1	0.76	0.362
Crude protein	18.4 ^a	17.0 ^b	15.9 ^b	0.30	<0.001
Lipid	9.60 ^b	12.2 ^a	12.0 ^a	0.30	<0.001
Fatty acids (% of total FA)					
Saturated fatty acids	61.4 ^a	56.5 ^b	53.4 ^c	0.60	<0.001
Monounsaturated fatty acids	31.3 ^c	36.3 ^b	39.0 ^a	0.72	<0.001
Polyunsaturated fatty acids (PUFA)	7.38	7.25	7.63	0.40	0.801
Oleic acid (C18:1)	35.2 ^c	38.2 ^b	40.3 ^a	0.06	<0.001
α-Linolenic acid (C18:3)	1.58 ^c	1.80 ^b	2.04 ^a	0.02	<0.001
n-6/n-3 PUFA ratio	6.45 ^a	5.61 ^b	5.54 ^b	0.05	<0.001
Amino acids (%)					
Leucine	1.80 ^a	1.41 ^b	1.51 ^b	0.03	<0.001
Methionine	0.58 ^a	0.47 ^b	0.50 ^b	0.01	<0.001
Phenylalanine	1.04 ^c	1.34 ^a	1.24 ^b	0.02	<0.001
Valine	1.07 ^b	1.11 ^a	1.11 ^a	0.01	0.039
Soluble Hydroxyproline	0.29	0.30	0.32	0.01	0.052
Insoluble Hydroxyproline	0.32 ^b	0.34 ^a	0.35 ^a	0.01	0.014
Soluble Collagen	2.13	2.27	2.35	0.06	0.052
Insoluble Collagen	2.35 ^b	2.57 ^a	2.57 ^a	0.05	0.014
Total Collagen	4.47 ^b	4.83 ^{ab}	4.92 ^a	0.10	0.025

SEM: standard error of the mean. ^{a-c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

Fatty acid composition was markedly modified by dietary treatment. Saturated FA (SFA) were significantly reduced in UCL and TCL, with the lowest values observed in TCL ($p < 0.001$). In contrast, monounsaturated FA (MUFA), including oleic acid (C18:1), increased significantly, with the highest values in TCL ($p < 0.001$). Polyunsaturated FA (PUFA) were not affected by treatment ($p = 0.801$).

The concentration of α -linolenic acid (C18:3) increased significantly in both UCL and TCL groups ($p < 0.001$), while the n-6/n-3 PUFA ratio was significantly reduced ($p < 0.001$), indicating an improved fatty acid profile in carob-fed lambs.

Essential amino acid composition was also affected. Leucine and methionine contents were significantly lower in carob-fed lambs compared with the control ($p < 0.001$), whereas phenylalanine was significantly increased ($p < 0.001$). Valine also showed lower values ($p = 0.039$) in carob-fed lambs compared with the control.

Regarding connective tissue components, insoluble hydroxyproline and insoluble collagen were significantly increased in carob-fed lambs ($p = 0.014$), leading to a significant increase in total collagen content ($p = 0.025$). Soluble hydroxyproline and soluble collagen were not significantly affected ($p > 0.05$).

3.5. Blood Biochemical Analysis

Serum biochemical parameters were significantly influenced by dietary treatment (Table 8). Total protein concentration increased in TCL and decreased in UCL compared with the control ($p < 0.001$). Albumin concentration was reduced in both UCL and TCL, with the lowest values observed in UCL ($p < 0.001$).

Table 8. Serum biochemical parameters of Assaf lambs fed diets containing untreated or NaOH-treated carob leaves and twigs.

