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Productive Performance, Carcass Traits, and Meat Quality in Finishing Lambs Supplemented with a Polyherbal Mixture

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1], Rufino López-Ordaz [1] and Pedro Abel Hernández-García [3]

1 Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo CP 56230, Mexico; al19132949@chapingo.mx (J.F.O.-O.); al20130495@chapingo.mx (G.D.-I.); lmirandar@chapingo.mx (L.A.M.-R.); rlopezo@chapingo.mx (R.L.-O.)
2 Departamento de Producción Agrícola Y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, Mexico City CP 04960, Mexico; gmendoza@correo.xoc.uam.mx
3 Centro Universitario UAEM Amecameca, Universidad Autónoma Del Estado De México, Amecameca CP 56900, Mexico; pahernandezg@uaemex.mx
* Correspondence: alarab@chapingo.mx

Abstract: The objective of this study was to evaluate the effects of dietary supplementation of a polyherbal mixture (HM) containing saponins, flavonoids, and polysaccharides on productive performance, carcass characteristics and meat quality of lambs during the final fattening period. Thirty-six Dorper × Katahdin lambs (23.27 ± 1.23 kg body weight (BW)) were housed in individual pens and were assigned to four treatments (n = 9) with different doses of HM: 0 (CON), 1 (HM1), 2 (HM2) and 3 (HM3) g of HM kg^{-1} of DM for 56 days. Data were analysed as a completely randomized design using the MIXED and GLM procedures of statistical analysis system (SAS), and linear and quadratic effects were tested to evaluate the effects of the HM level. DM digestibility decreased in lambs fed HM3 ($p < 0.05$). There was no effect of HM on daily weight gain, dry matter intake, final BW, feed conversion, carcass characteristics, colour (L* and a*) and meat chemical composition. Meat pH, cooking loss and drip loss increased linearly ($p < 0.05$) when the HM dose was increased. The Warner-Bratzler shear force (WBSF) of meat was lower ($p < 0.05$) in lambs fed HM3. In conclusion, dietary inclusion of 3 g HM kg^{1} of DM improves meat tenderness. However, high doses of HM in the diet may decrease the digestibility of DM and increase the cooking loss and drip loss of lamb meat during the final fattening period.

Keywords: fattening lamb; saponins; bioactive compounds; mutton tenderness

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1. Introduction

Antibiotics have been commonly used as growth promoters in animals. However, the emergence of bacteria resistant to these drugs has led to the search for alternative products with similar effects to antibiotics, but of natural origin [1]. Dietary supplementation with herbal products seems to be a promising strategy to improve the productive performance, carcass characteristics and meat quality of small ruminants [2]. Some polyherbal mixtures (HM) prepared with medicinal plants have shown positive effects on productive performance, meat and carcass quality characteristics of steers and lambs during the final fattening period [3–5]. On the other hand, in calves, it has been reported that the use of HM can improve growth and health status during the pre-ruminant period until weaning by modifying gene expression [6]. However, the effects of bioactive compounds (for example, saponins and flavonoids) of HM in biological systems, may depend on the efficiency of their absorption and extensive metabolic transformation [7].

Previous studies [8,9] have shown that some plants containing saponins, flavonoids and polysaccharides can improve antioxidant status, ruminal fermentation, immune response and productive performance in sheep. Likewise, some HM containing saponins,

flavonoids and tannins have been shown to have a positive impact on nutrient utilization efficiency in goats [10]. Other products containing saponins have shown positive impact on energy metabolism and on the duodenal flux of amino acids [11], ruminal fermentation rate [12,13], rumen microbial populations [14], and production of volatile fatty acids [12–14]. Similarly, flavonoids can modulate the ruminal microbiome, improve rumen fermentation and metabolic status to improve the productive performance and health of ruminants [15]. Some HM containing flavonoids have shown positive impact on antioxidant status [7], and ruminal microbial populations of lambs [16]. In addition, flavonoid supplementation modifies the expression of genes in the rumen epithelium that could be related to inflammation and animal behaviour modulation [17].

Some plant parts containing saponins have also been used to improve the meat quality of adult goats and kids [18,19]. However, there is limited information on the effects of plants or HM containing saponins, flavonoids, and polysaccharides on the productivity, carcass characteristics, and meat quality of lambs. The botanical origin, the dose, and the composition of the diet used can influence the biological response that saponins have on ruminants [20]. Although, the effects of using saponins in ruminant feed have been investigated in animals fed diets containing a high proportion of forage [11,13]; information on the effects of these bioactive metabolites in ruminants fed high concentrate diet is limited and inconsistent [19,21]. Some saponin extracts improve ruminal fermentation and increase the efficiency of energy use in the animals, which could result in better productive performance [11]. However, the effects of saponins on ruminal fermentation may differ depending on the ruminal pH [22], which varies according to the dietary level of concentrate. Due to the beneficial effects of herbal products and their secondary metabolites, it has been hypothesized that supplementation with HM as a source of saponins, flavonoids and polysaccharides can contribute to improving the productivity of the lambs during the final fattening period, without affecting the quality of the meat or the characteristics of the carcass. The objective of this study was to evaluate the effects of increasing doses of an HM containing saponins, flavonoids, and polysaccharides on the productive performance, carcass characteristics, and meat quality of lambs fed high-concentrate diets.

