

Carcass traits, fatty acid composition, gene expression, oxidative stability and quality attributes of different muscles in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination

Kifah Jumaah Odhaib^{1,2}, Kazeem Dauda Adeyemi^{1,3}, and Awis Qurni Sazili^{1,4,5,*}

* **Corresponding Author:** Awis Qurni Sazili
Tel: +60-3-89474870, **Fax:** +60-3-89381024,
E-mail: awis@upm.edu.my

¹ Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

² Department of Physiology, College of Veterinary Medicine, University of Basrah, 61004 Basrah, Iraq

³ Department of Animal Production, University of Ilorin, PMB 1515 Ilorin, Nigeria

⁴ Laboratory of Sustainable Animal Production and Biodiversity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

⁵ Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ORCID

Kazeem Dauda Adeyemi

<https://orcid.org/0000-0002-6719-2081>

Awis Qurni Sazili

<https://orcid.org/0000-0002-7362-0855>

Submitted Jun 17, 2017; Revised Jul 30, 2017;

Accepted Oct 22, 2017

Objective: This study examined the influence of dietary supplementation of *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination on carcass attributes, fatty acid (FA) composition, gene expression, lipid oxidation and physicochemical properties of *longissimus dorsi* (LD), *semitendinosus* (ST), and *supraspinatus* (SS) muscles in Dorper lambs.

Methods: Twenty-four Dorper lambs (18.68±0.6 kg, 4 to 5 months old) were randomly assigned to a concentrate mixture containing either, no supplement (control, T1), 1% *Rosmarinus officinalis* leaves (T2), 1% *Nigella sativa* seeds (T3), or 1% *Rosmarinus officinalis* leaves+1% *Nigella sativa* seeds (T4) on a dry matter basis. The lambs were fed the treatments with urea-treated rice straw for 90 days, slaughtered and the muscles were subjected to a 7 d *postmortem* chill storage.

Results: The T2 lambs had greater ($p<0.05$) slaughter and cold carcass weights than the control lambs. Dietary supplements did not affect ($p>0.05$) chill loss, dressing percentage, carcass composition, intramuscular fat and muscle pH in Dorper lambs. Meat from supplemented lambs had lower ($p<0.05$) cooking and drip losses, shear force, lightness, and lipid oxidation and greater ($p<0.05$) redness compared with the control meat. The impact of dietary supplements on muscle FA varied with muscle type. Diet had no effect ($p>0.05$) on the expression of stearoyl-CoA desaturase and lipoprotein lipase genes in LD and ST muscles in Dorper lambs. The T2 and T3 diets up regulated the expression of AMP-activated protein kinase alpha 2 gene in LD and ST muscles and up regulated the expression of sterol regulatory element-binding protein 1 in ST muscle in Dorper lambs.

Conclusion: Dietary supplementation of *Nigella sativa* seeds and *Rosmarinus officinalis* leaves had beneficial effects on meat quality in Dorper lambs.

Keywords: Dorper Lambs; Gene Expression; Meat Quality; *Nigella sativa*; *Rosmarinus officinalis*

INTRODUCTION

Ruminant meat is a good source of animal protein, which is valued in many cultural culinary traditions [1]. Nonetheless, in recent times, its consumption has been linked with the incidence of chronic diseases [2] in humans thereby triggering a lack of consumer confidence in ruminant meat. In addition, the meat industry has been adversely affected by food scares relating to the residual effects of antibiotic growth promoters used in animal nutrition [3]. Thus, enhancing the safety, nutritional and sensory quality of ruminant meat in order to meet the rapidly changing requirements of consumers have been the subject of research in recent times.

Dietary supplementation of medicinal plants to livestock has been advocated as an effective strategy for improving production performance [4] of livestock and the quality and storage stability of animal products [5]. It has been established that nutritional strategy is more effective in enhancing the oxidative stability of meat when compared to exogenous addition of antioxidants because dietary antioxidants are preferentially deposited where they are most needed [5,6]. In addition, dietary intervention remains the most effective strategy to modify the oxidative stability of intact muscle foods, where the use of exogenous antioxidant may be difficult or practically impossible [1,6]. Nonetheless, the effects of medicinal plants on livestock product quality are highly variable and inconsistent in the published literatures [5-7]. These scenarios have created the impetus for further research in diverse production systems to allow informed choices and tailored decisions in the use of medicinal plants for the improvement of the healthiness and storage stability animal products.

Nigella sativa (NS) and *Rosmarinus officinalis* (RO) contain myriad phytochemicals whose antioxidant, therapeutic, antimicrobial, antitumor and anti-inflammatory properties have been documented [8,9]. Dietary supplementation of RO and NS improved body weight gain and lean to fat ratio in lambs [10,11]. Nonetheless, there is limited investigation on the effects of dietary supplementation of NS seeds and RO leaves on the physicochemical properties and oxidative stability of meat in ruminants. The use of medicinal plants as antioxidant in foods is favoured due to the hazardous effects of synthetic antioxidants on human health [6,7].

There has been a renewed interest in the manipulation of the fatty acid (FA) composition of ruminant meat to meet the prevailing consumers' demands [1]. Plant polyphenols, such as those found in NS and RO, when supplemented in ruminant diets could manipulate rumen biohydrogenation of unsaturated FAs thereby modifying the FA composition of ruminant meat [12]. The changes in muscle FAs due to feeding strategies are implicated in the expression of lipogenic genes [13,14]. An improved understanding of the genes and the underlying mechanisms involved in fat metabolism would allow a better control of the content and composition of FA in ruminant meat [13,14]. Therefore, the objective of this study was to determine the effects of NS seeds, RO leaves and their combination on carcass traits, FA composition, expression of lipogenic genes, physicochemical properties and lipid oxidation in *longissimus dorsi* (LD), *semitendinosus* (ST), and *supraspinatus* (SS) muscles in Dorper lambs.

MATERIALS AND METHODS

Animal welfare

This study was conducted following the guidelines of the Research Policy of Universiti Putra Malaysia on Animal Welfare

and Ethics. The care of the Dorper lambs was in accordance to Malaysian standards.

Experimental diet and management of animals

Twenty-four, entire male Dorper lambs with average initial body weight of 18.68 ± 0.6 kg and 4 to 5 months old were used for the trial. Each lamb was housed in individual pens (1.3 m \times 0.9 m) provided with drinking and feeding facilities. The experimental diets were formulated to meet the nutritional requirements of lambs in line with NRC [15] recommendation. The lambs were randomly allotted to one of the four experimental diets namely, a concentrate mixture (55% yellow corn, 20% soybean meal, 20% rice bran, 3% palm oil, 1% CaCO₃, 0.5% NaCl, 0.5% minerals-vitamins mix) without an additive (control, T1), concentrate mixture+1% (dry matter [DM] of concentrate) *Rosmarinus officinalis* leaves (T2), concentrate mixture+1% (DM of concentrate) *Nigella sativa* seeds (T3), concentrate mixture+1% (DM of concentrate) *Rosmarinus officinalis* leaves+1% (DM of concentrate) *Nigella sativa* seeds (T4). Each lamb received concentrate at 1% of body weight with *ad libitum* urea-treated rice straw daily for 90 d following two weeks of acclimatization. The concentrate was offered to the lambs in equal proportion in two splits at 0800 and 1600 hours. All lambs had *ad libitum* access to water and mineral block.