	Treatments ¹			SEM	<i>p</i> -Value
	Control	UCL	TCL		
Total protein (g/dL)	6.23 ^b	5.85 ^c	6.51 ^a	0.05	<0.001
Albumin (g/dL)	3.59 ^a	2.87 ^c	3.29 ^b	0.05	<0.001
Blood urea nitrogen (mg/dL)	38.5 ^a	26.8 ^c	34.0 ^b	0.20	<0.001
HDL (mg/dL)	23.0 ^a	16.6 ^c	19.5 ^b	0.13	<0.001
LDL (mg/dL)	27.7 ^c	35.0 ^a	32.7 ^b	0.22	<0.001
GOT (U/L)	80.9 ^b	86.0 ^a	78.0 ^c	0.13	<0.001

SEM: standard error of the mean. ^{a–c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

Blood urea nitrogen was significantly decreased in carob-fed lambs ($p < 0.001$), with the greatest reduction observed in UCL. Lipid profile parameters were also affected, as HDL concentrations decreased and LDL concentrations increased in both UCL and TCL compared with the control ($p < 0.001$). Serum GOT activity differed among treatments ($p < 0.001$), with UCL showing the highest value and TCL the lowest.

3.6. Rumen Fermentation and Microbial Populations

Rumen pH and microbial populations were significantly affected by dietary treatment (Table 9). Rumen pH increased in carob-fed lambs ($p < 0.001$), with UCL showing the highest value (6.46), followed by TCL (6.20), compared with the control (5.98).

Table 9. Rumen pH and microbial populations ($\log_{10}$ CFU or cells/mL) of Assaf lambs fed different diets.

	Treatments ¹			SEM	<i>p</i> -Value
	Control	UCL	TCL		
pH	5.98 ^c	6.46 ^a	6.20 ^b	0.06	<0.001
Total bacteria	9.78 ^c	9.83 ^b	9.85 ^a	0.004	<0.001
Protozoa	5.67 ^a	4.40 ^c	5.45 ^b	0.01	<0.001
Starch-degrading bacteria	6.08 ^c	6.28 ^b	6.62 ^a	0.01	<0.001
Lactic acid bacteria	5.92 ^c	6.05 ^b	6.81 ^a	0.01	<0.001

SEM: standard error of the mean. ^{a–c} Means within a row with different superscripts differ ($p < 0.05$). ¹ Control: diets with no carob leaves; UCL: diets with untreated carob leaves; TCL: diets with NaOH-treated carob leaves.

Total bacterial counts increased significantly in both UCL and TCL, with TCL showing the highest population density ($p < 0.001$). Protozoal counts were significantly reduced in UCL and TCL compared with the control ($p < 0.001$), with the lowest values observed in

UCL. Populations of starch-degrading bacteria and LAB were significantly increased by carob inclusion ($p < 0.001$), particularly in the TCL group.

4. Discussion

4.1. Growth Performance

Improving growth performance through nutritional strategies remains a central objective in small ruminant production systems, particularly under conditions where feed efficiency and economic sustainability represent major constraints [22]. The dietary treatments applied in the present study were associated with higher daily weight gain compared with previous reports by Mohammed et al. [14], who replaced fiber sources with 10% and 20% carob pulp, as well as Bottegal et al. [13], who used a 20% replacement level in lamb diets.

It is important to acknowledge that initial BW differed among treatment groups, which may introduce potential bias due to compensatory growth, whereby lighter animals tend to exhibit higher growth rates. To address this limitation, additional growth indicators were evaluated, including absolute BW gain and relative growth rate. The consistency of treatment effects across all growth parameters (final BW, weight gain, relative growth rate, and ADG) supports the conclusion that the observed improvements in UCL and TCL lambs were not solely attributable to initial weight differences. In particular, the significantly higher relative growth rate in UCL and TCL groups indicates that growth enhancement occurred on a proportional basis, reinforcing the biological relevance of the dietary effect. Nevertheless, the potential influence of baseline imbalance cannot be entirely excluded and should be considered when interpreting the magnitude of treatment responses.

The improvement in growth performance observed in this study may be attributed, at least in part, to differences in nutrient utilization efficiency rather than increased voluntary intake. Since DMI remained consistent across treatments, the superior growth performance of the carob-fed lambs was likely associated with more efficient nutrient use. However, this interpretation is indirect, as digestibility measurements were limited to DM and OM, restricting full mechanistic resolution of nutrient utilization pathways.