2. Materials and Methods

2.1. Location

The experiment was conducted at the Teaching and Research Unit of Small Ruminants located at the Experimental Farm of the Universidad Autónoma Chapingo, Mexico, which is located at 19°22′ north latitude and 98°35′ west longitude, with an altitude of 2250 m. The climate is temperate subhumid, with rain during the summer and dry during the winter, with average annual precipitation and temperatures of 665 mm and 15.2 °C, respectively [23]. The study was conducted during the summer, under hot and rainy conditions. The care and handling procedures for the lambs were carried out following the guidelines of the Official Mexican Standard (NOM-062-ZOO-1995).

2.2. Polyherbal Mixture Characteristics

The HM used was Peptasan® (Nuproxa S. de RL. de CV. Querétaro, México), which is a commercial polyherbal formula labelled to contain 150 g kg^{-1} of saponins. In addition, Peptasan® is composed of parts from the *Saccharum officinarum*, *Balanites roxburghi* and *Acacia concinna* plants. *S. officinarum* contains polysaccharides with immunostimulating effects [24]; *B. roxburghii* contains saponins and flavonoids with antioxidant, anti-inflammatory, antimicrobial and antiviral properties [25]; and *A. concinna* contains saponins with immunomodulatory properties [26].

2.3. Diet Composition

HM was fed to the lambs through diets formulated to have weight gains of 300 g d^{-1} [27]. HM (1, 2 or 3 g kg^{-1} of diet DM basis) was premixed with minor ingredients (vitamin and mineral supplement, limestone and salt) before incorporation into complete mixed

diets. The lambs were fed a finishing diet (total mixed ration) comprised 30.3% ground corn, 24.1% ground sorghum, 8.1% soybean meal, 7.1% wheat bran, 7.4% corn gluten, 2.3% bypass fat, 19.4% oat straw, 0.5% vitamin and mineral supplement, 0.5% salt, and 0.3% limestone (DM basis). Oat straw was ground in a hammer mill (Azteca 20, Molinos Azteca, Guadalajara, México) with a 3.8 cm screen before incorporation into total mixed ration. The nutrient composition of the basal diet was 15.53% crude protein, 2.58% ether extract, 13.57% acid detergent fiber, 26.14% neutral detergent fiber, 5.47% ash and 2.8 Mcal of metabolizable energy according to NRC [27] DM basis.

2.4. Animals and Experimental Design

Thirty-six male Dorper × Katahdin lambs (23.27 ± 1.23 kg BW, 4–5 months old) were randomly distributed in four treatments: (1) basal diet without HM (CON); (2) HM1, CON + 1 g of HM kg^{-1} dry matter (DM); (3) HM2, CON + 2 g of HM kg^{-1} DM; and (4) HM3, CON + 3 g of HM kg^{-1} DM. The lambs were placed in individual pens (2.6 m × 0.8 m) equipped with automatic drinkers and individual feeders. Prior to the start of the experimental phase, lambs were vaccinated against *Clostridium* and *Pasteurella* (2.5 mL $lamb^{-1}$, Bobact® 8 MSD-Merck, Kenilworth, NJ, USA), and dewormed through an oral administration of Koptisin ovine® (10 mg kg^{-1} BW, Chinoin, Labs, Mexico City, Mexico). Additionally, 1 mL $lamb^{-1}$ of vitamins containing 500,000 IU of vitamin A, 75,500 IU of vitamin D and 50 mg of vitamin E (Vigantol® Bayer, Mexico City, Mexico) was provided on day 1 of the adaptation period. The lambs had an adaptation period to the basal diet of 14 days, and the experimental phase lasted 56 days. During the adaptation period, the lambs received oat straw as a ruminal pH buffer, and the experimental diets were administered at increasing levels (20, 40, 60, 80 and 100% of the total ration) for 14 days (3 days per level, except for 100%), until the oat straw was reduced to 0%. The feed was provided at 09:00 and 17:00 h, and the drinking water was supplied ad libitum. Individual BW was recorded before the morning feeding on days 1, 14, 28, 42 and 56, of the experimental phase. The amount of diet offered and refused was recorded daily to estimate dry matter intake (DMI, kg d^{-1}). The amount of feed offered was always 10% higher than the previous intake to ensure ad libitum intake. Daily weight gain (DWG, kg d^{-1}) was calculated between feeding period intervals. The feed conversion ratio (FCR) was expressed as feed consumption per unit of body weight gain. Figure 1 shows the experimental procedure.

