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Effects of Replacing Concentrate Feed with Carob (*Ceratonia siliqua*) Pods on Growth Performance, Carcass Characteristics, Meat Quality, and Rumen Fermentation in Assaf Lambs

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Abstract

This study examined the effects of replacing 25% (P25) or 50% (P50) of concentrate dry matter (DM) with sun-dried carob (*Ceratonia siliqua* L.) pods on growth performance, apparent nutrient digestibility, carcass traits, meat quality, serum biochemistry, and rumen microbiology in growing Assaf lambs. Twenty-four weaned male Assaf lambs (initial body weight [BW] 27.0 ± 0.5 kg; 2.5 months of age) were randomly assigned to three dietary treatments (n = 8 per group) in a completely randomized design and fed for 16 weeks. P50 achieved the highest ANCOVA-adjusted least squares mean final BW (53.0 kg) and average daily gain (ADG) (220.8 g/d), followed by P25 (51.1 kg; 203.3 g/d) and the control (46.2 kg; 160.2 g/d) ($p < 0.001$). Feed conversion ratio (FCR) improved from 8.99 in the control to 6.16 and 6.11 in P25 and P50, respectively, with no significant difference between the two carob-supplemented groups ($p < 0.001$). Apparent DM and organic matter (OM) digestibility increased with carob inclusion at both 3 and 6 months of age ($p \leq 0.0001$). Cold carcass weight (CCW) was higher in carob-supplemented lambs ($p < 0.001$), whereas carcass muscle proportion did not differ among treatments ($p = 0.688$), and carcass fat proportion was higher in P25 than in the control ($p = 0.039$). Warner–Bratzler shear force declined progressively with carob inclusion ($p < 0.001$), indicating improved meat tenderness. Serum total protein was highest in P25, whereas blood urea nitrogen (BUN), low-density lipoprotein (LDL), and glutamate oxaloacetate transaminase (GOT) decreased with carob inclusion ($p < 0.001$). Rumen pH was highest in P25 (6.40), total bacterial and lactic acid bacteria (LAB) counts increased, and protozoa counts declined

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($p < 0.001$). Because carob pods replaced concentrate, rather than being added to an isonitrogenous diet, the combined effects of pods per se, reduced crude protein supply, and altered energy density must all be considered when interpreting the results. These findings support carob pods as a practical, locally available partial substitute for concentrate feed in Assaf lamb production under Mediterranean and Near Eastern conditions.

Keywords: *Ceratonia siliqua*; Assaf lambs; concentrate substitution; condensed tannins; carcass quality; rumen microbiology; serum metabolites; sustainable feeding

1. Introduction

Sheep production is integral to agriculture and food security across the Mediterranean and Near East, underpinning rural livelihoods and sustaining extensive land management on terrain unsuitable for cropping [1–3]. These systems are now under intensifying pressure from climate change, rising concentrate prices, and growing competition between livestock and crop production for arable land [4,5]. The Assaf breed, a composite of East Friesian and Awassi genetics, is well adapted to both intensive and semi-intensive Mediterranean conditions and achieves annual milk yields of approximately 450 L while maintaining satisfactory growth and carcass output [6,7]. Maintaining or improving productive performance in Assaf lambs while reducing dependence on imported cereal-based concentrates is therefore a priority for producers and policy makers alike [8,9]. Under the constraints of rising concentrate prices and competition for arable land, partially substituting purchased concentrate with a locally available, drought-tolerant feed resource offers a direct route to lowering feed costs and import dependence without requiring additional cropland, providing the practical rationale for evaluating carob pods as a partial concentrate replacement in the present study.

The carob tree (*Ceratonia siliqua* L.) is a drought-tolerant species native to the Mediterranean basin, whose pods have been used as livestock feed for centuries [10]. Carob pods contain high concentrations of soluble sugars (48 to 56%) and carbohydrates (40 to 60%), appreciable amounts of dietary fiber (27 to 50%) and polyphenolic compounds, particularly condensed tannins (18 to 20%), and relatively low concentrations of protein (3 to 4%) and fat (0.2 to 0.6%). These compositional characteristics influence ruminal fermentation and protein metabolism [11,12]. The soluble carbohydrate fraction supplies rapidly fermentable energy that can support microbial protein synthesis and stabilize rumen pH [13], while CT at moderate concentrations forms reversible complexes with dietary protein, reducing ruminal degradation and increasing the flow of undegraded protein to the small intestine [14–16]. This protein-sparing mechanism, often evidenced by reduced blood urea nitrogen, is a central rationale for evaluating tannin-containing feeds in growing ruminants [17,18]. This mechanism has been proposed in previous studies and may have contributed to the present observations; however, because ruminal ammonia, nitrogen balance, and protein degradability were not measured, this explanation remains speculative. However, the increasing cost and competition for conventional concentrate ingredients, such as cereal grains and protein sources, have encouraged the search for locally available agro-industrial by-products and non-conventional feed resources that can partially replace concentrate feeds without compromising animal performance. Carob pods represent a promising alternative because they are abundant in Mediterranean regions, contain substantial amounts of fermentable carbohydrates, and provide bioactive compounds, including condensed tannins and phenolic compounds. Their use as a partial substitute for concentrate may reduce dependence on purchased feed ingredients, enhance

the utilization of locally available biomass, and contribute to more sustainable feeding systems. Annual pruning generates large quantities of carob biomass in Mediterranean agro-silvo-pastoral systems, and utilization of this resource within circular bioeconomy frameworks can reduce feed costs and environmental impacts simultaneously [19,20].

Previous in vivo studies have generally used low to moderate inclusion levels of carob pulp or pod fractions, typically 10–20% of dietary DM, and have reported moderate improvements in growth performance and feed efficiency, with inconsistent effects on carcass traits and meat quality [21,22]. In a companion study from the same experimental platform [23], chemical composition, in vitro gas production, and nutrient degradability of carob leaves from Tunisia and Palestine were characterized, providing the nutritional framework for the present feeding trial. Nevertheless, the potential of carob pods as a partial replacement for concentrate, particularly at relatively high substitution levels, remains insufficiently investigated. However, a direct, controlled comparison of two inclusion levels of carob pods (25% and 50% of concentrate DM) within a single experiment, covering growth performance over a full 16-week finishing cycle, digestibility at two physiological stages, detailed carcass and meat quality evaluation, serum biochemistry, and rumen microbiology, has not been reported for Assaf lambs.

Beyond growth performance, evaluation of meat quality responses is essential when assessing alternative feed resources because dietary modifications can influence carcass characteristics, muscle composition, fatty acid profile, and technological properties of meat [24–26]. Dietary condensed tannins can modulate ruminal biohydrogenation, potentially affecting the transfer of unsaturated fatty acids to muscle lipids [27–29]. In addition, the phenolic compounds in carob pods possess antioxidant properties that may contribute to the preservation of meat quality during post mortem storage; however, direct measurements of muscle antioxidant status are required to confirm this potential mechanism [10,30]. Therefore, assessing meat quality responses provides important information on whether carob pod inclusion can improve production efficiency while maintaining or enhancing the nutritional and commercial value of lamb meat.

The present study therefore compared two inclusion levels of sun-dried carob pods (25% and 50% of concentrate DM, designated P25 and P50) in diets of growing Assaf lambs to test the hypothesis that replacing part of the concentrate with carob pods would enhance dietary energy utilization through their high fermentable sugar content and condensed tannins, thereby improving growth and feed efficiency, while favorably modifying meat fatty acid composition by modulating ruminal biohydrogenation, with the magnitude of response depending on inclusion level. The present study therefore evaluated the overall biological response to practical dietary substitution, rather than the isolated effect of carob pods themselves.