Determination of chemical composition and phytochemical contents of dietary treatments

The feed samples were dried at 60°C for 48 h to determine the DM content, ground to pass a through a 1 mm screen and analysed for protein, ether extract, crude protein and ash according to the method of AOAC [16]. The acid detergent fibre and neutral detergent fibre were analysed by the protocol of Van Soest et al [17]. The total phenol and tannin contents were determined following the procedure of Makkar et al [18]. The chemical composition and phytochemical contents of the dietary treatments, additives and urea treated rice straw are shown in Table 1.

Determination of fatty acid composition of dietary treatments

The total lipids in dietary treatments were extracted in chloroform:methanol (2:1, v/v) mixture following the protocol described by Adeyemi et al [1]. The extracted lipid was transmethylated to fatty acid methyl esters using 2 mL 14% BF₃ and 2 mL 0.66 N KOH in methanol following the protocol of AOAC [16]. The chromatography settings, the column and the standard used were as described by Adeyemi et al [1]. The FA composition of the dietary treatments is presented in Table 2.

Slaughtering and carcass analysis

On the last day of the feeding trial, the lambs were fasted over-

Table 1. Chemical composition of dietary treatments, urea treated rice straw, *Nigella sativa* seeds and *Rosmarinus officinalis* leaves

Parameter	T1 ¹⁾	T2	T3	T4	UTRS	NS	RO
Chemical composition (% DM)							
Dry matter	90.00	90.38	90.05	90.46	96.58	92.62	91.63
Organic matter	94.83	94.85	94.76	94.83	87.06	96.09	93.95
Ash	5.15	5.12	5.24	5.16	12.94	3.91	6.05
Crude protein	16.96	16.86	17.03	16.92	4.98	22.70	5.59
Ether extract	3.74	3.80	3.70	3.70	1.63	9.034	4.23
Crude fibre	3.07	3.08	3.15	3.13	36.25	6.60	13.40
Neutral detergent fibre	38.93	39.25	46.59	47.16	80.75	35.30	36.64
Acid detergent fibre	8.88	7.38	6.98	8.99	48.57	21.24	19.08
Phytochemical compounds							
Total polyphenol (mg/g)	3.16	12.35	19.08	34.86	-	37.69	43.29
Non-tannin polyphenol (mg/g)	0.98	4.30	3.61	7.88	-	2.16	10.71
Tannin polyphenol (mg/g)	2.18	8.05	15.47	26.98	-	35.53	32.58

NS, *Nigella sativa* seeds; RO, *Rosmarinus officinalis* leaves; UTRS, urea treated rice straw; DM, dry matter.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

night with *ad libitum* access to water and slaughtered according to the Halal procedure as described in MS1500:2009 [19]. After

Table 2. Fatty acid composition (% of total FA) of dietary treatments

Fatty acid	Dietary treatment			
	T1 ¹⁾	T2	T3	T4
C14:0	0.89	1.22	0.76	0.82
C16:0	36.44	31.24	31.89	32.53
C16:1	0.57	0.72	0.62	0.67
C18:0	8.67	7.61	7.89	8.16
C18:1n-9	40.30	46.59	46.62	46.59
C18:2n-6	7.14	7.19	6.18	5.44
C18:3n-3	1.70	1.43	1.47	1.49
C20:4n-6	1.53	1.09	1.33	1.23
C20:5n-3	0.10	0.30	0.92	0.71
C22:5n-3	1.94	1.36	1.50	1.52
C22:6n-3	0.75	0.93	0.81	0.89
Sum and ratio of FA ²⁾				
ΣSFA	45.99	40.07	40.54	41.51
ΣUFA	54.00	59.92	59.45	58.49
ΣMUFA	40.87	47.32	47.24	47.22
ΣPUFA	13.13	12.62	12.21	11.27
Σn-3	4.47	4.01	4.70	4.61
Σn-6	8.66	8.60	7.51	6.67
n-6:n-3	1.94	2.13	1.62	1.44
UFA:SFA	1.18	1.50	1.47	1.41
PUFA:SFA	0.29	0.32	0.30	0.27
Total FA (mg/g)	1,679.61	1,230.10	1,372.04	1,655.40

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

²⁾ ΣSFA = C14:0+C16:0+C18:0; ΣMUFA = C16:1+C18:1+C18:1 trans-11; ΣUFA = C16:1+C18:1+Σn-3+Σn-6; ΣPUFA = Σn-3+Σn-6; Σn-3 = C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; Σn-6 = C18:2n-6+C20:4n-6; n-6:n-3 = (C18:2n-6+C20:4n-6)÷(C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3).

visceration, each carcass was split along the vertebra column into right and left halves. The right half was used for carcass analyses as described by Adeyemi et al [20].

Muscle sampling and storage of meat

Meat samples were left intact on the left half of each carcass until a particular *postmortem* storage was reached. The SS muscle was sampled from the right forelimb. The right LD muscle was excised from the 6th to 8th lumbar vertebra. The ST muscle was sampled at the posterior face of the left hind limb. On day 0, 90 g of each muscle sample was removed from each carcass, trimmed free of epimyseal connective tissue and external fat and divided into three parts. The first part (10 g) was pulverized in liquid nitrogen with porcelain mortar and pestle to produce a homogenous powder, stored at -80°C until analysis and assigned for the determination of muscle pH, FA composition and lipid oxidation. The second part (30 g) was vacuum packaged and stored in a chiller at 4°C±1°C and used to determine drip loss. The third part (50 g) was used to determine cooking loss, colour, and shear force on d 0. Upon the completion of each storage period, muscle cuts (60 g) were removed from the carcass, trimmed free of epimyseal connective tissue and external fat and sectioned into two parts. The first part (10 g) was pulverized in liquid nitrogen and assigned as described earlier. The second portion (50 g) was used to determine colour coordinates, cooking loss and shear force.

Determination of muscle pH, colour coordinates, drip and cooking losses, shear force, lipid oxidation and fatty acid composition

Muscle pH, meat colour coordinates, drip loss, cooking loss and shear force were determined following the protocol described by Lokman et al [21]. Lipid oxidation in the muscle samples was quantified as 2-thiobarbituric acid reactive sub-

stances (TBARS) using QuantiChrom™ TBARS Assay Kit (DTBA-100, BioAssay Systems, Hayward, CA, USA) in line with the manufacturer's procedure. The muscle FA composition was determined as described earlier.