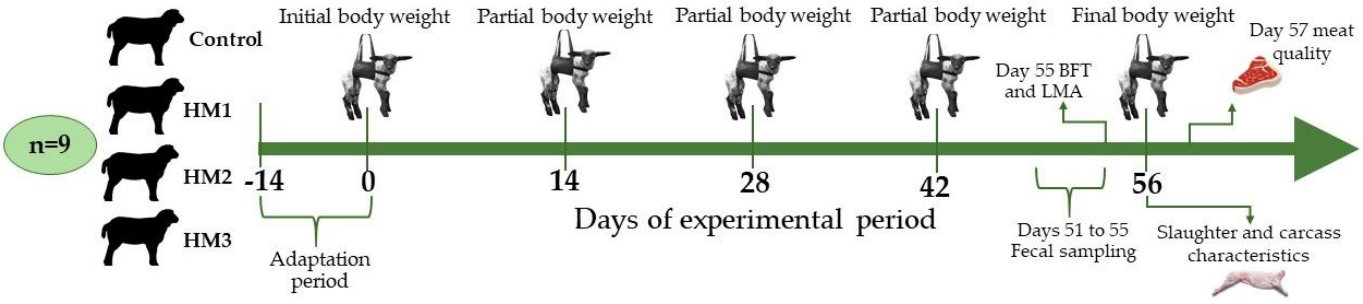

Figure 1. Completely randomized design and sampling times of lambs supplemented with a polyherbal mixture (HM) during the final fattening period; *n* = 9—indicate the number of animals sampled in each treatment; Control—basal diet without HM; HM1—basal diet + 1 g of HM kg^{-1} of dry matter (DM); HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; BFT—backfat thickness; LMA—*longissimus* muscle area.

2.5. Sampling and Analyses of Feeds

Samples of feed provided and rejected were collected daily to determine the chemical composition. Prior to the analysis, the food samples were dried at 55 °C in a forced air oven and then ground in a Wiley mill (model 4, Arthur Thomas Co. Philadelphia, PA, USA). The variables determined were dry matter, crude protein, ether extract and ash [28]. Acid

detergent fibre and neutral detergent fibre was determined using the procedures described by Van Soest et al. [29].

2.6. Apparent Dry Matter Digestibility

Faecal samples were collected from each animal during five consecutive days (in the morning at 08:00 a.m. and in the afternoon at 16:00 p.m. before feed delivery) starting on day 51, directly from the rectum [5]. Feed and orts were collected daily during the same period. Acid-insoluble ash was employed as a marker of internal tract digestibility to analyse the apparent total tract DM digestibility [30].

2.7. Carcass Characteristics

The *longissimus* muscle area (LMA) and the backfat thickness (BFT) located between the 12th and 13th ribs of the lamb were measured on day 55 of the experiment using a Sonovet 600 (Medison, Inc., Cypress, CA, USA) with a 7.5 Mhz transducer [31]. After the last weighing (day 56 of the experiment) the lambs were fasted for 18 h before being slaughtered. All lambs were slaughtered on the same day. The slaughter process was conducted in a commercial slaughterhouse in accordance with standard procedures of the Official Mexican Standard (NOM-033-SAG/ZOO-2014). Lambs were stunned (captive bolt), exsanguinated and skinned. Immediately after the slaughter, the hot carcass weight was registered (HCW). The hot carcass yield (HCY) was determined through HCY = (HCW/FBW) $*$ 100, as it was described by Zimerman et al. [32]. In addition, the skin, head, legs, testicles, rumen (empty), liver, spleen, kidneys, heart, lungs, small intestine (empty), and large intestine (empty) were each weighed separately.

2.8. Meat Quality

After 1 h post-mortem, the right *Longissimus thoracis* (LT) muscle between the 7th and 11th ribs was removed from the carcass with a scalpel and used for pH, colour, Warner-Bratzler shear force (WBSF), chemical composition, drip loss and cooking loss analysis. Samples of *LT* muscle (approximately 600 g) were collected from the carcass and then frozen at −20 °C for a subsequent meat quality analysis.