2. Materials and Methods

2.1. Animals, Experimental Design, and Diets

All experimental procedures were approved by the Palestinian Association of Agricultural Engineers–Jerusalem Branch (Ethical Approval No. 3/A.E.A/2026) and conducted in compliance with Directive 2010/63/EU and the ARRIVE guidelines for reporting animal research. Informed consent was obtained from the herd owner. Ethical approval was granted in March 2024, before the experiment began in April 2024; the approval number follows the institution's internal registration sequence, rather than the calendar year of issue, which is why it does not numerically match the approval date.

The experiment was conducted from April 2024 on a private sheep farm in Jenin, Palestine. Twenty-four weaned male Assaf lambs (2.5 months of age; initial BW 27.0 $\pm$ 0.5 kg) were randomly allocated to three dietary treatments using a completely randomized de-

sign, with eight lambs per treatment. Randomization used a random number generator without prior stratification by BW. In retrospect, stratifying animals by initial BW before random allocation would have been preferable to minimize baseline imbalance; this was not conducted in the present trial, which is acknowledged as a design limitation. Initial BW was subsequently included as a covariate in the statistical model for final BW and ADG to correct for baseline differences. The 16-week feeding trial (from 2.5 to 6.5 months of age) included a 14-day adaptation period.

Three dietary treatments were evaluated: (1) a control diet comprising wheat straw as the sole roughage source, supplemented with a conventional concentrate; (2) P25, in which 25% of concentrate DM was replaced with sun-dried carob pods while wheat straw was retained at the control level; and (3) P50, in which 50% of concentrate DM was replaced with sun-dried carob pods. The experimental diets were not formulated to be isonitrogenous or isoenergetic; as carob pods replaced concentrate, dietary crude protein (CP) fell from approximately 13.2% in the control to 8.9% in P50, and energy density changed correspondingly. The diets were therefore designed to evaluate the practical consequences of partial concentrate substitution under field conditions, not to isolate the effect of carob pods *per se*. Diet composition and the amount of each feed ingredient were adjusted monthly to meet the nutritional requirements of the lambs (Table 1), and the chemical compositions of the dietary ingredients are presented in Table 2.

Table 1. Compositions of the diets (g/lamb/day, as-fed basis; corresponding dry matter intake values in parentheses, g DM/lamb/day) offered to the lambs during the 16 weeks of the study.

		Treatment [1]		
		Control	**P25**	**P50**
Week 0 to week 4	Straw	400 (372)	400 (372)	400 (372)
	P25	0	125 (112)	0
	P50	0	0	250 (225)
	Concentrate	500 (450)	375 (337)	250 (225)
	Total offered and consumed	900 (822)	900 (822)	900 (822)
Week 4 to week 8	Straw	400 (372)	400 (372)	400 (372)
	P25	0	175 (157)	0
	P50	0	0	350 (314.6)
	Concentrate	700 (629)	525 (472)	350 (314.6)
	Total offered and consumed	1100 (1001)	1100 (1001)	1100 (1001)
Week 8 to week 12	Straw	500 (465)	500 (465)	500 (465)
	P25	0	200 (180)	0
	P50	0	0	400 (359.6)
	Concentrate	800 (719)	600 (539)	400 (359.6)
	Total offered and consumed	1300 (1184)	1300 (1184)	1300 (1184)
Week 12 to week 16	Straw	700 (651)	700 (651)	700 (651)
	P25	0	225 (202.2)	0
	P50	0	0	450 (404.5)
	Concentrate	900 (809)	675 (606.8)	450 (404.5)
	Total offered and consumed	1600 (1460)	1600 (1460)	1600 (1460)

Control, no carob pods; P25, 25% of concentrate DM replaced by carob pods; P50, 50% of concentrate DM replaced by carob pods. Values are presented as g/lamb/day on an as-fed basis, with corresponding DM values in parentheses (g DM/lamb/day). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

Lambs were housed individually in shaded pens with continuous access to drinking water. Feed was offered twice daily (07:00 and 16:00 h), and the amount of feed offered to each lamb was recorded daily. As the offered feed was fully consumed, and no measurable

refusals were observed, daily DM intake (DMI) was calculated based on the amount of feed offered and the DM content of the individual feed ingredients. Body weight was recorded every 10 days throughout the experimental period, and ADG was calculated from the slope of BW on time; FCR was calculated as kg total DM consumed per kg BW gained. Animal health and general condition were monitored daily throughout the study, and any observable health problems or abnormal clinical signs were recorded.

Table 2. Chemical composition of dietary ingredients (% of DM, unless otherwise stated).

Item	Wheat Straw	Carob Pods	Concentrate [1]
DM, % of fresh matter	92.9	89.9	89.9
Organic matter	88.6	88.1	86.7
Crude protein	5.01	5.24	18.0
Crude fiber	37.5	9.7	6.6
Neutral detergent fiber	39.3	28.3	25.3
Acid detergent fiber	32.4	20.5	18.5
Acid detergent lignin	11.4	9.5	4.2
Condensed tannins [2]	NM	3.86	NM
Total phenols [3]	NM	12.4	NM

[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: 45.8 g dicalcium phosphate, 15 g magnesium sulfate, 6.15 g ferrous sulfate, 0.393 g potassium iodide, 0.753 g copper sulfate, 0.248 g cobalt sulfate, 0.373 g zinc sulfate, and 0.022 g sodium selenite; 10,000 IU vitamin A, 1.5 IU vitamin D3, and 60 mg vitamin E]. [2] Tannin contents: catechin equivalent/100 g DM, NM = not measured. [3] Polyphenol contents in mg equivalent gallic acid (GAE)/g DM.

2.2. Carob Pod Preparation

Carob pods were collected during the annual pruning season from local *Ceratonia siliqua* trees in Jenin. Freshly harvested pods were sun-dried for 10 days at 27–30 °C, with the pods turned manually twice daily to ensure uniform drying on clean cement surfaces, coarsely chopped and ground through a hammer mill fitted with a 5 mm screen before incorporation into the diets, blended for uniformity, and stored in airtight containers at room temperature until use. No washing or chemical treatment was applied.

2.3. Nutrient Digestibility

Two in vivo digestibility trials were conducted during the last week of the third and sixth months of life, while lambs continued to receive their assigned treatments. Digestibility measurements were conducted over seven consecutive days during the last week of the third month and repeated during the final week of the sixth month of age. Because lambs entered the trial at 2.5 months of age and underwent a 14-day dietary adaptation period, the first digestibility trial (last week of the third month) began approximately 2 to 3 weeks after the end of adaptation, and the second trial (last week of the sixth month) was conducted near the end of the 16-week feeding period. Lambs were housed individually and fed twice daily at fixed times, with feed allowances adjusted for age. During each digestibility trial, representative samples of the experimental diets were offered, and orts (feed refusals) were collected daily, composited by animal over the 7-day sampling period, oven-dried at 55 °C for 48 h, ground through a 1 mm screen, and stored until chemical analysis. Fecal samples were collected directly from the rectum once daily for seven consecutive days. After each collection, samples were placed in labeled plastic bags and stored at −20 °C until completion of the 7-day sampling period. The daily samples from each lamb were then pooled by animal on an equal-weight basis, thoroughly mixed, and a representative composite sample was oven-dried at 55 °C for 48 h, ground through a 1 mm screen, and stored pending analysis. Feed and fecal samples were analyzed for DM and OM [31].