Nevertheless, the observed improvements in DM and OM digestibility, together with enhanced growth performance, suggest a positive effect of carob inclusion on overall nutrient utilization. This interpretation is supported by previous studies indicating that the inclusion of Mediterranean browse species at moderate dietary levels can improve nutrient availability and optimize rumen fermentation [42,43]. Another contributing factor may be the presence of CTs in carob leaves and their role in enhancing nitrogen utilization [44,45]. Condensed tannins in untreated carob leaves form reversible complexes with dietary proteins at the near-neutral pH of the rumen, thereby protecting proteins from excessive microbial deamination [46,47]. This mechanism is supported indirectly by the reduction in BUN, suggesting improved nitrogen retention rather than increased protein intake. These complexes dissociate under the acidic conditions of the abomasum, releasing high-quality protein for digestion and absorption in the small intestine. The marked reduction in BUN observed in UCL lambs further supports improved nitrogen retention and reduced ruminal ammonia losses, indicating more efficient conversion of dietary protein into body tissue rather than urinary nitrogen excretion.

The similar final BW achieved with both carob-based diets suggests potentially different but overlapping physiological mechanisms rather than a single causal pathway. In UCL diets, improved nitrogen utilization may have been more prominent, whereas TCL likely enhanced energy availability via fiber degradation. Comparable dual-mechanism responses have been reported in studies evaluating tree foliage supplementation in small ruminants [48].

The superior final BW observed in the TCL group appears linked to structural modifications of fibrous material through NaOH treatment, which disrupts lignocellulosic bonds and enhances the availability of fermentable carbohydrates [17]. However, alkaline treatment also partially reduces tannin activity, which could attenuate protein-protective effects observed in UCL diets. Performance benefits in NaOH-treated materials were likely derived mainly from improved energy availability rather than from a combined energy and protein-protection effect [49].

Dry matter intake remained unchanged among treatments, indicating that growth differences were not driven by feed intake variation [50]. Feed conversion ratio improved significantly, with carob leaves-supplemented diets showing the lowest values. However, feed efficiency (gain:feed) provides a more direct biological index than FCR, and its interpretation should be aligned with energy utilization efficiency rather than feed intake alone. These results confirm that diets supplemented with both untreated and treated carob leaves promoted a greater feed efficiency than with the control diet. Therefore, the present results support the use of carob leaves as a sustainable fibrous feed resource capable of improving feed efficiency under Mediterranean production conditions.

4.2. Nutrient Digestibility

Replacing a portion of wheat straw with TCL markedly improved several aspects of nutrient digestibility compared with both the control and UCL diets. Across both 3- and 6-month sampling points, TCL consistently enhanced the apparent digestibility of DM and OM. These findings are consistent with previous research on alkali-treated fibrous feeds, where NaOH has been reported to disrupt the lignocellulosic matrix, to saponify ester linkages between lignin and structural carbohydrates, swells fiber, and facilitates microbial enzyme access [18,20,21]. Such mechanisms, including disruption of ester linkages between lignin and structural carbohydrates and increased fiber swelling, are widely documented in the literature; however, they were not directly evaluated in the present experiment. In a similar context, studies on NaOH-treated almond hulls and other cereal straws have shown that although the responses to treatments may vary, the treatment often enhances microbial accessibility to both carbohydrate and nitrogen fractions, improving DM and OM utilization [18,19,51,52]. These observations emphasize that TCL improves digestibility via structural disruption of lignocellulosic components.

4.3. Carcass Characteristics and Meat Quality

Improvements in carcass traits further indicate that NaOH-treated carob leaves enhanced nutrient partitioning toward tissue deposition. However, because initial BW differed among groups, carcass responses may partly reflect baseline differences despite ANCOVA adjustment. These responses were greater than those reported by Bottegal et al. [13]. The superior carcass characteristics observed in the TCL group can be attributed to enhanced energy extraction from fiber fractions due to alkaline treatment [53,54]. Increased volatile fatty acid production, particularly acetate, may support lipogenesis [55]. The stable DMI suggests that differences in carcass output were driven by differences in nutrient utilization efficiency rather than intake level [56].