Prior to the analysis of cooking losses (CL) and Warner-Bratzler shear force (WBSF), the samples were thawed for 24 h at 4 °C in a cooler protected from drafts and the meat samples were analysed in triplicate. CL was determined according by Vazquez-Mendoza et al. [33]; for this purpose, fillets with 2.5 cm thick were roasted on a grill (Toastmaster cool-edge-grill, Macon, MO, USA) until they reached an internal temperature of 70 °C, which was monitored with a thermometer (Brannan & Sons, Cleator Moor, Cumbria, UK). When the temperature reached 70 °C, the fillets were removed from the grill and allowed to cool to room temperature (20–25 °C). To calculate the percentage of CL, each fillet was weighed before and after the procedure (weight of raw meat−weight of cooked meat)/weight of raw meat ×100), as it was described by Vazquez-Mendoza et al. [33]. In order to measure the WBSF, 2.5 cm thick meat fillets (three per lamb) were cooked at 70 °C using the CL method, as sited above. WBSF was measuring using an Instron® universal testing machine (model 1132, Instron, Canton, MA, USA) with a Warner-Bratzler accessory [34]. Meat colour was determined on cuts of the *longissimus dorsi* muscle 24 h after slaughter using a Minolta CM-2006d spectrophotometer (Konica model, Minolta Holdings Inc., Osaka, Japan). Lightness (L*), redness (a*) and yellowness (b*) as meat quality attributes were evaluated using the procedure described by Miltenburg et al. [35]. With the values of a* and b*, the Chroma (C*) and Hue (H*) indices were calculated using the equations: Chroma = $(a^* 2 + b^* 2)^{0.5}$ and Hue = $\tan^{-1}$ (b*/a*) × 57.29 both expressed in degrees [36]. Colour coordinate values were obtained using the average of three measurements of colour for each sample. Meat pH was measured following the procedure described by Negrete et al. [37]. This was measured in triplicate on 3 g of *longissimus dorsi* muscle homogenized in 20 mL of deionized water using a blender Waring 51BL32 (model 700, Torrington, CT, USA), and using a Hanna® pH meter (Model HI 98127, Waterproof Tester, Woonsocket, RI, USA). Drip loss value was calculated

as weight loss of the fresh meat sample (90 g) placed in a plastic bag after storage for 24 h at 4 °C. Drip loss was determined in triplicate as percentage of water released from fresh muscle [38].

Prior to the proximate analysis of meat, the samples were thawed for 24 h at 4 °C. The subcutaneous fat and connective tissue were separated from the muscle using a scalpel, and the meat was ground and homogenized for 5 min with a mixer. Meat samples were analysed in triplicate to determine the moisture, lipid, protein and ash content as a percentage of the muscle sample following AOAC procedures [28].

2.9. Statistical Analysis

All statistical analyses were performed using the SAS statistical program [39]. First, it was performed the normality test on all variables using the UNIVARIATE procedure. BW, DMI, DWG and FCR data were analysed for each period with a completely randomized design with repeated measures over time, using the MIXED procedure. Initially, initial BW was included as a covariate to adjust the variables DWG, DMI and final BW. However, this covariate was removed from the model because it was not significant ($p > 0.05$). Different variance–covariance structures were verified to fit the statistical model, and the compound symmetry structure showed the best fit according to the criteria of the lowest values of BIC and AIC [40]. The full statistical model used was:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + A_k + e_{ijk} \quad (1)$$

where Y_{ijk} represents the value measured at period j and treatment i for the lamb k, μ represents the overall mean, T_i represents the fixed effect of HM treatments (i = 1, 2, 3, 4), P_j represents the fixed effect of the period within four feeding periods (j = period 1: 1–14, period 2: 15–28, period 3: 29–42 and period 4: 43–56 d), $(T \times P)_{ij}$ represents the fixed effect of interaction between treatment and period, A_k represents the random effect of lambs provided different diets (k = 1, 2, 3, ... 36), and e_{ijk} represents the random residual error.

On the other hand, data on carcass characteristics, animal organs and meat quality were analysed using the GLM procedure. Each lamb was considered an experimental unit. Initially, final BW was included as a covariate to adjust all variables (carcass characteristics, organs and meat quality). However, this covariate was removed from the model because it was not significant ($p > 0.05$). The statistical model used was: $Y_{ijk} = \mu + T_i + e_{ij}$, in which μ is the mean value, T_i is the treatment effect (fixed), and e_{ij} is the error term.

Linear and quadratic orthogonal polynomials were used to evaluate the effects of HM level on all variables evaluated. Means of treatments were compared using the Tukey test, and significant differences were considered when $p \leq 0.05$. In addition, a trend was considered when $p > 0.05$ and ≤ 0.10.

3. Results

3.1. Productive Performance and Digestibility

Final body weight (FBW) was not affected by treatments (Table 1). For dry matter intake (DMI), no significant differences were found among the treatments during the experimental period. On the other hand, DWG showed a tendency of linear decrease ($p = 0.06$), and the lambs that were supplemented with HM3 performed lower than the lambs fed with the other diets. However, the feed conversion ratio was not affected by the level of HM added to the diet. On the other hand, the dry matter digestibility (DMD) decreased linearly ($p = 0.03$) as the dose of HM in the diet increased. The lowest digestibility of DM was observed in lambs fed HM3 diet (Table 1).