Acid-insoluble ash (AIA) was used as an internal marker [32], and apparent digestibility was calculated following Dhanoa et al. [33]:

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

2.4. Slaughter, Carcass Traits, and Meat Quality

At the end of the 16-week trial, all lambs were fasted for 24 h, with water *ad libitum*, and slaughtered by standard halal procedures. Hot carcass weight (HCW) was recorded immediately after evisceration; cold carcass weight (CCW) was recorded after 24 h chilling at 4 °C. Dressing percentage was calculated as (HCW/slaughter BW) × 100. Carcass composition (bone, muscle, fat proportions) was assessed by dissection of the right half-carcass. Warner–Bratzler shear force (WBSF) was measured on cooked (75 °C internal temperature) cylindrical cores (1.27 cm diameter) cut from the LD muscle between the 12th and 13th ribs. Shear force values below 40 N are generally considered indicative of acceptable tenderness [34]. Meat color (L*, a*, b*) was measured on the LD cut surface 30 min post-cutting with a calibrated portable colorimeter. Muscle pH was recorded at 0, 6, and 24 h post-slaughter by direct electrode insertion. Water-holding capacity (WHC) was determined by the filter paper press method [35].

2.5. Blood Sampling and Serum Biochemistry

Jugular blood was collected monthly into plain vacutainer tubes, allowed to clot at room temperature, and centrifuged at 3000× *g* for 15 min at 4 °C; serum was stored at −20 °C for no longer than three months before biochemical analysis. Serum total protein, albumin, BUN, high-density lipoprotein (HDL), LDL, and GOT were measured with commercial enzymatic kits (Spinreact, Girona, Spain) on an automated biochemical analyzer. Because blood was drawn repeatedly on the same animals, these data were analyzed as repeated measures.

2.6. Rumen Fluid Sampling and Microbial Enumeration

Rumen fluid was collected 3 h after morning feeding by esophageal tube connected to a vacuum pump, with the first 100 mL discarded to reduce saliva contamination. Sampling at a fixed post-prandial time limits temporal variation, but provides only a single timepoint estimate, so results reflect relative treatment differences, rather than diurnal fermentation dynamics. Rumen pH was measured immediately with a calibrated portable meter. Total anaerobic bacteria were cultured on Wilkins–Chalgren anaerobic agar (39 °C, 48 h) [36]. Starch-degrading bacteria were enumerated on starch agar. LAB were cultured on de Man, Rogosa, and Sharpe (MRS) agar (37 °C, 48 h). Protozoa were counted microscopically following Saro et al. [37]. VFA, lactate, ammonia-N, and buffering capacity were not measured, which limits mechanistic interpretation of the rumen data.

2.7. Chemical Analysis

All laboratory chemical analyses were performed in triplicate. Dry matter (AOAC 934.01), CP (AOAC 984.13; N × 6.25), ether extract (AOAC 920.39), and ash (AOAC 942.05) were determined according to AOAC [31]. Crude fiber was determined following Thiex [38]. NDF and ADF were measured on an Ankom fiber analyzer (ANKOM Technology, Macedon, NY, USA) according to Van Soest et al. [39]. Acid-insoluble ash (AIA) was determined according to Van Keulen and Young [40] and used as an internal marker for estimating apparent nutrient digestibility. Total phenolics were quantified by the Folin– Ciocalteu method and expressed as GAE per gram DM. Condensed tannins were measured by the butanol-HCl assay and expressed as catechin equivalents per 100 g DM [41].

Fatty acid methyl esters were prepared by acid-catalyzed transesterification and analyzed using Perkin–Elmer gas chromatography (Model 8420, Beaconsfield, UK) equipped with a flame-ionization detector on a 100 m CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands) following Perez-Palacios et al. [42]. Fatty acids were identified by comparing their retention times with those of a certified FAME reference standard mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, USA). Fatty acids, including saturated, monounsaturated, and polyunsaturated fatty acids and their positional isomers, were identified and quantified. Intramuscular lipid was extracted by the Folch et al. [43] chloroform–methanol (2:1, v/v) method.

Collagen fractions were quantified via hydroxyproline content following acid hydrolysis [44]. Essential amino acids (leucine, methionine, phenylalanine, valine) were determined by HPLC after acid hydrolysis (AOAC 982.30) [31].

2.8. Statistical Analysis

Data were analyzed using the PROC GLM of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Variables measured at a single timepoint were analyzed using a general linear model, with dietary treatment as the main fixed effect. Because treatments represented practical dietary substitutions of concentrate with carob pods, rather than isonitrogenous and isoenergetic diets, the treatment effect was interpreted as the response to the complete dietary formulation. The model $Y_{ij} = \mu + T_i + e_{ij}$ was used, 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. Growth performance variables, including final body weight, average daily gain, carcass traits, and feed conversion ratio, were analyzed using analysis of covariance (ANCOVA) to adjust for baseline differences among animals. Initial BW was included as a covariate for BW and ADG according to the model $Y_{ij} = \mu + T_i + \beta(X_{ij} - \overline{X}) + e_{ij}$, where X_{ij} is the initial BW, $\overline{X}$ is the overall mean initial BW, and β is the regression coefficient associated with the covariate. The assumption of homogeneity of regression slopes was verified before conducting the ANCOVA. Residual plots were examined to confirm model adequacy, and no data transformation was required. Differences among treatments were considered significant at $p < 0.05$, whereas values of $0.05 \leq p < 0.10$ were considered trends. When a significant treatment effect was detected, least squares means were separated using Tukey's honestly significant difference (HSD) test to control the experiment-wise Type I error rate. Variables measured repeatedly on the same animals over time were analyzed using a repeated-measures mixed model (PROC MIXED, SAS Institute) with the structure $Y_{ijk} = \mu + T_i + A(T)_{ij} + M_k + TM_{ik} + e_{ijk}$, where T_i is the fixed effect of treatment, M_k is the fixed effect of month or age, TM_{ik} is the treatment $\times$ time interaction, and $A(T)_{ij}$ is the random effect of animal nested within treatment. Covariance structures evaluated for the residual matrix included first-order autoregressive [AR(1)], compound symmetry (CS), and unstructured (UN); the best-fitting structure was selected by minimizing the Akaike information criterion (AIC). The Kenward–Roger method was applied to approximate degrees of freedom.

3. Results

3.1. Growth Performance, Dry Matter Intake, and Feed Conversion

Growth performance data are presented in Table 3. Initial BW differed significantly among groups ($p = 0.042$): the control lambs were heavier than P25 lambs (29.0 vs. 27.0 kg), while P50 lambs were intermediate (28.9 kg). Because this baseline imbalance could bias unadjusted comparisons through compensatory growth, final BW and ADG were reanalyzed by ANCOVA, with initial BW as the covariate. After adjustment, treatment effects

on both variables remained highly significant ($p < 0.001$). The covariate was significant for final BW ($p < 0.001$), but not for ADG ($p = 0.442$), confirming that the growth rate advantage in carob-supplemented lambs was not explained by initial weight differences. ANCOVA-adjusted final BW ranked P50 > P25 > control (53.0, 51.1, and 46.2 kg). ANCOVA-adjusted ADGs were 220.8, 203.3, and 160.2 g/d for P50, P25, and control, corresponding to increases of 37.8% and 26.9% relative to control. Likewise, FCR improved from 8.99 in the control to 6.16 and 6.11 in P25 and P50, respectively, with no significant difference between the two carob-supplemented groups ($p < 0.001$).

Table 3. Growth performance and feed conversion ratios of Assaf lambs fed carob pods at two inclusion levels, presented as least squares means from one-way ANOVA (first value per variable) and as ANCOVA-adjusted least squares means with initial body weight as covariate (second value, where applicable).