Regarding meat tenderness, carob supplementation significantly reduced shear force values, where the TCL group exhibited the lowest shear force. These improvements in tenderness are likely associated with greater intramuscular fat deposition facilitated by improved fiber and DM digestibility in TCL and enhanced protein utilization in UCL, which enhances lubrication of muscle fibers. However, post-mortem proteolytic activity was not directly measured, alterations in post-mortem proteolytic enzyme activity, particularly calpains, may also contribute, influenced by improved energy status and tannin-mediated

nitrogen protection [23]. These findings indicate that TCL maximizes both carcass yield and meat quality by improving the digestibility of structural fibers, whereas UCL enhances protein efficiency and meat tenderness.

Concerning meat color, the absence of significant differences in L^* and a^* values indicates that the inclusion of carob leaves did not affect meat lightness or redness, suggesting no major influence on myoglobin stability or oxidation state. This agrees with previous findings that moderate inclusion of tannin-rich feeds does not markedly alter lamb meat color characteristics [57]. Although b^* values tended to decrease in UCL lambs, this effect was not statistically significant and therefore should be interpreted with caution. The numerical reduction in yellowness may be associated with minor changes in lipid oxidation or pigment deposition, possibly influenced by polyphenolic compounds present in carob leaves. Similar observations were reported by Vasta et al. [58], who noted that natural antioxidants in the diet can slightly modify meat color parameters without causing pronounced changes.

The lack of significant differences in pH0 and pH6 suggests that pre-slaughter glycogen reserves and early post-mortem glycolysis were not affected by dietary treatments. However, the significant reduction in pH24 in UCL lambs indicates a greater extent of post-mortem glycolysis. Importantly, the observed pH24 values remained within the normal physiological range, indicating acceptable meat quality.

Water-holding capacity was not influenced by dietary treatment, which is consistent with previous reports showing that moderate levels of tannins do not impair muscle water retention [59].

In contrast, meat tenderness was significantly improved in carob-fed lambs, as evidenced by reduced shear force values, particularly in the TCL group. This enhancement in tenderness is unlikely to be directly associated with collagen quantity alone, but rather with changes in muscle proteolytic activity during post-mortem aging. Polyphenolic compounds in carob leaves may enhance antioxidant status, thereby preserving proteolytic enzyme activity and promoting muscle protein degradation. Similar improvements in lamb meat tenderness with phenolic-rich diets have been attributed to enhanced proteolysis and reduced oxidative damage to muscle proteins [27].

4.4. Meat Chemical Composition and Fatty Acid Profile

One of the most notable findings of this study was the marked modification of the FA profile of the LD muscle. Carob supplementation significantly altered muscle chemical composition, with higher moisture and intramuscular fat contents and slightly reduced CP levels. The slight reduction in CP concentration in UCL and TCL meat is most plausibly explained by a dilution effect due to higher fat and water deposition, rather than impaired muscle protein synthesis, and is consistent with the digestibility and nitrogen utilization data. Lambs fed UCL and TCL exhibited numerically higher muscle moisture content (69.4%, and 69.1%, respectively) compared with the control group (67.9%); however, these differences were not statistically significant, indicating that dietary treatment had no measurable effect on muscle moisture content.

Although CP content decreased slightly in the meat of UCL (17.0%) and TCL (15.9%) compared with control lambs (18.4%), protein levels remained within the normal physiological range, indicating no compromise to muscle quality [60].

Intramuscular fat content increased significantly in carob-fed lambs, reaching 12.2% (UCL) and 12.0% (TCL) compared with 9.6% in controls (increases ~25% for TCL and 27% for UCL). The higher intramuscular fat in TCL lambs aligns with enhanced fiber digestibility (NDF, ADF) and energy extraction, whereas UCL lambs benefited from improved post-ruminal protein utilization, which can indirectly support lipogenesis. Similar responses

have been reported by Arshad et al. [61] and Ponnampalam et al. [23], who attributed these effects to tannin-mediated shifts in rumen fermentation and lipid metabolism.

The FA profile shifted toward a healthier composition, with a significant reduction in SFA from control (61.4%) to UCL (56.5%) and TCL (53.4%). Monounsaturated FA increased significantly, reaching 39.0% in TCL and 36.3% in UCL compared with 31.3% in controls. Oleic acid (C18:1) showed a similar upward trend, with TCL exhibiting the highest concentration (40.3%), followed by UCL (38.2%) and control (35.2%). These changes reflect tannin-mediated modulation of ruminal biohydrogenation and improved escape of UFA from the rumen, consistent with the digestibility and microbial efficiency patterns observed [29,30].