Table 1. Productive performance of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Initial body weight (IBW) kg	23.15	23.45	22.93	23.55	1.233	0.90	0.90
Final body weight (FBW) kg	41.93	39.88	40.13	38.80	1.608	0.21	0.82
Dry matter intake (DMI) kg d^{-1}	1.161	1.083	1.059	1.034	0.056	0.12	0.64
Daily weight gain (DWG) kg d^{-1}	0.335 *	0.293	0.307	0.272 *	0.020	0.06	0.85
Feed conversion ratio (FCR) DMI/DWG	3.49	3.74	3.54	3.91	0.196	0.23	0.76
Dry matter Digestibility (DMD) %	75.71 ^{a}	74.72 ^{ab}	72.31 ^{ab}	70.39 ^{b}	1.528	0.03	0.76

[1] Peptasan® based on *Saccharum officinarum, Balanites roxburghi and, Acacia concinna*. CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$; *—indicates a tendency.

3.2. Carcass Traits

No differences were observed in hot carcass weight, hot carcass yield, backfat thickness, *longissimus dorsi* muscle area, weight of internal organs (empty rumen, small intestine, large intestine, lungs and trachea, heart, liver, kidneys, spleen), nor in the weight of testicles, skin, feet and head by the effect of supplementation with the HM (Table 2).

Table 2. Carcass traits and organ weights of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Hot carcass weight kg	20.73	19.43	19.38	18.88	0.757	0.11	0.22
Hot carcass yield %	49.47	48.72	48.28	48.95	0.767	0.57	0.54
Backfat thickness mm	3.00	3.11	3.00	3.11	0.114	0.67	0.99
Muscle area *longissimus dorsi* cm^2	11.24	10.90	10.92	10.66	0.312	0.22	0.90
Rumen (empty) kg	1.188	1.152	1.134	1.139	0.047	0.43	0.47
Small intestine (empty) kg	0.882	0.839	0.896	0.913	0.046	0.47	0.34
Large intestine (empty) kg	1.046	1.042	1.024	1.045	0.053	0.93	0.86
Lungs and Trachea kg	0.699	0.686	0.679	0.638	0.040	0.30	0.41
Heart kg	0.198	0.172	0.176	0.192	0.009	0.74	0.92
Liver, kg	0.823	0.842	0.839	0.800	0.034	0.64	0.71
Kidneys kg	0.337	0.352	0.328	0.316	0.019	0.31	0.24
Spleen kg	0.076	0.079	0.083	0.078	0.006	0.70	0.60
Testicles kg	0.690	0.717	0.718	0.634	0.055	0.50	0.62
Skin kg	2.914	2.718	2.834	2.527	0.159	0.15	0.40
Feet kg	0.882	0.824	0.833	0.807	0.041	0.25	0.44
Head kg	1.967	2.025	1.986	1.937	0.072	0.69	0.64

[1] Peptasan® based on *Saccharum officinarum, Balanites roxburghi* and, *Acacia concinna*. CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means.

3.3. Meat Quality

Meat pH, cooking loss and drip loss increased linearly ($p < 0.05$) as the dose of HM in the diet increased (Table 3). The WBSF of meat decreased linearly as the level of HM in the diet increased ($p = 0.02$). On the other hand, no significant changes were observed in meat colour variables, with the exception of yellowness (b*), which decreased as dietary HM dose increased ($p = 0.04$). The chemical composition (moisture, protein, fat and ash) of the meat was not affected by the dose of HM in the diet.

Table 3. Meat characteristics of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Meat pH (24 h)	5.50 ^{ab}	5.36 ^{b}	5.69 ^{a}	5.84 ^{a}	0.14	0.04	0.32
WBSF kg cm^{-2}	6.47 ^{a}	6.29 ^{a}	5.53 ^{ab}	4.73 ^{b}	0.57	0.02	0.58
Cooking loss (%)	16.89	18.72	19.28	20.13	1.09	0.04	0.65
Dripp loss (%)	3.55 ^{b}	4.07 ^{ab}	4.84 ^{a}	4.81 ^{a}	0.38	0.01	0.48
Lightness (*L**)	36.22	36.20	33.45	34.77	1.27	0.22	0.60
Redness (*a**)	9.23	8.45	9.05	9.23	0.44	0.75	0.28
Yellowness (*b**)	10.28 ^{a}	9.11 ^{b}	9.45 ^{ab}	8.73 ^{b}	0.45	0.04	0.62
Chroma	13.87	12.46	13.12	12.74	0.51	0.25	0.33
Hue °	47.81 ^{a}	47.12 ^{ab}	46.48 ^{ab}	43.40 ^{b}	1.65	0.07	0.47
Moisture, g 100 g^{-1}	73.70	73.69	73.69	73.58	0.48	0.97	0.99
Crude protein, g 100 g^{-1}	20.38	20.47	20.59	20.48	0.38	0.94	0.88
Fat, g 100 g^{-1}	2.45	2.46	2.45	2.49	0.07	0.99	0.98
Ash, g 100 g^{-1}	1.34	1.33	1.33	1.32	0.03	0.82	0.98