Parameter	Treatment [1]			SEM	*p*-Value	
	Control	P25	P50		Treatment	Covariate
Initial body weight (kg)	29.0 ^{a}	27.0 ^{b}	28.9 ^{ab}	0.58	0.042	N/A
Final body weight (kg)	46.9 ^{c}	49.9 ^{b}	53.5 ^{a}	0.64	<0.001	<0.001
Final body weight, adjusted (kg)	46.2 ^{c} (0.41)	51.1 ^{b} (0.44)	53.0 ^{a} (0.41)			
Average daily gain (g/d)	160 ^{c}	205 ^{b}	220 ^{a}	3.51	<0.001	0.438
Average daily gain, adjusted (g/d)	160 ^{c} (3.66)	203 ^{b} (3.93)	221 ^{a} (3.62)			
Feed conversion ratio (kg feed/kg gain)	9.02 ^{a}	6.11 ^{b}	6.13 ^{b}	0.177	<0.001	0.571
Feed conversion ratio, adjusted (kg feed/kg gain)	8.99 ^{a} (0.186)	6.16 ^{b} (0.200)	6.11 ^{b} (0.184)			

SEM, standard error of the mean. LS: ANCOVA least squares 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 pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

Table 4 presents the calculated daily nutrient intake of lambs in the different experimental groups during each feeding period. Dry matter intake (DMI) and the intake of individual nutrients increased progressively from 2 to 5 months of age across all treatments. At 2 and 3 months of age, DMI was similar among treatments, whereas the replacement of concentrate with carob pods resulted in a gradual decrease in crude protein intake (CPI) and corresponding increases in neutral detergent fiber intake (NDFI), acid detergent fiber intake (ADFI), acid detergent lignin intake (ADLI), and crude fiber intake (CFI). The same pattern was observed during the subsequent feeding periods (4 and 5 months of age), with nutrient intake increasing as feed allowance increased, while the relative differences among treatments reflected the progressive inclusion of carob pods in the diets. Organic matter intake (OMI) also increased with advancing age and remained generally comparable among treatments within each feeding period.

Table 4. Nutrient feed intake (kg/d/lamb) of Assaf lambs fed carob pods at three inclusion levels and different experimental periods.

Groups	DMI	OMI	CPI	NDFI	ADFI	ADLI	CFI
Period				2 months (0–4 weeks)			
Control	0.821	0.788	0.110	0.284	0.222	0.067	0.183
P25	0.821	0.790	0.094	0.288	0.225	0.073	0.187
P50	0.821	0.791	0.078	0.291	0.227	0.080	0.191
Period				3 months (4–8 weeks)			
Control	1.001	0.961	0.146	0.334	0.259	0.075	0.196
P25	0.997	0.959	0.123	0.338	0.262	0.084	0.201
P50	1.001	0.966	0.101	0.345	0.266	0.094	0.207

Table 4. *Cont.*

Groups	DMI	OMI	CPI	NDFI	ADFI	ADLI	CFI
Period				4 months (8–12 weeks)			
Control	1.184	1.136	0.169	0.399	0.310	0.091	0.240
P25	1.184	1.139	0.143	0.405	0.314	0.101	0.247
P50	1.184	1.142	0.118	0.411	0.318	0.112	0.253
Period				5 months (12–16 weeks)			
Control	1.460	1.400	0.197	0.503	0.393	0.118	0.322
P25	1.460	1.404	0.162	0.511	0.399	0.132	0.331
P50	1.460	1.406	0.140	0.517	0.402	0.142	0.336

DMI: dry matter intake; OMI: organic matter intake; CPI: crude protein intake; NDFI: neutral detergent fiber intake; ADFI: acid detergent fiber intake; ADLI: acid detergent lignin intake; CFI: crude fiber intake.

3.2. Apparent Dry and Organic Matter Digestibility

Digestibility results are presented in Table 5. At 3 months, apparent DM digestibility rose from 63.6% in the control to 71.2% and 71.5% in P25 and P50, respectively (p = 0.0001); the two carob groups did not differ from one another, but both significantly exceeded the control. At 6 months, digestibility increased in a graded fashion, 70.2% (P50) > 65.2% (P25) > 63.6% (control) ($p < 0.0001$), with all three groups differing. Apparent OM digestibility followed the same pattern, 72.6 and 72.9% vs. 64.8% in control at 3 months (p = 0.0001), and 73.3, 68.5, and 66.9% at 6 months ($p < 0.0001$). Notably, digestibility in P25 was lower at 6 months than at 3 months, whereas the control remained stable and P50 increased; this within-treatment decline does not fit a straightforward ruminal adaptation explanation and warrants verification of the digestibility measurements before the data are interpreted further.

Table 5. Apparent digestibility (%) of dry matter (DM) and organic matter (OM) at 3 and 6 months of age in Assaf lambs fed carob pods at two inclusion levels.

Digestibility	Treatment [1]			SEM	*p*-Value
	Control	P25	P50		
DM at 3 months (%)	63.6 ^{b}	71.2 ^{a}	71.5 ^{a}	0.493	0.0001
DM at 6 months (%)	63.6 ^{c}	65.2 ^{b}	70.2 ^{a}	0.698	<0.0001
OM at 3 months (%)	64.8 ^{b}	72.6 ^{a}	72.9 ^{a}	0.449	0.0001
OM at 6 months (%)	66.9 ^{c}	68.5 ^{b}	73.3 ^{a}	0.635	<0.0001

SEM: standard error of the mean; DM: dry matter; OM: organic matter. $^{a–c}$ Within a row: means with different superscripts differ significantly ($p < 0.05$). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

3.3. Carcass Characteristics and Meat Tenderness

Carcass traits are presented in Table 6. CCW differed significantly among treatments ($p < 0.001$); ANCOVA-adjusted means were 20.6, 23.9, and 25.5 kg for control, P25, and P50, respectively (+16.0% and +23.8% vs. control), with P25 and P50 not differing from one another. Dressing percentage also differed significantly (p = 0.002); adjusted means were 43.2, 46.9, and 48.2% for control, P25, and P50, respectively, again with no difference between P25 and P50, indicating that non-carcass components were proportionally smaller in carob-supplemented lambs. Carcass muscle proportion did not differ among treatments (65.7, 66.1, and 65.6% for control, P25, and P50; p = 0.688). Carcass fat proportion differed among treatments (p = 0.039), being higher in P25 (16.2%) than in the control (15.6%); P50 (15.9%) did not differ significantly from either group. Tenderness improved progressively, as reflected by WBSF values of 33.6, 32.0, and 28.6 N for control, P25, and P50, respectively ($p < 0.001$; −4.8% and −14.9% vs. control), indicating improved tenderness at both inclusion levels.

Table 6. Carcass characteristics and Warner–Bratzler shear force (WBSF) of Assaf lambs fed carob pods at two inclusion levels, presented as least squares means from one-way ANOVA (first value per variable), and as ANCOVA-adjusted least squares means with initial body weight as covariate (second value, where applicable).

Parameter	Treatment [1]			SEM	*p*-Value	
	Control	P25	P50		Treatment	Covariate
Cold carcass weight (kg)	20.4 [c]	23.3 [b]	25.8 [a]	0.59	<0.001	0.032
Cold carcass weight (kg)	20.6 [b] (0.56)	23.9 [a] (0.60)	25.5 [a] (0.55)			
Dressing percentage (%)	43.4 [b]	46.7 [a]	48.3 [a]	0.85	0.002	0.679
Dressing percentage (%)	43.2 [b] (0.90)	46.9 [a] (0.97)	48.2 [a] (0.89)			
Carcass bone (%)	18.4	18.5	18.3	0.13	0.665	0.805
Carcass bone (%)	18.4 (0.13)	18.5 (0.14)	18.3 (0.13)			
Carcass muscle (%)	65.7	66.1	65.6	0.46	0.688	0.999
Carcass muscle (%)	65.7 (0.48)	66.1 (0.52)	65.6 (0.48)			
Carcass fat (%)	15.6 [b]	16.2 [a]	15.9 [ab]	0.14	0.039	0.951
Carcass fat (%)	15.6 (0.15)	16.2 (0.16)	15.9 (0.14)			
WBSF (N)	33.6 [a]	32.0 [b]	28.6 [c]	0.23	<0.001	<0.001

CCW: cold carcass weight; WBSF: Warner–Bratzler shear force; SEM: standard error of the mean. Lower WBSF indicates more tender meat. [a–c] Within a row: means with different superscripts differ significantly ($p < 0.05$); rows with no superscripts indicate no significant difference ($p \geq 0.05$). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

3.4. Chemical Composition and Fatty Acid Profile of Longissimus Dorsi Muscle

The chemical composition, fatty acid profile, and selected amino acid concentrations of the *Longissimus dorsi* muscle are presented in Table 7. Muscle moisture content was greater in P50 (69.8%) than in the control (67.9%) and P25 (66.7%), which did not significantly differ from each other ($p < 0.001$). In contrast, crude protein concentration was lower in P50 (15.4%) than in the control (18.4%) and P25 (17.7%; $p < 0.001$). Intramuscular lipid content increased progressively with dietary carob pod inclusion, reaching 10.4% in P25 and 11.2% in P50 compared with 9.6% in the control ($p < 0.001$). Soluble collagen, insoluble collagen, total collagen, and both soluble and insoluble hydroxyproline concentrations were not affected by dietary treatment ($p \geq 0.386$).