RNA extraction from muscle samples and quantitative real-time polymerase chain reaction

Total RNA from LD and ST muscles (pulverized in liquid nitrogen and stored at -80°C) was extracted and purified using The RNeasy Fibrous Tissue Kit (cat. no. 74704) following the manufacturer's protocol. The concentration and purity of the RNA was assessed using a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 260/280 nm absorbance. The purified RNA was kept at -80°C until further analysis. The reverse transcription of total RNA to complementary DNA was done using Quantitate Reversed Transcription Kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol. Gene expression was carried out using Quantitative real-time polymerase chain reaction (PCR). The PCR reaction was performed on a total volume of 20 μL using the iQTMSYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Each 20 μL PCR reaction contained 10 μL 2 \times SYBR Green Master Mix, 1 μL forward primer, 1 μL reverse primer, 5 μL template cDNA and 3 mL RNase-free water. The PCR conditions for all genes were, initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s, annealing for 30 s, and extension at 72°C for 30 s with a single fluorescence detection point at the end of the relevant annealing section. At the end of the PCR run, the temperature was increased from 70°C to 95°C at the rate of $0.5^{\circ}\text{C}/\text{min}$, and the fluorescence was measured at every 5 s interval to construct the melting curve. The comparative CT method ($\Delta\Delta\text{CT}$) expression of the investigated genes was normalized with the endogenous control hypoxanthine phosphoribosyltransferase 1. CT values are means of duplicate measurements. Comparative CT quantification was determined by the $\Delta\Delta\text{CT}$ method. The primers used are

shown in Table 3.

Statistical analysis

The experiment followed a completely randomized design. The gene expression data was checked for normality prior to subjecting it to the generalized linear model (GLM) of SAS [22]. Data obtained from carcass traits and muscle FA were subjected to the GLM procedure of SAS [22]. Data for physicochemical properties were analyzed using the PROC MIXED procedure of SAS [22] in which diet and *postmortem* storage days and their first order interaction were fitted as fixed effects in a repeated measure. Means were separated using the "PDIFF" option of the "LSMEANS" statement of the MIXED procedure. Tukey HSD test was used to adjust the means. The level of significance difference was set at $p < 0.05$.

RESULTS

Carcass traits

The final body weight and carcass characteristics of Dorper lambs fed different medicinal plants are shown in Table 4. Dorper lambs fed 1% RO leaves had greater ($p < 0.05$) final body weight compared with those fed other diets. Dietary supplementation of medicinal plants had no effect ($p > 0.05$) on the hot carcass weight, chill loss, dressing percentage, percentages of shoulder, legs, breast, loin and neck in Dorper lambs. The proportion of lean, bone and fat in the neck, loin and breast cuts in Dorper lambs were similar ($p > 0.05$) between the diets. Dietary treatments had no effect ($p > 0.05$) on the proportion of bone and fat in the shoulder and leg cuts of Dorper lambs. The T3 lambs had greater ($p < 0.05$) lean in the leg cut compared with lambs fed other dietary treatments.

Muscle fatty acid composition

The FA composition of LD muscle in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination is shown in Table 5. Except for the concentration of C18:1n-9,

Table 3. Target genes and sequences of primers

Gene No.	Targets genes	Primers	Amplicon (bp)	Annealing temperature ($^{\circ}\text{C}$)	Accession No.
1	<i>LPL</i>	F-5'aatgaagatgaacggaacg-3' R-5'gcactttccaaccaggatgt-3'	119	60	NM_001009394
2	<i>SCD</i>	F- 5'cccagctgtcagagaaaagg- 3' R- 5'gatgaagcacaacagcagga- 3'	115	60	AJ001048
3	<i>SREBF1</i>	F-5'ctgctatgcaggcagcac- 3' R- 5'ggttgatggcagcttgt- 3'	99	60	GU206528
4	<i>YWHAZ</i>	F-5'tgtaggagcccaggtcatct-3' R-5'ttctctctgtattctcagccatct-3'	102	60	AY970970
5	<i>PRKAA2</i>	F-5'accctccatttgatgatga-3' R-5'tggcaacagaacgattgaga-3'	97	60	NM_001112816

F: forward, R: reverse; *LPL*, lipoprotein lipase; *SCD*, stearoyl-CoA desaturase; *SREBF1*, sterol regulatory element-binding transcription factor 1; *YWHAZ*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; *PRKAA2*, AMP-activated protein kinase alpha 2.

Table 4. Carcass traits in Dorper lambs fed diets containing *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination

Parameter	T1 ¹⁾	T2	T3	T4	SEM	p value
Slaughter weight (kg)	31.97 ^b	34.80 ^a	31.85 ^b	33.00 ^b	0.388	0.001
Hot carcass weight (kg)	13.15	15.10	13.90	14.65	0.500	0.072
Cold carcass weight (kg)	12.37 ^b	14.10 ^a	13.17 ^{ab}	13.95 ^a	0.161	0.009
Chill loss (%)	5.60	6.62	5.28	4.69	0.788	0.792
Dressing (%)	41.08	43.39	43.58	44.38	0.325	0.337
Neck (%)	8.44	7.12	7.23	7.37	0.302	0.423
Legs (%)	29.36	30.00	27.79	30.43	0.917	0.758
Shoulder (%)	22.00	23.13	23.59	23.30	0.632	0.823
Loin (%)	18.20	20.46	22.55	18.56	0.903	0.342
Breast and flank (%)	22.77	20.83	20.64	20.04	0.608	0.444
Composition of prime cuts (%)						
Leg lean	67.49 ^b	68.36 ^b	72.15 ^a	68.99 ^b	0.55	0.001
Leg bone	21.89	20.91	19.56	21.77	1.01	0.396
Leg fat	10.61	10.73	8.28	9.22	0.330	0.146
Neck lean	54.10	52.97	57.74	58.49	0.722	0.122
Neck bone	42.34	39.90	39.47	37.67	0.688	0.295
Neck fat	3.55	3.96	2.78	3.83	0.210	0.342
Shoulder lean	56.09	60.81	56.51	58.45	0.803	0.126
Shoulder bone	33.11	26.73	35.70	34.29	1.026	0.202
Shoulder fat	10.79	11.11	9.72	9.25	0.428	0.416
Loin lean	56.64	60.18	58.18	57.88	1.073	0.791
Loin bone	29.38	25.65	24.07	26.06	0.46	0.358
Loin fat	12.85	13.60	13.51	13.30	0.389	0.499
Breast lean	63.27	67.05	65.33	62.95	0.547	0.153
Breast bone	25.56	21.11	21.97	24.19	0.718	0.286
Breast fat	11.16	11.82	12.69	12.85	0.219	0.141

SEM, standard error of means.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.^{a,b,c} Means having different superscripts along the same row are significantly different ($p < 0.05$).

which differed between the diets, supplementation of medicinal plants did not affect the composition of most FA and the intramuscular fat (IMF) in LD muscle in Dorper lambs. The percentage of C18:1n-9 in the LD muscle of lambs fed RO leaves was greater ($p < 0.05$) than that of the control lambs. The LD muscle of the T3 and T4 lambs had similar percentage of C18:1n-9, which did not differ from those of lambs, fed other dietary treatments.