[1] Peptasan® based on *Saccharum officinarum*, *Balanites roxburghi* and, *Acacia concinna*. WBSF—Warner-Bratzler shear force; CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$.

4. Discussion

Some plants containing saponins, polysaccharides and flavonoids have shown positive effects on antioxidant capacity and immune status in ruminants [8,41]. In addition, saponins have been reported to improve the energy utilization efficiency and increase the duodenal flux of amino acids and microbial protein [11]. Consequently, lambs supplemented with herbal products containing saponins, polysaccharides, and flavonoids would be expected to have higher growth rates. However, although in our study FBW and DWG were not affected by HM, a linear reduction trend was observed in DWG of lambs fed the HM3 diet, which could be a consequence of the lower dry matter digestibility observed with HM3. This suggests that high doses of HM in the diet could affect the growth rate of lambs when it is used for prolonged periods. Similar results were previously reported by Liu et al. [42] in lambs supplemented with *Medicago sativa* saponin extracts (0, 0.5, 1, 2 and 4 g kg^{-1} DM for 90 days); and by Nasri et al. [43] who examined the effects of increasing doses of *Quillaja saponaria* saponin extracts (0, 30, 60 and 90 mg kg^{-1} DM for 57 days) in lambs fed high concentrate diets. In the latter investigation, BW and DWG was similar among treatments, regardless of the dose of saponins used. In another study, Wang et al. [9] investigated the effects of supplementing lambs with *Astragalus membranaceus* roots (0, 20, 50 and 80 g kg^{-1} DM for 56 days) containing saponins, polysaccharides and flavonoids. In that study, BW was not affected, but DWG was higher in the treatments supplemented with *Astragalus membranaceus*, perhaps as a consequence of the beneficial effects that the saponins, flavonoids and polysaccharides of the plant had on the antioxidant and immune status, and on the serum concentration of growth hormone in the animals.

Some plants containing saponins, polysaccharides and flavonoids increase the relative abundance of fibre-degrading bacteria in the rumen [41]. This could result in higher fibre and feed digestibility and could also increase ruminal passage rate and dry matter intake. However, in our study, DMI was similar among lambs of all treatments during the experimental period. Although HM could increase the rate of passage, saponins are natural surfactant glycosides, which may have a bitter and astringent taste for animals [44]. This can cause low palatability of the diet, which would partially explain the absence of changes observed in DMI. In a similar study, Liu et al. [42] investigated the effects of extracts of *Medicago sativa* saponins (0, 0.5, 1, 2 and 4 g kg^{-1} DM for 90 days) on the productive performance of lambs. In that study, DMI increased linearly as the dose of saponins in the diet increased. This suggests that the lambs are able to adapt to consume saponins, but this adaptation could require long periods of supplementation.

Some plant-extracted saponins have shown promising effects on improving feed utilization efficiency because they can suppress enteric methane emissions through direct effects on ruminal microorganisms [12,14]. In the present study, FCR was similar among treatments, suggesting that HM did not affect feed utilization efficiency. The absence of significant changes in FCR could be explained by the fact that DMI and DWG were also not affected by the treatments. Similar results were previously reported by Mandal et al. [18] in goats supplemented with 5 g d^{-1} of *Acacia concinna* pods for 90 days, and by Liu et al. [42] in lambs supplemented with alfalfa saponin extracts (0.5, 1, 2 and 4 g kg^{-1} DM for 90 days). In their study, they observed that FCR was similar among treatments, even though feed digestibility was higher in lambs supplemented with saponins.

Previous studies have reported that digestion and utilization of nutrients in the diet of ruminants could be improved by dietary supplementation of saponins [12,45], and plants containing saponins, polysaccharides and flavonoids [41]. However, in our study, a negative effect of HM on DM digestibility was observed. Similar results were previously reported by Nasri et al. [43] in lambs supplemented with saponin extracts from *Quillaja saponaria* at dietary concentrations of 30, 60 and 90 mg kg^{-1} DM; and by Nasehi et al. [21] in lambs supplemented with increasing doses (0, 6.1, 8.7 and 11.3 g kg^{-1} DM) of saponins from the green tea plant (*Camellia sinensis)*. Their results showed that saponins reduced the digestibility of DM of the lambs but did not affect their productive performance.