Dietary treatment significantly altered the fatty acid composition of the muscle. Stearic acid (C18:0) remained unchanged among treatments ($p = 0.385$). Oleic acid (C18:1) was highest in the control (35.2%), intermediate in P50 (34.6%), and lowest in P25 (33.8%; $p < 0.001$). Linoleic acid (C18:2) followed a similar pattern, decreasing from 10.2% in the control to 9.4% in P50 and 9.1% in P25 ($p < 0.001$). Alpha-linolenic acid (C18:3) showed a tendency to decrease with increasing carob pod inclusion, although the effect did not reach statistical significance ($p = 0.058$). The n-6/n-3 PUFA ratio was lower in P25 (5.85) than in the control (6.45) and P50 (6.25; $p < 0.001$). Total saturated fatty acids were reduced in both carob-fed groups (54.6 to 55.9%) compared with the control (61.4%; $p < 0.001$), whereas monounsaturated fatty acids increased from 31.3% in the control to 37.3% in P25 and 35.4% in P50 ($p < 0.001$). Polyunsaturated fatty acids were greatest in P50 (8.75%), intermediate in P25 (8.13%), and lowest in the control (7.38%; $p = 0.018$).

Among the selected amino acids, leucine concentration was greater in P25 (1.85%) than in the control (1.80%) and P50 (1.78%; $p < 0.001$). Methionine was lower in both carob-supplemented groups (0.52 to 0.53%) than in the control (0.58%; $p < 0.001$). Phenylalanine

was highest in P25 (1.10%), intermediate in P50 (1.06%), and lowest in the control (1.04%; $p < 0.001$). Valine concentration declined progressively with increasing dietary carob pod inclusion, from 1.07% in the control to 1.05% in P25 and 1.03% in P50 ($p < 0.001$).

Table 7. Chemical composition, fatty acid profile (% of total fatty acids), and selected amino acids of *Longissimus dorsi* muscle from Assaf lambs fed carob pods at two inclusion levels.

Parameter	Treatment [1]			SEM	*p*-Value
	Control	P25	P50		
Moisture (%)	67.9 [b]	66.7 [b]	69.8 [a]	0.43	<0.001
Crude protein (%)	18.4 [a]	17.7 [a]	15.4 [b]	0.38	<0.001
Soluble collagen (%)	2.13	2.14	2.05	0.060	0.478
Insoluble collagen (%)	2.35	2.31	2.26	0.050	0.386
Total collagen (%)	4.48	4.45	4.31	0.110	0.446
Lipids (%)	9.6 [c]	10.4 [b]	11.2 [a]	0.21	<0.001
Stearic acid (C18:0, %)	15.7	15.7	15.7	0.014	0.385
Oleic acid (C18:1, %)	35.2 [a]	33.8 [c]	34.6 [b]	0.053	<0.001
Linoleic acid (C18:2, %)	10.2 [a]	9.1 [c]	9.4 [b]	0.041	<0.001
α-Linolenic acid (C18:3, %)	1.58	1.55	1.50	0.021	0.058
n-6/n-3 PUFA ratio	6.45 [a]	5.85 [b]	6.25 [a]	0.070	<0.001
Saturated fatty acids (%)	61.4 [a]	54.6 [b]	55.9 [b]	0.530	<0.001
Monounsaturated fatty acids (%)	31.3 [c]	37.3 [a]	35.4 [b]	0.510	<0.001
Polyunsaturated fatty acids (%)	7.38 [b]	8.13 [ab]	8.75 [a]	0.310	0.018
Leucine (%)	1.80 [b]	1.85 [a]	1.78 [b]	0.010	<0.001
Methionine (%)	0.58 [a]	0.52 [b]	0.53 [b]	0.010	<0.001
Phenylalanine (%)	1.04 [c]	1.10 [a]	1.06 [b]	0.010	<0.001
Valine (%)	1.07 [a]	1.05 [a]	1.03 [b]	0.010	<0.001
Soluble hydroxyproline (%)	0.29	0.29	0.28	0.010	0.478
Insoluble hydroxyproline (%)	0.32	0.31	0.30	0.300	0.386

PUFA: polyunsaturated fatty acids; SEM: standard error of the mean. [a–c] Within a row: means with different superscripts differ significantly ($p < 0.05$); rows with no superscripts indicate no significant difference ($p \geq 0.05$). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

3.5. Meat Color, pH, and Water-Holding Capacity

Meat color, pH decline kinetics, and WHC are presented in Table 8. None of the color parameters (L*, a*, or b*) differed significantly among dietary treatments ($p = 0.793$, 0.872, and 0.848, respectively). Post-slaughter muscle pH levels at 0, 6, and 24 h were unaffected by diet ($p = 0.962$, 0.917, and 0.733). WHC did not differ among treatments ($p = 0.171$). Carob pod inclusion at either level therefore had no detectable effect on meat color, pH trajectory, or water retention capacity.

Table 8. Meat color, post-slaughter pH, and water-holding capacity (WHC) of Longissimus dorsi from Assaf lambs fed carob pods.

Trait	Treatment [1]			SEM	*p*-Value
	Control	P25	P50		
L* (lightness)	44.6	44.6	44.9	0.290	0.793
a* (redness)	22.7	22.9	22.7	0.310	0.872
b* (yellowness)	3.98	3.87	3.96	0.140	0.848
pH at 0 h	6.98	6.92	6.92	0.190	0.962
pH at 6 h	6.35	6.30	6.35	0.100	0.917
pH at 24 h	5.91	5.86	5.95	0.080	0.733
WHC (%)	90.9	89.5	90.5	0.520	0.171

SEM: standard error of the mean; WHC: water-holding capacity. No trait differed significantly among treatments ($p \geq 0.05$). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

3.6. Serum Biochemical Parameters

Serum biochemical data (end-of-trial treatment least squares means from the repeated-measures analysis) are in Table 9. All six parameters were significantly affected by treatment

($p < 0.001$). Serum total protein was highest in P25 (7.24 g/dL) and lowest in the control (6.23 g/dL; +16.2% in P25 vs. control). Albumin declined progressively with carob inclusion: 3.59, 3.26, and 2.90 g/dL for control, P25, and P50. Compared with the control, BUN was significantly lower in both carob groups (33.8 and 32.3 mg/dL for P25 and P50 vs. 38.5 mg/dL; −12.2% and −16.1%). By contrast, HDL was highest in P25 (24.2 mg/dL) and did not differ between the control (23.0) and P50 (22.6). In parallel, LDL declined progressively: 27.7, 25.5, and 21.7 mg/dL for control, P25, and P50, respectively (−7.9% and −21.7%). Similarly, GOT was significantly lower in carob groups (71.5 and 69.9 U/L for P25 and P50 vs. 80.9 U/L in the control; −11.6% and −13.6%).

Table 9. Serum biochemical parameters of Assaf lambs fed carob pods, analyzed as repeated measures over four monthly samplings (end-of-trial treatment least squares means shown).