Alpha-linolenic acid (C18:3 n-3) increased significantly in carob-fed lambs (1.80–2.04%) compared with control (1.58%), resulting in a significant reduction in the n-6/n-3 PUFA ratio. Both UCL (5.61) and TCL (5.54) groups approached the recommended ratio for human health, supporting the functional benefit of carob supplementation mediated via improved nutrient digestibility and ruminal microbial modulation [9]. These effects likely result from tannin-induced modulation of ruminal biohydrogenation pathways, allowing greater transfer of dietary omega-3 FAs to muscle tissue [62,63].

The observed changes in amino acid composition and connective tissue characteristics suggest that the inclusion of carob leaves may influence protein metabolism and muscle composition. The significant reductions in leucine and methionine in UCL and TCL lambs indicate alterations in amino acid availability or utilization. These effects may be associated with tannins present in carob leaves, which are known to bind dietary proteins and reduce their ruminal degradation, thereby modifying the flow of amino acids available for absorption and subsequent tissue deposition [59,64].

In contrast, the marked increase in phenylalanine and the slight increase in valine suggest a differential response among amino acids to dietary treatments. The rise in phenylalanine may reflect shifts in microbial protein synthesis or post-absorptive metabolism, potentially influenced by plant secondary compounds. Similar patterns have been reported in studies evaluating alternative plant-based feeds, where changes in amino acid profiles were linked to variations in rumen fermentation and nutrient absorption [65]. However, given the relatively small change in valine, its biological relevance should be interpreted cautiously.

Regarding connective tissue components, the increases in insoluble hydroxyproline and insoluble collagen, along with the rise in total collagen content, indicate an accumulation of less soluble collagen fractions in carob-fed lambs. While this may suggest enhanced collagen cross-linking or maturation, such interpretations should be made cautiously, as direct measures of cross-linking were not assessed. The lack of significant changes in soluble hydroxyproline and soluble collagen further supports the notion that dietary effects were more pronounced in the insoluble fraction. Polyphenolic compounds in carob leaves may play a role in modulating collagen metabolism and stability, as suggested by previous studies examining the influence of plant bioactive compounds on muscle tissue characteristics [66].

4.5. Blood Metabolites, Rumen pH, and Microbial Ecology

Total serum protein concentration was highest in TCL lambs (6.51 g/dL), representing a ~5% increase compared with control lambs (6.23 g/dL). This aligns with improved digestibility of DM, OM, and fiber fractions, leading to enhanced amino acid supply [28,67]. Blood urea nitrogen was lowest in UCL lambs (26.8 mg/dL), representing a ~30% reduction compared with controls, confirming tannin-mediated protein sparing and post-ruminal absorption [44].

The ruminal pH results revealed that the incorporation of carob leaves into the diets influenced ruminal fermentation conditions. The ruminal pH in the control group (5.98) was slightly below the conventional threshold of 6.0; however, this value should be interpreted cautiously, as ruminal pH is subject to normal diurnal fluctuations and may transiently decrease after feeding. Therefore, although the control group approached the lower physiological range, it cannot be conclusively interpreted as subacute ruminal acidosis in the absence of additional diagnostic indicators [68]. No clinical or metabolic signs associated with ruminal acidosis, such as reduced feed intake, abnormal behavior, or impaired health status, were observed in control lambs during the experimental period.

Nevertheless, the lower ruminal pH observed in the control group may indicate a less favorable environment for fibrolytic activity, which could partly explain the reduced nutrient digestibility and growth performance in this group. In contrast, diets containing carob leaves and twigs appeared to promote a more stable ruminal fermentation pattern, as reflected by higher pH values and improved nutrient utilization [68].

The increased ruminal pH value obtained from the UCL sheep could be an indication that the fermentation patterns were moderated, possibly due to the suppression of protozoal populations. An increased ruminal pH value, which falls within the physiological range (6.2–6.6), suggesting moderated fermentation likely due to suppression of protozoa and improved nitrogen retention, which enhances microbial protein synthesis [69,70].