Regarding carcass characteristics, HCW and HCY were not affected by dietary supplementation of HM. No information is available on the effects of HM containing saponins, polysaccharides and flavonoids on sheep or goat carcass characteristics. However, results that are congruent with our findings were previously reported by Nasri et al. [43] on lambs supplemented with increasing doses of saponin extracts from *Quillaja saponaria* (0, 30, 60 and 90 mg kg^{-1} DM for 57 days); and by Abdallah et al. [46] on sheep supplemented with 10 and 15% dried *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. Their results showed that HCW and HCY were not affected by dietary supplementation of saponins, and neither were they affected by the mixture of saponins, polysaccharides and flavonoids from *Astragalus membranaceus*. The limited information on the effects of HM on ruminant carcass characteristics makes it difficult to explain the results observed in this and other studies. However, the similarity of BFT in the carcass of lambs from all treatments may partially explain the absence of changes in HCY in the present study.

BFT and LMA were also not affected by the HM dietary supplementation. The mechanism of action of herbal products and their bioactive compounds on lipogenesis has not been studied in lambs [4]. However, Liang et al. [47] observed that, in beef cattle fed with high-grain rations, supplementation of flavonoid extracts in the diet increased BFT through changes in the differential expression of genes involved in lipid metabolism. In the present study BFT was not affected by the inclusion of HM in the diet, even though it contains parts of the plant *Balanites roxburghii*, which contains flavonoids [25]. This suggests that the effects of flavonoids on BFT are dependent on botanical origin. Given that fat deposition, physical and chemical carcass characteristics of lambs are influenced by breed, sex, age and weight [48,49], the homogeneity of these characteristics in the lambs used in the present study partially explains the absence of changes in LMA and BFT.

Regarding the internal and external organs of lambs, similar results were previously reported by Hundal et al. [19] in goats supplemented with 2% of *Macrotyloma uniflorum* seeds containing saponins; and by Abdallah et al. [46] in sheep supplemented with 10 and 15% of *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. They observed that the weight of the kidneys on sheep supplemented with the highest dose of saponins, flavonoids and polysaccharides from *A. membranaceus* was higher than that on sheep from the other treatments, but there was no effect on the other internal organs. Information on the effects of herbal products or their bioactive compounds on the size and weight of internal organs in ruminants is still limited, which makes it difficult to explain the results observed in this study. However, differences in the internal organs of sheep are

influenced by the breed, sex and age of the animals [50], and by the feeding regime [51]. In the present study all these factors were controlled, which would partially explain the absence of significant changes.

The lowest pH of the meat was observed in the lambs with the HM1 treatment, while in the animals of the other treatments the pH was similar, within the normal range of 5.5 to 5.8 suggested by Sañudo et al. [52]. Abdallah et al. [46] did not observe pH changes in the meat of lambs supplemented with 10 and 15% *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. In another study, Nasri et al. [43] also did not observe pH changes in the meat of lambs supplemented with saponin extracts from *Quillaja saponaria* at concentrations of 30, 60 and 90 mg kg^{-1} DM. However, it was observed that the pH of the meat in lambs of all treatments was below the normal range, similar to what was observed in our study with the HM1 treatment. Therefore, the effects of HM on the pH of the meat observed in the present study could be related to the presence of bioactive compounds. The pH is important for preserving meat during storage. A low pH has a bacteriostatic effect, while a pH above the normal range favours the growth of proteolytic microorganisms [53,54]. This suggests that supplementation of low doses of HM in the diet could promote favourable bacteriostatic effects in lamb meat, and thus increase its shelf life.

Ponnampalam et al. [55] mentioned that an ultimate pH > 5.8 is associated with alterations in drip loss and WBSF. In addition, in sheep meat, Watanabe et al. [56] reported a curvilinear association between ultimate pH and WBSF values, with a toughness peak at pH around 6.0 and improvements in tenderness at pH below and above 6.0. In our study, WBSF decreased as the dose of HM increased; however, this result must be carefully interpreted considering the low number of replicates used and the high coefficient of variation observed (30.74%, data not shown). Similar results were previously reported by Qin et al. [57] in lambs fed pomace (7.8 and 16% for 80 days) obtained from *Hippophae rhamnoides* fruits, which contained 0.69 and 1.02% flavonoids, respectively. In that experiment, WBSF decreased when the flavonoid dose increased. In another study, Abdalla et al. [46] observed no significant changes in WBSF of meat from lambs supplemented with saponins, flavonoids and polysaccharides from *Astragalus membranaceus* roots. WBSF is a well-known method for estimating the meat tenderness [57], consequently, the lower WBSF observed in the present study suggests that dietary supplementation of HM could improve the lamb meat tenderness. Although the exact mechanism is unknown, the changes in WBSF observed in this and other studies suggest that bioactive compounds contained in some plants facilitate the activation of some peptidases such as calpains and cathepsins, which help prevent and delay post-mortem muscle fibre stiffening [58]. It is also possible that these bioactive compounds act by reducing calpastatin activity, allowing a higher rate of myofibril protein degradation [59]. This hypothesis is supported by the observed linear increase in drip loss as WBSF decreased, because drip water losses may increase when calpastatin activity decreases [60]. Furthermore, Webb and Agbeniga [61] reported a linear relationship between WBSF and drip loss, in which higher drip loss was associated with rapid tenderisation and lower WBSF of the meat.