Parameter	Treatment [1]			SEM	*p*-Value
	Control	P25	P50		
Total protein (g/dL)	6.23 [c]	7.24 [a]	6.57 [b]	0.022	<0.001
Albumin (g/dL)	3.59 [a]	3.26 [b]	2.90 [c]	0.053	<0.001
Blood urea nitrogen (mg/dL)	38.5 [a]	33.8 [b]	32.3 [c]	0.21	<0.001
High-density lipoprotein (mg/dL)	23.0 [b]	24.2 [a]	22.6 [b]	0.16	<0.001
Low-density lipoprotein (mg/dL)	27.7 [a]	25.5 [b]	21.7 [c]	0.14	<0.001
Glutamic-oxaloacetic transaminase (U/L)	80.9 [a]	71.5 [b]	69.9 [c]	0.13	<0.001

BUN: blood urea nitrogen; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GOT: glutamate oxaloacetate transaminase; SEM: standard error of the mean. [a–c] Within a row: means with different superscripts differ significantly ($p < 0.05$). Data were analyzed by a mixed model (treatment, time, treatment × time; animal as a random repeated subject). [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

3.7. Rumen pH and Microbial Populations

Rumen pH and microbial counts (repeated-measures treatment means) are presented in Table 10. Rumen pH was highest in P25 (6.40), followed by P50 (6.13) and the control (5.98; $p < 0.001$). Total anaerobic bacteria were highest in P50 (9.90 log10 CFU/mL), then P25 (9.86) and control (9.78; $p < 0.001$). Starch-degrading bacteria were higher in P25 and P50 (6.63 and 6.65 log10 CFU/mL) than in the control (6.08; $p < 0.001$). In a comparable pattern, LAB counts were highest in P50 (6.85), intermediate in P25 (6.80), and lowest in the control (5.92 log10 CFU/mL; $p < 0.001$; +14.9% and +15.7% vs. control). Protozoa counts were highest in the control (5.67) and significantly lower in P25 (5.55; −2.1%) and P50 (5.62; −0.9%; $p < 0.001$). These results should be interpreted with caution because rumen fluid was collected at a single post-prandial timepoint by esophageal tube, and VFA, lactate, ammonia-N, and buffering capacity were not measured.

Table 10. Rumen pH and microbial populations (log10 CFU or cells/mL) of Assaf lambs fed carob pods, analyzed as repeated measures.

Parameter	Treatment [1]			SEM	*p*-Value
	Control	P25	P50		
pH	5.98 [b]	6.40 [a]	6.13 [b]	0.059	<0.001
Total bacteria	9.78 [c]	9.86 [b]	9.90 [a]	0.005	<0.001
Starch-degrading bacteria	6.09 [b]	6.63 [a]	6.65 [a]	0.013	<0.001
Lactic acid bacteria	5.92 [c]	6.80 [b]	6.85 [a]	0.007	<0.001
Protozoa	5.67 [a]	5.56 [c]	5.62 [b]	0.009	<0.001

SEM: standard error of the mean; CFU: colony-forming units. [a–c] Within a row: means with different superscripts differ significantly ($p < 0.05$). Rumen fluid collected at a single post-prandial timepoint by esophageal tube; VFA, lactate, ammonia-N, and buffering capacity were not measured. [1] Control: diets with no carob pods; P25: diets with carob pods replacing concentrate feed mixture at 25%; P50: diets with carob pods replacing concentrate feed mixture at 50%.

4. Discussion

4.1. Growth Performance, Dry Matter Intake, and Feed Conversion

Despite random allocation of animals to treatments, initial BW differed significantly among groups, indicating that baseline balance was not fully achieved by randomization alone. This necessitated statistical adjustment using ANCOVA, as described below. A key methodological consideration in this study is the significant difference in initial BW among groups. The control lambs were heavier than P25 lambs at the start of the trial (29.0 vs. 27.0 kg), while P50 was intermediate (28.9 kg). Such imbalances can distort growth comparisons through compensatory growth: lighter animals may gain weight at proportionally higher rates, simply because they have more tissue growth potential relative to their current size [23]. In the present study, ANCOVA was applied with initial BW as a covariate to mathematically remove its confounding influence before comparing treatment effects on final BW and ADG. The homogeneity-of-regression-slopes assumption was satisfied, confirming that the relationship between initial and final BW was parallel across treatments, a prerequisite for valid ANCOVA. Crucially, the initial BW covariate was significant for final BW, but not for ADG. The non-significance of the covariate for ADG means that, after accounting for baseline weight, the initial BW of an individual lamb did not predict its rate of gain; therefore, the growth rate advantage of carob-supplemented lambs reflects a true dietary treatment effect, rather than a regression artifact. This result is consistent with findings from a companion study using carob leaves and twigs in Assaf lambs [45], where initial BW also differed among groups, and ANCOVA similarly confirmed that adjusted growth differences were attributable to the dietary treatment, rather than baseline weight. The consistency of growth advantages across adjusted final BW, ADG, and FCR across both studies strengthens the conclusion that carob inclusion confers genuine productive benefits in this breed under these conditions.

It must nevertheless be acknowledged that the diets were not isonitrogenous or isoenergetic: dietary CP fell from approximately 13.2% in the control to 8.9% in P50 as carob pods replaced concentrate. The growth advantages in carob-supplemented lambs must therefore be interpreted as the net result of carob pod inclusion, combined with a reduced CP and energy supply from concentrate, rather than as an effect of carob pods alone. Because VFA, protein digestibility, and energy balance were not directly measured, it is not possible to partition the contributions of individual dietary changes to the observed growth outcomes. This limitation is acknowledged throughout the discussion and in the conclusions.

After ANCOVA adjustment, ADG in P50 (220.8 g/d) and P25 (203.3 g/d) substantially exceeded the control (160.2 g/d; +37.8% and +26.9%, respectively). These improvements are larger than those reported for lower inclusion levels of carob pulp in Moroccan Deroua lambs [22], and substantially exceed the 5-15% improvements typically associated with 10-20% inclusion of carob by-products reviewed by Bottegal et al. [21]. The greater magnitude observed here may reflect the higher inclusion levels (up to 50% of concentrate DM), the longer trial duration (16 weeks), and the replacement of part of the starch-rich concentrate with carob pods, which provide readily fermentable sugars and condensed tannins that can modify ruminal fermentation and improve nitrogen utilization, thereby enhancing the efficiency of nutrient use [46,47].

The soluble carbohydrate fraction of carob pods provides rapidly fermentable energy for rumen microbes, stimulating microbial protein synthesis and supporting energy–nitrogen synchrony in the rumen [11,13]. The concurrent reduction in BUN in P25 and P50 (from 38.5 to 33.8 and 32.3 mg/dL) is consistent with CT-mediated protection of dietary protein from ruminal degradation [14–16], which would increase the supply of amino acids at the intestinal level and support tissue deposition. However, because tannin activity was

not directly measured, and dietary CP also declined, both mechanisms are plausible, and their relative contributions cannot be separated from the present data. The improvement in FCR (8.99 to 6.11 kg DM per kg gain) is consistent with findings from Bampidis and Robinson [48] for citrus by-products, which share with carob pods the combination of soluble carbohydrates and polyphenols. The observation that DMI expressed as a proportion of BW was unchanged (2.71–2.76%) confirms that the higher absolute DMI in carob groups was driven by their greater body mass, rather than by heightened appetite, suggesting that the productive response was mediated by improved nutrient utilization efficiency, rather than simply by increased feed consumption.