The significant reduction in protozoal populations could be attributed to the increased bacterial populations, which compete with and displace protozoal populations, thereby altering the balance of overall microbial activity. In ruminants, the suppression of protozoal populations has been attributed to the improved nitrogen retention and increased efficiency of microbial protein synthesis [71]. Although the individual phenolic compounds were not identified, the bioactive compounds that are naturally present in the carob leaves could have been responsible for the altered ruminal microorganisms, resulting in the bacterial-dominated fermentation patterns.

The increase in total bacterial population from control, then to UCL, and finally to TCL lambs indicates that alkaline treatment improved substrate accessibility, particularly structural carbohydrates. This response was observed experimentally in the present study. Sodium hydroxide treatment has been shown to break hydrogen bonds in lignocellulose, which increases surface area for microbial attachment and degradation. However, these structural modifications were not directly assessed in this study, and their contribution to the observed microbial changes is inferred from existing literature rather than directly demonstrated here. The increase in starch-degrading bacteria and LAB in TCL lambs also indicates that alkaline treatment improved carbohydrate availability and, subsequently, carbohydrate fermentation. The increase in amylolytic and LAB populations typically indicates that carbohydrate fermentation is efficient and that carbohydrate turnover is enhanced [72,73]. However, it is noteworthy that despite the increase in total population of microorganisms that contribute to fermentation, TCL lambs had moderate pH levels, indicating that fermentation end-products were either buffered or absorbed.

A limitation of the present study is the absence of detailed rumen fermentation end-products, particularly volatile fatty acids, as well as fiber-specific digestibility measurements (NDF and ADF). Volatile fatty acids represent the primary metabolic output of rumen microbial fermentation and are essential for a comprehensive evaluation of rumen energy metabolism. The lack of these measurements restricts mechanistic interpretation of the observed effects of carob inclusion on rumen function. Future studies incorporating complete digestibility profiling and volatile fatty acids analysis are therefore warranted to better elucidate the underlying fermentation pathways.

5. Conclusions

This study showed that partial replacement of wheat straw with carob leaves and twigs (*Ceratonia siliqua*) effectively enhances growth, carcass traits, nutrient utilization, and meat quality in Assaf lambs under Mediterranean production conditions. Untreated carob leaves improved growth performance and feed efficiency compared with the control diet. Sodium hydroxide-treated carob leaves increased the digestibility of dry matter and organic matter, promoting higher carcass weights, greater dressing percentage, and more efficient conversion ratio. Both untreated and sodium hydroxide-treated diets improved meat quality. Sodium hydroxide-treated leaves enhanced the fatty acid profile by reducing saturated fatty acids, increasing monounsaturated fatty acids, and lowering the ratio of omega-6 to omega-3 polyunsaturated fatty acids. Untreated leaves maintained higher ruminal pH and decreased protozoal counts. Incorporating 25% untreated carob leaves into lamb diets is recommended for enhancing growth performance and protein utilization; however, this value should be interpreted as specific to the tested conditions rather than as a universal recommendation. Further studies are needed to optimize inclusion levels, treatment methods, and long-term effects on health and product quality. The absence of measurements such as volatile fatty acids, fiber fraction digestibility, and rumen functional profiling limits full mechanistic interpretation of the observed responses.

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Abbreviations

The following abbreviations are used in this manuscript:

ADF	Acid detergent fiber
ADG	Average daily gain
BW	Body weight
AIA	Acid-insoluble ash
BUN	Blood urea nitrogen
CP	Crude protein

CTs	Condensed tannins
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acids
FCR	Feed conversion ratio
GOT	Glutamic-oxaloacetic transaminase
HCW	Hot carcass weight
HDL	High-density lipoprotein
LAB	Lactic acid bacteria
LD	<i>Longissimus dorsi</i>
LDL	Low-density lipoprotein
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
OM	Organic matter
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TCL	NaOH-treated carob leaves and twigs
UCL	Untreated carob leaves and twigs
WHC	Water-holding capacity

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