Drip loss is associated with the capacity to retain water in the muscle, with the juiciness and the tenderness of the meat [46,49]. In the present study, the drip loss of meat increased when the dose of HM increased, indicating that high doses of HM could affect the water retention capacity, tenderness and juiciness of meat. Abdallah et al. [46] investigated the effects of dietary supplementation with dried *Astragalus membranaceus* roots containing saponins, flavonoids, and polysaccharides, and observed that meat drip loss decreased in response to *A. membranaceus* supplementation. However, WBSF was similar in the meat of lambs from all treatments. Although the exact mechanism involved is unknown, the higher drip loss observed in the meat analysed in the present study could be related to the observed changes in WBSF, as previously discussed.

Colour is an important attribute of meat quality because it is the first aspect that attracts consumers when choosing fresh meat [62]. A variety of secondary compounds

from plants can improve oxidative stability and prevent discolouration of meat of small ruminants [2]. In the present study, HM did not affect the values of L*, a*, Chroma and Hue °. However, b* decreased in response to supplementation of HM in the diet. This result could be positive because consumers generally do not expect to find high b* in fresh meat [63]. Similarly, previous studies [46,64] reported that supplementation with medicinal plants containing saponins, polysaccharides, and flavonoids also did not affect the colouration of meat from lambs and goats.

There is little information on the use of HM containing saponins, polysaccharides and flavonoids as a colour preservative in ruminant meat. The pigment content of meat can modify its colouration [35]. Likewise, the inclusion of some medicinal plants containing flavonoids increases the hypertrophy of muscle fibres in lambs [57], which could dilute the content of muscle pigments and consequently alter meat colour [65,66]. These findings suggest that the HM used could increase muscle hypertrophy, which would partially explain the observed reduction in b*. On the other hand, Luo et al. [64] reported that dietary supplementation of medicinal plants containing saponins, polysaccharides and flavonoids altered the pigment content on the meat of small ruminants. Similar effects of consumption of these metabolites would partially explain the b* changes in the meat of lambs supplemented with HM in the present study.

In the present study, the chemical composition of lamb meat was similar in all treatments, perhaps as a consequence of the low impact of HM supplementation on the nutritional composition of the diet. In a similar study, Abdallah et al. [46] investigated the effects of *Astragalus membranaceus* roots (0, 10 and 15% for 47 days) containing saponins, polysaccharides and flavonoids on sheep meat quality. In that research, the moisture, protein and ash content of the meat was similar among treatments. However, they observed that fat content decreased in sheep that ate *A. membranaceus* roots. Furthermore, in our study, HM supplementation had little impact on the final BW of the lambs, all being of the same breed and age, which partially explains the absence of significant changes in the chemical composition of the meat [48,49,54].

5. Conclusions

The results of this study indicate that dietary supplementation with HM reduces dry matter digestibility (linear effect). However, the inclusion of up to 3 g HM kg^{-1} DM does not affect productive performance, carcass characteristics, chemical composition, and meat colouration (lightness and redness) of lambs fed high concentrate diets during the final fattening period. Meat yellowness decreases (linear effect) in response to HM supplementation in the diet, which could be positive because consumers, in general, do not expect to find high yellowness in fresh meat. On the other hand, meat pH, cooking loss and drip loss increase linearly as the dose of HM in the diet increases (linear effect). In addition, Warner-Bratzler shear force decreases as the dose of HM increases (linear effect). Thus, Peptasan® HM could be used to improve meat tenderness of lambs fed high concentrate diets. However, this result must be carefully interpreted considering the low number of replicates used. In addition, the increased drip loss in response to HM supplementation could be a risk of microbial spoilage during meat storage. Therefore, it is convenient to carry out meat quality analyses at the muscle level to evaluate the impact of other doses of this HM in rations with different proportion of concentrate for lambs in different experimental periods and physiological stages.

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