4.2. Apparent Dry and Organic Matter Digestibility

Apparent DM and OM digestibility were consistently higher in carob-supplemented lambs at both digestibility periods, with a noteworthy pattern: at 3 months, P25 and P50 did not differ from each other, but both exceeded the control, whereas, at 6 months, all three treatments were statistically distinct, and the advantage of P50 over P25 had widened. The greater divergence between treatment groups at 6 months may reflect age-related maturation of the digestive system and/or gradual adaptation of the rumen microbial community to the polyphenol-rich substrate, although these mechanisms were not directly evaluated in the present study [37,49,50]. As the rumen microbiome matures and adapts, its capacity to degrade both soluble and structural fractions of carob pods likely improves, yielding incrementally higher digestibility in P50 lambs, who received a greater total polyphenol and soluble carbohydrate load.

Multiple non-exclusive mechanisms may underlie the digestibility improvements. The soluble sugars in carob pods stimulate microbial activity and improve energy–nitrogen synchrony, which enhances microbial efficiency [10,13]. Moderate CT concentrations can reshape the microbial community by reducing protozoa (which engulf and lyse bacteria, representing a net loss of microbial nitrogen) and shifting the bacterial community toward more efficient fibrolytic and proteolytic populations [15,41,51]. The progressive replacement of concentrate with carob pods did not reduce the dietary lignin fraction, because carob pods contained a higher acid detergent lignin content (ADL; 9.5% DM) than the concentrate mixture (ADL; 4.2% DM) they replaced. Therefore, the improvement in apparent digestibility may instead be associated with differences in carbohydrate composition and fermentability between carob pods and the concentrate ingredients, along with possible effects of carob pod bioactive compounds on ruminal fermentation processes. Because only DM and OM digestibility were measured, these results cannot be extrapolated to fiber-specific (NDF, ADF) or protein digestibility without further analysis, which is acknowledged as a limitation.

4.3. Carcass Traits and Meat Tenderness

The improvement in CCW in carob-supplemented lambs is a direct downstream consequence of improved growth performance and feed efficiency. Greater live weight gain at slaughter produces heavier carcasses, and the proportional increase in carcass fat proportion in P25 (16.2 vs. 15.6% in the control; +3.8%) indicates that additional nutrients were partitioned toward fat deposition, consistent with a state of positive energy balance; carcass muscle proportion, by contrast, did not differ among treatments (65.7, 66.1, and 65.6% for control, P25, and P50), indicating that the additional carcass weight reflected proportional growth of all tissue components, rather than a shift in tissue partitioning. This pattern of carcass weight gain without a corresponding change in muscle proportion has been observed in lambs supplemented with Mediterranean browse and fibrous by-products at moderate dietary levels, where improved energy extraction from fermentable carbohy-

drates supported tissue deposition [21,50]. The significant increase in dressing percentage with carob inclusion indicates that non-carcass components (gastrointestinal tract fill, hide, head, and feet) were proportionally smaller in carob-supplemented lambs, in contrast to some previous carob pod studies, in which dressing percentage was unaffected [22].

The progressive decline in WBSF from 33.6 N in the control to 28.6 N in P50 (−14.9%) indicates a practically meaningful improvement in meat tenderness. All three groups remained below the 40 N threshold commonly associated with consumer-acceptable tenderness [34], but the difference between P50 and the control is commercially significant. Two mechanisms most plausibly account for this improvement. First, the higher intramuscular lipid content in P50 (11.2% vs. 9.6% in the control) lubricates myofibrillar structures, reducing the resistance to shearing [24,26]. Second, because stearic acid did not differ among treatments, while oleic and linoleic acid were lower in carob-supplemented lambs, the fatty acid shift does not correspond to a simple decrease in SFA or increase in MUFA; any contribution of altered fat composition, as distinct from the increase in total intramuscular lipid content, to the physical properties of the fat and to shear force therefore remains uncertain and should not be attributed to an SFA-to-MUFA shift [52]. Polyphenolic compounds in carob pods could be associated with improved post mortem muscle antioxidant status, which has been linked in other studies with reduced oxidative inactivation of the calpain proteolytic system and, thereby, with sustained tenderization [30,34]. However, because antioxidant status, TBARS, calpain activity, and post mortem proteolysis were not measured in the present study, this antioxidant mechanism remains speculative.

4.4. Muscle Composition and Fatty Acid Profile

The decline in muscle CP from 18.4% in the control to 15.4% in P50 is most parsimoniously explained by a combination of dietary CP dilution and intramuscular fat accretion. As carob pods replaced concentrate, dietary CP decreased (the concentrate supplied 18.0% CP vs. 5.24% for carob pods), providing less substrate for muscle protein synthesis in P50. Simultaneously, the greater intramuscular lipid content in P50 dilutes the relative proportion of protein within the muscle [53,54]. The maintenance of satisfactory growth performance despite these changes suggests that the efficiency of dietary protein utilization improved, consistent with the BUN data, but the CP concentration in P50 muscle (15.4%) is at the lower end of the normal range for lamb and warrants consideration when evaluating the nutritional quality of the product.

The modification of the fatty acid profile is a notable outcome of the study, although the direction of change does not match the pattern classically attributed to CT-mediated inhibition of ruminal biohydrogenation. Condensed tannins are generally reported to suppress the ruminal bacteria responsible for converting unsaturated fatty acids to stearic acid (C18:0), which would be expected to increase the outflow of unsaturated intermediates to the small intestine and their incorporation into muscle lipid [27–29,55]. In the present study, however, stearic acid did not differ among treatments, and both oleic acid and linoleic acid were lower in the carob-supplemented groups than in the control, a pattern that does not support enhanced ruminal escape of unsaturated fatty acids. Because rumen biohydrogenation intermediates were not measured, this discrepancy cannot be resolved from the present data, and mechanisms other than, or in addition to, CT-mediated inhibition of biohydrogenation, such as diet-related changes in the fatty acid substrate reaching the rumen, more plausibly account for the observed profile; it cannot be concluded that carob pod inclusion improved the fatty acid profile through reduced ruminal biohydrogenation. The non-significant reduction in C18:3n-3 and the significant reduction in the n-6/n-3 ratio in P25 (5.85 vs. 6.45 in control) nevertheless suggest a modest improvement in the omega-3 fatty acid status of P25 meat, beneficial for consumer health [24].

Regarding amino acids, the significant reduction in methionine in P25 and P50 relative to the control, and reduction in valine in P50, may reflect lower supply of sulfur-containing amino acids consequent upon reduced dietary CP and possible tannin–protein binding reducing the post-ruminal availability of specific amino acids. The increase in phenylalanine in P25 likely reflects shifts in microbial protein amino acid profile, rather than a specific dietary effect. The absence of significant differences in total collagen and collagen fractions confirms that intramuscular connective tissue structure was not compromised by carob inclusion, consistent with similar slaughter age and physiological maturity across groups.

4.5. Serum Biochemical Parameters

The reduction in serum BUN from 38.5 mg/dL in the control to 32.3 mg/dL in P50 (−16.1%) is a hallmark biochemical response to diets containing CT, reflecting reduced ammonia production in the rumen and decreased hepatic urea synthesis [14–18]. When CT forms complexes with dietary protein, it slows or prevents microbial proteolysis in the rumen, reducing the rate of ammonia release and absorption across the rumen wall. This CT-mediated nitrogen-sparing is generally interpreted as an improvement in nitrogen use efficiency, rather than as a sign of protein deficiency, particularly when growth performance is simultaneously maintained or improved, as in the present study. However, the lower dietary CP in P50 independently reduces the pool of ruminally degradable protein available for ammonia production, so both the tannin effect and the reduced dietary CP likely contribute to the lower BUN in P50 relative to P25, and their contributions cannot be partitioned from the present data.