The FA composition of ST muscle in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination is presented in Table 6. The concentration of C18:0 was greater ($p < 0.05$) in the ST muscle of the control lambs compared with those fed the NS seeds. The concentration of C18:0 in the meat of T2 and T4 lambs did not differ from those fed other dietary treatments. The ST muscle of T2 and T3 lambs had similar concentration of C18:1n-9 and total monounsaturated FAs, which were significantly different ($p < 0.05$) from those of lambs fed the T1 and T4 diets. The ST muscle of T3 lambs had greater ($p < 0.05$) concentration of C18:3n-3 compared with the control lambs. The concentration of C18:3n-3 in the ST muscle of T4 lambs did not differ from those fed

other treatments. Diet had no effect ($p > 0.05$) on IMF in ST muscle in Dorper lambs.

The FA composition of SS muscle in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination is presented in Table 7. Dietary supplements had no significant effect ($p > 0.05$) on the IMF and FA composition of SS muscle in Dorper lambs.

Physicochemical traits of different muscles in Dorper lambs

The physicochemical properties and oxidative stability of LD, ST, and SS muscles in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination are presented in Table 8. Dietary supplements had no significant effect ($p > 0.05$) on the muscle pH in different muscles in Dorper lambs. Regardless of muscle type, the pH on d 0 was greater ($p < 0.05$) than that observed on d 1 and 7 *postmortem*. The interaction between diet and *postmortem* storage on muscle pH was not significant ($p > 0.05$).

The percentage drip loss in the LD and SS muscles of the control lambs was greater ($p < 0.05$) than those of the supple-

Table 5. Fatty acid composition (% of total FA) and intramuscular fat (IMF) of *longissimus dorsi* muscle in Doper lambs fed diets containing *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination

Parameter	Treatment				SEM	p value
	T1 ¹⁾	T2	T3	T4		
C14:0	2.39	2.65	2.64	3.55	0.450	0.132
C16:0	25.16	25.77	25.42	25.72	1.783	0.906
C16:1	2.84	2.01	2.49	2.66	0.452	0.377
C17:0	1.02	0.95	1.04	1.09	0.009	0.328
C18:0	21.73	19.93	21.54	21.44	5.321	0.679
C18:1n-9	36.78 ^b	40.85 ^a	37.85 ^{ab}	37.67 ^{ab}	5.386	0.026
C18:2n-6	5.33	3.85	4.42	4.37	1.942	0.530
C18:3n-3	0.39	0.32	0.37	0.35	0.012	0.675
C20:4n-6	3.04	2.28	2.65	1.94	1.273	0.570
C20:5n-3	0.40	0.32	0.59	0.32	0.104	0.608
C22:5n-3	0.34	0.48	0.49	0.21	0.058	0.348
C22:6n-3	0.32	0.43	0.34	0.26	0.056	0.795
Sum and ratio of FA ²⁾						
ΣSFA	50.56	49.46	50.81	52.23	11.159	0.711
ΣUFA	49.45	50.53	49.19	47.78	11.164	0.715
ΣMUFA	39.62	42.86	40.34	40.33	6.074	0.309
ΣPUFA	9.83	7.67	8.86	7.45	9.59	0.682
Σn-3	1.46	1.53	1.79	1.13	0.584	0.688
Σn-6	8.36	6.13	7.06	6.30	5.99	0.578
n-6:n-3	5.78	4.26	4.58	5.55	1.35	0.239
UFA:SFA	0.98	1.03	0.97	0.92	0.017	0.693
PUFA:SFA	0.19	0.15	0.18	0.14	0.005	0.743
IMF (g/100 g)	5.14	5.24	5.20	5.04	0.012	0.119

SEM, standard error of means; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

²⁾ ΣSFA = C14:0+C16:0+C18:0; ΣMUFA = C16:1+C18:1+C18:1 trans-11; ΣUFA = C16:1+C18:1+Σn-3+Σn-6; ΣPUFA = Σn-3+Σn-6; Σn-3 = C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; Σn-6 = C18:2n-6+C20:4n-6; n-6:n-3 = (C18:2n-6+C20:4n-6)÷(C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3).

^{a,b,c} Means having different superscripts along the same row are significantly different (p<0.05).

mented lambs. In ST muscle, the control lambs had similar (p>0.05) drip loss as those fed dietary RO leaves. The ST muscle in the T3 and T4 lambs had lower (p<0.05) drip loss than those fed the T1 and T2 diets. The percentage drip loss decreased (p<0.05) over *postmortem* storage of LD, ST, and SS muscles. There was no significant interaction between diet and *postmortem* storage days for drip loss in different muscles in Dorper lambs.

Dietary treatments had no effect (p>0.05) on the cooking loss of SS muscles in Dorper lambs. Cooking loss in LD and ST muscles of the control lambs was greater (p<0.05) than that of the supplemented lambs. Cooking loss in LD, ST, and SS muscle in Dorper lambs increased (p<0.05) over *postmortem* storage. There was no significant interaction (p>0.05) between diet and *postmortem* storage for cooking loss in different muscles in Dorper lambs.

Table 6. Fatty acid composition (% of total FA) and intramuscular fat (IMF) of *semitendinosus* muscle in Doper lambs fed diets containing *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination

Parameter	Treatment				SEM	p value
	T1 ¹⁾	T2	T3	T4		
C14:0	2.99	3.31	4.11	4.63	0.761	0.075
C16:0	26.41	26.31	26.89	26.43	1.050	0.854
C16:1	2.35 ^b	3.20 ^a	3.03 ^a	2.59 ^{ab}	0.168	0.045
C17:0	1.02	1.06	1.05	1.08	0.018	0.926
C18:0	23.76 ^a	19.79 ^b	19.44 ^b	21.89 ^b	6.357	0.010
C18:1n-9	37.09 ^b	38.86 ^a	39.27 ^a	34.58 ^b	4.770	0.039
C18:2n-6	3.63	4.03	3.17	4.65	1.538	0.417
C18:3n-3	0.28 ^b	0.29 ^b	0.42 ^a	0.37 ^{ab}	0.0049	0.046
C20:4n-6	1.74	2.12	1.56	2.270	0.394	0.387
C20:5n-3	0.13	0.16	0.15	0.357	0.025	0.20
C22:5n-3	0.24	0.26	0.31	0.270	0.019	0.933
C22:6n-3	0.24	0.34	0.20	0.262	0.007	0.193
Sum and ratio ²⁾						
ΣSFA	54.29	50.74	51.90	54.65	8.209	0.214
ΣUFA	45.69	49.25	48.10	45.35	8.194	0.212
ΣMUFA	39.44 ^b	42.05 ^a	42.29 ^a	37.17 ^b	5.58	0.030
ΣPUFA	6.25	7.19	5.81	8.18	3.55	0.33
Σn-3	0.89	1.05	1.08	1.26	0.050	0.20
Σn-6	5.36	6.15	4.73	6.92	3.056	0.35
n-6:n-3	5.99	5.91	4.34	5.54	2.270	0.417
UFA:SFA	0.85	0.98	0.94	0.83	0.011	0.21
PUFA:SFA	0.12	0.14	0.12	0.15	0.001	0.54
IMF (g/100 g)	5.34	5.09	5.23	5.03	0.117	0.220

SEM, standard error of means; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

²⁾ ΣSFA = C14:0+C16:0+C18:0; ΣMUFA = C16:1+C18:1+C18:1 trans-11; ΣUFA = C16:1+C18:1+Σn-3+Σn-6; ΣPUFA = Σn-3+Σn-6; Σn-3 = C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; Σn-6 = C18:2n-6+C20:4n-6; n-6:n-3 = (C18:2n-6+C20:4n-6)÷(C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3).

^{a,b,c} Means having different superscripts along the same row are significantly different (p<0.05).

The shear force in the LD and ST muscles of the control lambs was greater (p<0.05) than that of the supplemented lambs. Dietary treatments had no effect (p>0.05) on the sheer force of SS muscle in Dorper lambs. Regardless of muscle, the shear force decreased (p<0.05) over *postmortem* storage. Interaction between diet and *postmortem* storage on shear force of different muscles in Dorper lambs was not significant (p>0.05).

The LD, ST, and SS muscles of the control lambs had lower (p<0.05) redness than the muscles of the supplemented lambs. Meat redness decreased (p<0.05) as *postmortem* storage progressed. There was no significant interaction (p>0.05) between diet and *postmortem* storage for the redness of meat in Dorper lambs. The lightness of the LD muscle in the control lambs was greater (p<0.05) than that of lambs fed other dietary treatments. The lightness of the ST and SS muscles in the T4 lambs

Table 7. Fatty acid composition (% of total FA) and intramuscular fat (IMF) of *supraspinatus* muscle in Dorper lambs fed diets containing *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination

Parameter	Treatment				SEM	p value
	T1 ¹⁾	T2	T3	T4		
C14:0	3.71	2.72	3.00	4.05	0.558	0.090
C16:0	26.03	24.29	24.03	25.64	2.010	0.176
C16:1	2.97	2.56	2.07	2.98	0.362	0.162
C17:0	1.10	1.07	1.00	1.08	0.015	0.693
C18:0	21.29	19.61	22.01	20.37	10.364	0.738
C18:1n-9	37.59	40.10	38.49	36.09	11.566	0.437
C18:2n-6	3.97	5.70	5.68	5.78	3.620	0.494
C18:3n-3	0.36	0.37	0.39	0.38	0.007	0.970
C20:4n-6	1.78	2.37	2.18	2.41	0.638	0.672
C20:5n-3	0.12	0.28	0.25	0.06	0.027	0.227
C22:5n-3	0.392	0.35	0.35	0.31	0.033	0.947
C22:6n-3	0.24	0.30	0.24	0.42	0.025	0.362
Sum and ratio ²⁾						
ΣSFA	52.55	47.94	50.33	51.54	11.405	0.294
ΣUFA	47.44	52.05	49.67	48.45	11.405	0.294
ΣMUFA	40.57	42.67	40.56	39.07	13.173	0.590
ΣPUFA	6.86	9.38	9.10	9.37	8.663	0.577
Σn-3	1.11	1.31	1.24	1.18	0.114	0.866
Σn-6	5.75	8.07	7.86	8.20	7.224	0.547
n-6:n-3	5.03	6.17	6.44	6.91	2.430	0.407
UFA:SFA	0.91	1.09	0.99	0.94	0.014	0.240
PUFA:SFA	0.13	0.19	0.18	0.18	0.003	0.506
IMF (g/100 g)	5.34	5.32	5.23	5.23	0.310	0.310

SEM, standard error of means. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

²⁾ ΣSFA = C14:0+C16:0+C18:0; ΣMUFA = C16:1+C18:1+C18:1 trans-11; ΣUFA = C16:1+C18:1+Σn-3+Σn-6; ΣPUFA = Σn-3+Σn-6; Σn-3 = C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; Σn-6 = C18:2n-6+C20:4n-6; n-6:n-3 = (C18:2n-6+C20:4n-6)÷(C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3).

was greater than those of lambs fed other dietary treatments. Lightness increased ($p < 0.05$) over *postmortem* storage. No significant interaction ($p > 0.05$) between diet and *postmortem* storage on meat lightness was observed. Dietary treatments had no significant effects ($p > 0.05$) on muscle yellowness in Dorper lambs. The muscle yellowness in LD, ST, and SS muscles on d 7 was lower than that observed on d 0 and 1 *postmortem*. There was no significant interaction ($p > 0.05$) between diet and *postmortem* storage for meat yellowness in Dorper lambs.

The TBARS value in the LD, ST, and SS muscles of the control lambs was greater ($p < 0.05$) than those of supplemented lambs. The concentration of TBARS in LD, ST, and SS muscles of Dorper lambs increased ($p < 0.05$) as *postmortem* storage progressed. Interaction between diet and *postmortem* storage was not significant ($p > 0.05$) for muscle lipid oxidation in Dorper lambs.

Gene expression in muscles

The mRNA expression of lipoprotein lipase (LPL) in LD and ST muscles in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination are presented in Figure 1 and 2, respectively. Dietary supplementation of NS seeds, RO leaves and their combination did not have significant effect ($p > 0.05$) on the mRNA expression of *LPL* gene in the LD (Figure 1) and ST (Figure 2) muscles in Dorper lambs.

The mRNA expression of stearoyl-CoA desaturase (SCD) in LD and ST muscles in Dorper lambs fed diet supplemented with NS seeds, RO leaves and their combination is presented in Figure 3 and 4, respectively. The mRNA expression of SCD in LD (Figure 3) and ST (Figure 4) muscles in Dorper lambs did not differ ($p > 0.05$) among dietary treatments.