The elevation of total serum protein in P25 (+16.2% vs. control), combined with declining albumin, indicates an expansion of the globulin fraction at moderate carob inclusion. The globulin increase may reflect enhanced immune activity or acute-phase protein responses. The progressive decline in albumin with increasing carob inclusion (3.59 to 2.90 g/dL) remains within the reference range for sheep, but approaches its lower limit at P50, suggesting that very high inclusion levels could eventually compromise albumin-dependent oncotic pressure and nutrient transport. This trend is most likely related to reduced methionine availability (methionine is a precursor for hepatic albumin synthesis), and possibly to tannin-induced reduction in the bioavailability of sulfur amino acids [14,56].

The progressive decline in LDL (from 27.7 to 21.7 mg/dL in P50; −21.7%) and the improvement in HDL in P25 (+5.2%) are consistent with a favorable modification of lipid metabolism. Polyphenolic constituents of carob pods have been associated, in other studies, with inhibition of intestinal cholesterol absorption and upregulation of hepatic LDL receptor expression; however, as hepatic function, intestinal cholesterol transport, and LDL receptor activity were not directly assessed in the present study, these mechanisms remain possible but unconfirmed explanations [10,11]. The reduction in GOT (from 80.9 to 69.9 U/L in P50; −13.6%) indicates reduced hepatocellular stress, consistent with a lower hepatic ammonia detoxification burden (supported by lower BUN), improved antioxidant protection from carob polyphenols, or reduced lipid peroxidation-related damage to hepatocyte membranes [30].

4.6. Rumen pH and Microbial Ecology

The rumen pH of 5.98 in the control, measured at a single post-prandial timepoint, is close to the conventional threshold of 6.0 associated with reduced fibrolytic activity [57]. However, a single esophageal tube sample cannot confirm sub-acute ruminal acidosis (SARA), and no control lambs showed clinical signs of acidosis or reduced intake throughout the trial. The reduction in concentrate (the primary source of rapidly fermentable starch) in carob-supplemented diets is the most plausible explanation for the higher post-feeding

pH in P25 (6.40) and P50 (6.13): as starch supply decreased, the rate of volatile acid production in the immediate post-feeding period was reduced, allowing rumen pH to recover more rapidly and to a higher equilibrium level [58]. The intermediate pH in P50 (6.13), despite containing more carob and less starch than P25, may reflect the higher absolute DMI in P50 generating a larger total fermentable substrate load per unit time, partially offsetting the starch-reduction benefit [57]. The absence of VFA, Lactate, ammonia-N, and buffering capacity measurements prevents fuller mechanistic interpretation, which is a recognized limitation.

The reduction in protozoa in carob-supplemented groups (from 5.67 to 5.55 log10 cells/mL in P25) is consistent with well-documented CT-mediated partial defaunation. Condensed tannins, particularly those from leguminous sources, suppress protozoal populations through direct toxicity, or through the formation of tannin–protein complexes that reduce the protein substrate available for protozoa [41,51]. Partial defaunation improves nitrogen use efficiency because protozoa engulf and lyse a substantial proportion of rumen bacteria, representing a significant net loss of microbial nitrogen that would otherwise be digested post-ruminally [15]. The concurrent increases in total bacteria, starch-degrading bacteria, and LAB populations in carob groups reflect the greater availability of fermentable substrates from carob soluble carbohydrates, which selectively support rapid-fermenting bacterial populations [13,59]. Higher LAB counts may also reflect the prebiotic-like properties of carob polyphenols, which selectively promote beneficial fermentative bacteria while inhibiting certain pathogens, consistent with patterns reported for other polyphenol-rich dietary supplements [14,46]. These rumen microbiology results should be interpreted as indicative, rather than definitive, given the single-timepoint, culture-based sampling approach. Future studies should incorporate molecular profiling (e.g., 16S rRNA amplicon sequencing) and multi-timepoint rumen cannula sampling to provide a mechanistically comprehensive characterization of the rumen microbial response to carob supplementation.

4.7. Limitations

Several limitations of the present study should be considered when interpreting the results. First, and most importantly, the diets were neither isonitrogenous nor isoenergetic; as carob pods replaced concentrate, dietary CP fell from approximately 13.2% in the control to 8.9% in P50, and energy density changed in parallel. All reported differences therefore reflect the combined effect of increasing carob pods and decreasing concentrate supply, rather than the effect of carob pods per se. Future studies comparing carob pods against a suitable control with matched CP and energy, or incorporating a PEG (polyethylene glycol) group to neutralize tannin activity, would allow cleaner attribution of effects. Second, rumen assessment was limited to pH and culture-based microbial counts from a single esophageal tube sample per occasion; VFA, ammonia-N, lactate, methane, and buffering capacity were not measured, and molecular microbiome profiling was not conducted. Third, the digestibility trials were limited to DM and OM; NDF, ADF, and CP digestibility levels were not determined. Fourth, antioxidant status, TBARS, post mortem proteolytic activity, and calpain activity in muscle were not measured, limiting mechanistic interpretation of the tenderness results. Fifth, systemic antioxidant capacity and inflammatory markers were not assessed in the lambs; consequently, although no clinical signs of illness or reduced intake were observed at either inclusion level, the present data cannot establish that high-dose carob pod supplementation is entirely free of subclinical antioxidant or inflammatory effects, and this question warrants dedicated evaluation in future work. These gaps represent important priorities for future research.

5. Conclusions

Partial replacement of concentrate feed with sun-dried carob pods at 25% (P25) or 50% (P50) of concentrate DM improved ANCOVA-adjusted ADG by 26.9% and 37.8%, and reduced FCR from 8.99 to 6.16 and 6.11 kg DM/kg gain, respectively, in growing Assaf lambs over a duration of 16 weeks. Initial BW was included as a covariate to account for pre-trial weight differences, and the growth advantage remained after adjustment. Apparent DM and OM digestibility increased at both 3 and 6 months, while carcass weight and fat proportion increased and WBSF declined with carob inclusion. Serum BUN, LDL, and GOT decreased, whereas rumen pH, total bacteria, and LAB counts increased, suggesting potential changes in nitrogen metabolism, lipid metabolism, and ruminal microbial ecology. However, condensed tannin-mediated nitrogen sparing, polyphenol-related hepatic effects, nitrogen balance, and liver function were not directly assessed and require further investigation. Because carob pods replaced concentrate, rather than being included in an isonitrogenous and isoenergetic diet, the observed responses represent the combined effects of carob pod inclusion, altered dietary CP, and energy density. P25 appears to be a practical inclusion level for Assaf lamb production because it improved growth performance and feed efficiency, without the reductions in muscle CP and serum albumin observed at P50. The effect of additional protein supplementation in high carob pod diets remains unknown and should be evaluated in future studies using balanced diets, integrating rumen fermentation and microbiome analyses.

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Abbreviations

The following abbreviations are used in this manuscript:

ADF	Acid detergent fiber
ADFI	Acid detergent fiber intake
ADLI	Acid detergent lignin intake
ADG	Average daily gain
ADL	Acid detergent lignin
AIA	Acid-insoluble ash
BUN	Blood urea nitrogen
BW	Body weight
CCW	Cold carcass weight
CFI	Crude fiber intake
CP	Crude protein
CPI	Crude protein intake
CT	Condensed tannins
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FBW	Final body weight
FCR	Feed conversion ratio
GAE	Gallic acid equivalents
GOT	Glutamate oxaloacetate transaminase
HCW	Hot carcass weight
HDL	High-density lipoprotein
LAB	Lactic acid bacteria
LD	Longissimus dorsi
LDL	Low-density lipoprotein
MRS	de Man, Rogosa, and Sharpe (medium)
MUFA	Monounsaturated fatty acid
NDF	Neutral detergent fiber
NDFI	Neutral detergent fiber intake
OM	Organic matter
OMI	Organic matter intake
PEG	Polyethylene glycol
PUFA	Polyunsaturated fatty acids
SARA	Sub-acute ruminal acidosis
SFA	Saturated fatty acids
VFA	Volatile fatty acids
WBSF	Warner-Bratzler shear force
WHC	Water-holding capacity

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