The relative expression of sterol regulatory element binding transcription factor 1 (SREBF1) in LD muscle in Dorper lambs was not influenced ($p > 0.05$) by dietary supplementation of RO leaves, NS seeds and their combination (Figure 5). Contrarily, the relative expression of SREBF1 in ST muscle was influenced by dietary supplements (Figure 6). The mRNA expression of SREBF1 in the SM muscle of Dorper lambs fed T2 and T3 diets was greater ($p < 0.05$) than in the SM muscle of the control lambs. The mRNA expression of SREBF1 in the SM muscle of Dorper lambs fed diet supplemented with blend of RO leaves and NS seeds was not significantly different ($p > 0.05$) from those fed other dietary treatments.

The expression of the AMP-activated protein kinase alpha 2 (*PRKAA2*) gene in the LD (Figure 7) and SM (Figure 8) muscles of Dorper lambs differ ($p < 0.05$) among the dietary treatments. The relative expression of *PRKAA2* in LD muscle of Dorper lambs fed T2 and T3 diets was greater ($p < 0.05$) than in the LD muscle of the control lambs. The mRNA expression of *PRKAA2* in the LD muscle of Dorper lambs fed diet supplemented with blend of RO leaves and NS seeds was not significantly different ($p > 0.05$) from those fed other dietary treatments. The relative expression of *PRKAA2* was greater ($p < 0.05$) in the ST muscle of lambs fed diet supplemented with NS seeds compared with those fed the control diet and T4 diet. The expression of *PRKAA2* in the ST muscle of lambs fed diet supplemented with RO leaves did not differ ($p > 0.05$) from that in the ST muscle of lambs fed other dietary treatments.

DISCUSSION

Dorper lambs fed 1% RO leaves had greater final body weight compared with those fed other diets. This observation could be attributed to the greater feed intake and efficiency in the T2 lambs as observed during the feeding trial. The greater slaughter weight in the T2 lambs could be responsible for their greater cold carcass weight. The current observation concurs with the findings of Allam et al [11] who observed that dietary RO improved final body weight in Awassi lambs. Despite the

Table 8. Physicochemical properties and lipid oxidation in *longissimus dorsi*, *semitendinosus* and *supraspinatus* muscles in Dorper lambs fed diet supplemented with *Rosmarinus officinalis* leaves, *Nigella sativa* seeds and their combination

Parameter	Muscle	Dietary treatments				SEM	Storage days			SEM	p value		
		T1 ¹⁾	T2	T3	T4		0	1	7		Diet (D)	Storage (S)	DxS
pH (unit)	LD	5.92	5.99	5.99	5.92	0.03	6.11 ^a	5.90 ^b	5.86 ^b	0.03	0.228	<0.0001	0.956
	ST	5.95	6.05	6.01	6.00	0.03	6.10 ^a	6.00 ^b	5.91 ^c	0.03	0.223	0.001	0.838
	SS	6.15	6.19	6.18	6.12	0.03	6.20 ^a	6.10 ^b	5.95 ^c	0.03	0.4626	<0.0001	0.982
Drip loss (%)	LD	5.19 ^a	3.28 ^b	3.27 ^b	2.95 ^c	0.08	-	3.80 ^a	2.40 ^b	0.23	0.002	0.002	0.240
	ST	3.51 ^a	3.50 ^a	2.16 ^b	2.11 ^b	0.05	-	3.93 ^a	2.58 ^b	0.12	0.032	0.006	0.112
	SS	3.64 ^a	2.36 ^b	2.89 ^b	2.25 ^b	0.07	-	3.95 ^a	2.00 ^b	0.23	0.003	0.015	0.321
Cooking loss (%)	LD	33.26 ^a	31.18 ^b	29.95 ^b	30.44 ^b	0.81	24.72 ^c	27.69 ^b	41.21 ^a	0.70	0.034	<0.0001	0.783
	ST	39.54 ^a	37.81 ^b	37.95 ^b	36.72 ^b	0.92	27.75 ^c	37.01 ^b	47.01 ^a	0.79	0.019	<0.0001	0.079
	SS	32.67	31.33	32.84	34.61	1.20	27.83 ^b	30.56 ^b	40.19 ^a	1.03	0.305	<0.0001	0.148
Shear force (kg)	LD	1.10 ^a	1.03 ^b	1.03 ^b	0.93 ^c	0.05	1.26 ^a	1.00 ^b	0.80 ^c	0.04	0.031	<0.0001	0.439
	ST	1.25 ^a	1.13 ^b	1.15 ^b	0.98 ^b	0.06	1.22 ^a	1.20 ^a	0.97 ^b	0.05	0.027	0.002	0.414
	SS	0.76	0.76	0.71	0.76	0.03	0.90 ^a	0.70 ^b	0.49 ^c	0.03	0.551	0.166	0.260
Yellowness (b*)	LD	11.07	11.91	11.46	11.32	0.13	11.77 ^a	11.87 ^a	9.93 ^b	0.11	0.271	<0.0001	0.091
	ST	10.69	9.92	9.60	10.10	0.19	10.73 ^a	10.73 ^a	9.55 ^b	0.17	0.201	<0.0001	0.210
	SS	11.70	11.34	11.04	11.65	0.26	11.11 ^b	12.13 ^a	11.05 ^b	0.23	0.2587	0.0023	0.672
Lightness (L*)	LD	38.62 ^a	35.44 ^b	34.41 ^b	32.67 ^c	0.53	31.43 ^c	36.29 ^b	38.14 ^a	0.46	<0.0001	<0.0001	0.148
	ST	41.74 ^a	40.97 ^a	40.69 ^a	39.00 ^b	0.57	39.97 ^b	40.18 ^b	41.65 ^a	0.48	0.012	0.041	0.551
	SS	38.11 ^a	38.83 ^a	38.97 ^a	36.71 ^b	0.42	34.58 ^b	39.52 ^a	40.36 ^a	0.37	0.0021	<0.0001	0.174
Redness (a*)	LD	12.98 ^b	13.95 ^a	13.72 ^a	14.08 ^a	0.24	14.39 ^a	12.67 ^b	11.45 ^b	0.21	0.012	<0.0001	0.142
	ST	13.18 ^b	14.55 ^a	14.64 ^a	14.83 ^a	0.24	14.63 ^a	13.68 ^b	12.34 ^c	0.21	<0.0001	<0.0001	0.094
	SS	13.02 ^b	14.72 ^a	14.26 ^a	14.49 ^a	0.21	14.94 ^a	13.95 ^b	12.73 ^c	0.18	<0.0001	<0.0001	0.250
TBARS (mg MDA/kg)	LD	0.53 ^a	0.42 ^b	0.39 ^b	0.41 ^b	0.01	0.32 ^b	0.32 ^b	0.72 ^a	0.04	0.0428	<0.0001	0.992
	ST	0.56 ^a	0.40 ^b	0.37 ^b	0.41 ^b	0.01	0.34 ^a	0.34 ^a	0.57 ^b	0.05	0.039	0.014	0.877
	SS	0.58 ^a	0.45 ^b	0.41 ^b	0.44 ^b	0.01	0.41	0.41	0.55	0.02	0.033	0.100	0.993

SEM, standard error of means; LD, *longissimus dorsi*; ST, *semitendinosus*; SS, *supraspinatus*.

¹⁾ T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

^{a,b,c} Means having different superscripts along the same row for each factor are significantly different (p < 0.05).

changes in slaughter and cold carcass weights among the treatments, chill loss, dressing percentage, percentages of shoulder, breast, neck and legs and the proportion of lean, bone and fat in the primal cuts of Dorper lambs did not differ. This observation suggests that the dietary supplements did not affect tissue partitioning in Dorper lambs. The current observation

is consistent with that of Hassan et al [10] who observed that dietary supplementation of NS (7.5 g NS/kg DM) had no effect on the carcass traits in Karadi lambs.

Herein, dietary supplementation of medicinal plants did not affect IMF and carcass fatness in Dorper lambs. This suggests that the muscle FA composition was not confounded by IMF and carcass fatness. The similar IMF and carcass fat-

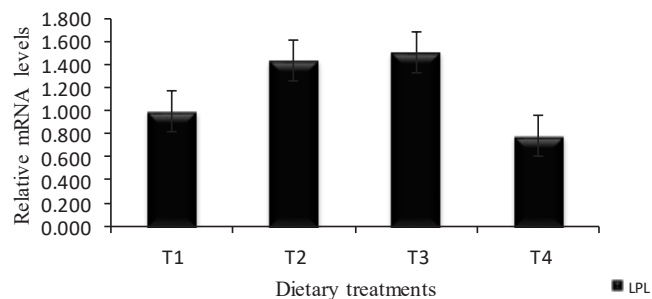


Figure 1. The relative expressions of lipoprotein lipase (LPL) target gene in *longissimus dorsi* of Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

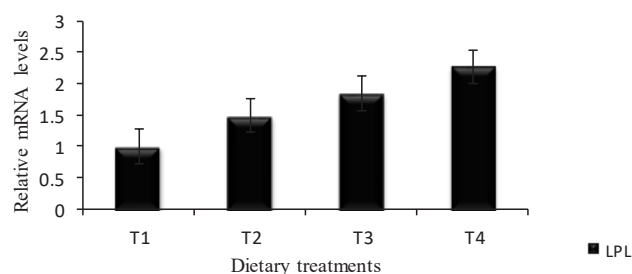


Figure 2. The relative expressions of lipoprotein lipase (LPL) target gene in *semitendinosus* muscle in of Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

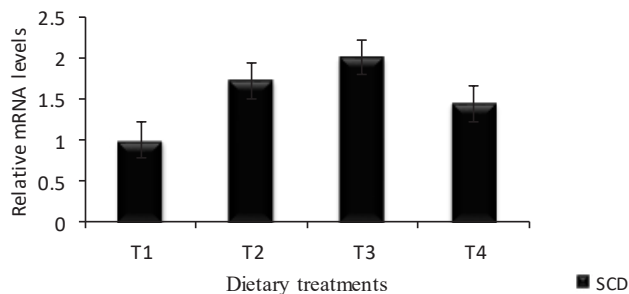


Figure 3. The relative expressions of stearoyl-CoA desaturase (*SCD*) target gene in *longissimus dorsi* in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

ness could be due to the similar energy content of the dietary treatments. Irrespective of dietary treatment and muscle type, C18:1n-9 was the most abundant FA followed by C16:0 and C18:0. Similar observation was documented in chevon [23] and lamb meat [6].

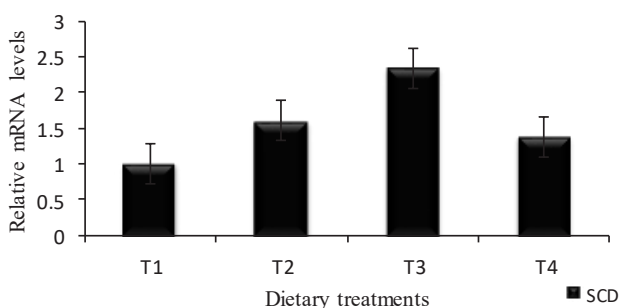


Figure 4. The relative expressions of stearoyl-CoA desaturase (*SCD*) target gene in *semitendinosus* muscle in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

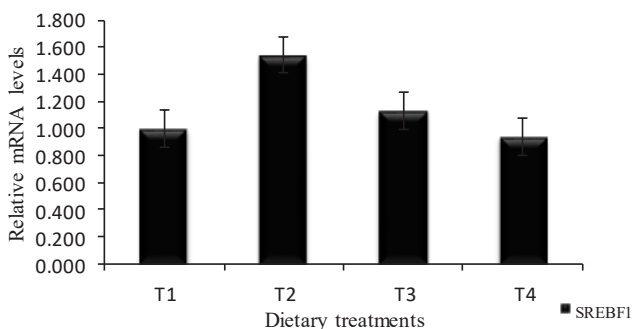


Figure 5. The relative expressions of sterol regulatory element-binding transcription factor 1 (*SREBF1*) target gene in *longissimus dorsi* of Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves.

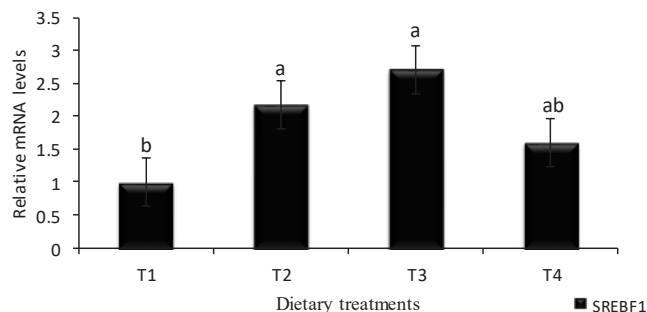


Figure 6. The relative expressions of sterol regulatory element-binding transcription factor 1 (*SREBF1*) target gene in *semitendinosus* muscle in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves. ^{a,b,c} Means with different superscript are significantly different ($p < 0.05$).

The muscle FA of Dorper lambs fed diets supplemented with medicinal plants was inconsistent. The FA content of SS muscle was unaffected by dietary supplements. This observation is

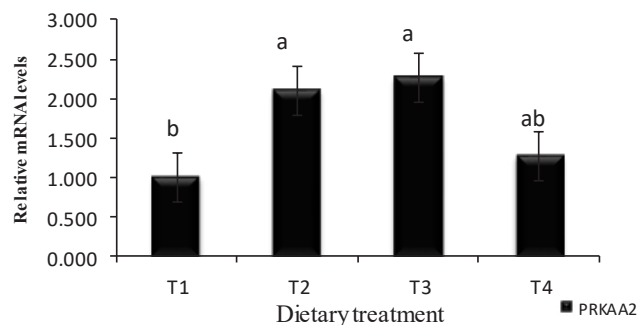


Figure 7. The relative expressions of AMP-activated protein kinase alpha 2 (*PRKAA2*) target gene in *longissimus dorsi* muscle in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves. ^{a,b,c} Means with different superscript are significantly different ($p < 0.05$).

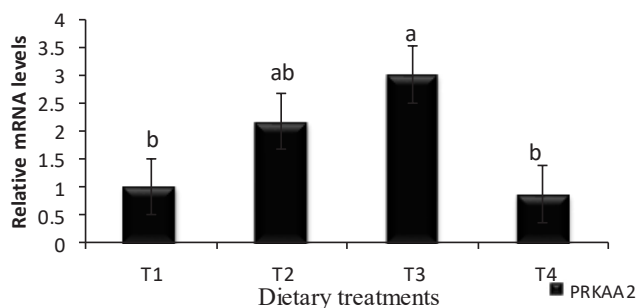


Figure 8. The relative expressions of AMP-activated protein kinase alpha 2 (*PRKAA2*) target gene in *semitendinosus* muscle in Dorper lambs fed *Nigella sativa* seeds, *Rosmarinus officinalis* leaves or their blend. T1, basal diet; T2, basal diet+1% *Rosmarinus officinalis* leaves; T3, basal diet+1% *Nigella sativa* seeds; T4, basal diet+1% *Nigella sativa* seeds+1% *Rosmarinus officinalis* leaves. ^{a,b,c} Means with different superscript are significantly different ($p < 0.05$).

consistent with that of Karami et al [5], who reported that dietary supplementation of turmeric and *Andrographis paniculata* leaves had minimal impact on the muscle FA composition of *longissimus dorsi* muscle in Kacang goats. In contrast, dietary thyme in pregnant and lactating Segurena ewes increased the concentration of polyunsaturated FAs in the meat from the lambs [6].

The changes in the concentration of C18:1n-9 in the LD muscles of Dorper lambs could be attributed to the changes in the ruminal concentration of the FA. The ruminal concentration of C18:1n-9 was greater in the rumen of the T2 lambs as observed during the feeding trial. Dietary supplementation of medicinal plants reduced the concentration of C18:0 and increased the concentration of C18:1 in the ST muscle of Dorper lambs. This observation could be attributed to the phenolic compounds in the supplements, which have the capacity to reduce the biohydrogenation of FAs in the rumen. Similar observation was reported in the LD muscle of goats fed different parts of *Andrographis paniculata* [12].

Diets had no effect on the muscle pH in Dorper lambs. This could be attributed to the similar energy content of the dietary treatments and the similar management and slaughter conditions employed during the trial. The pH values observed in the current study fall within the pH of normal meat as reported in goats [1] and beef [24]. The current observation corroborates the findings of Karami et al [5] who observed that dietary *Andrographis paniculata* and turmeric powder had no effect on muscle pH in chevon. Contrarily, dietary supplementation of quercetin increased the pH of *longissimus* muscle in Holstein Friesian cattle [24]. *Postmortem* storage influenced muscle pH in Dorper lambs. The pre-rigor pH was greater than the post-rigor pH. This observation could be attributed to *postmortem* glycolysis, which requires the conversion of glycogen to lactic acid [25]. Similar observation was observed in chevon [1].

The supplementation of NS seeds, RO leaves and their blend reduced drip loss in different muscles in Dorper lambs. However, the impact of dietary medicinal plants on cooking loss in mutton was muscle dependent. Dietary supplements reduced cooking loss in LD and ST muscles but had no effect on the cooking loss in SS muscle in Dorper lambs. The reduction in drip and cooking losses could be due to the presence of antioxidant compounds in the supplements, which reduced the oxidation of myofibrillar proteins during *postmortem* chill storage. The current finding is consistent with that of Yusuf [12] who observed that dietary supplementation of *Andrographis paniculata* in goats reduced cooking loss in chevon. In contrast, dietary quercetin did not affect the drip and cooking losses in *longissimus* muscle of beef cattle [24]. Cooking and drip losses increased over *postmortem* chill storage. This observation could be due to the loss of the structural integrity of the myofibrils [25]. At rigor, the muscle pH nears the iso-

electric point of most proteins thereby affecting their ability to hold water [26]. This observation could also be due to stearic effects, in which there is a reduction in the available space for water resulting from the formation of crosslinks between thin and thick filaments during the development of rigor [25,26]. The increase in drip loss over chill storage is in tandem with the report in goats [26]. However, cooking loss was reduced [26] during *postmortem* storage of chevon.

The LD and ST muscles in supplemented lambs had lower shear force than those from the control lambs. The higher tenderness in the meat of supplemented lambs could be due to the lower cooking loss of the meat samples. Adequate water in muscle increases juiciness on mastication, which enhances tenderness [25,26]. Reduced cooking loss would possibly enhance tenderness because a given cross-sectional area of a meat sample would have less structural components and more water [26]. In line with the current observation, Yusuf [12] observed that dietary *Andrographis paniculata* improved tenderness in chevon. In addition, dietary supplementation of quercetin improved the tenderness of *longissimus* muscle of beef cattle [24]. Contrarily, dietary treatments had no effect on the shear force value of SS muscle in Dorper lambs. The shear force of different muscles reduced over chill storage. This observation could be attributed to the weakening of myofibrillar structures by endogenous muscle proteinases [25,26].

The meat from the supplemented lambs had greater redness than the meat from the control lambs. This observation could be due to the antioxidant effect of the polyphenols in the supplements, which prevented oxidative deterioration as depicted in the TBARS data. Similarly, dietary *Moringa oleifera* [7] turmeric and *Andrographis paniculata* [5] leaves improved the redness of chevon. Contrarily, dietary quercetin did not affect the colour coordinates of *longissimus* muscle in Holstein Friesian cattle [24]. Chill storage influenced the colour coordinates of mutton. The redness and yellowness of chevon decreased while the lightness increased over chill storage. This finding could be due to the oxidative deterioration of myoglobin during chill storage. A decrease in the concentration of myoglobin and an increase in the concentration of met-myoglobin play a major role in the loss of redness in meat during chill storage [26].

Dietary supplementation of medicinal plants reduced lipid oxidation in different muscles in Dorper lambs. This observation could be attributed to the presence of polyphenols in the medicinal plants, which exert anti-oxidative effect. Similar findings were observed in goats fed turmeric and *Andrographis paniculata* [5] and *Moringa oleifera* [7] leaves. However, dietary quercetin did not affect the TBARS values in *longissimus* muscle in Holstein Friesian cattle [24]. Lipid oxidation increased over chill storage. This could be due to the loss of endogenous antioxidants in the meat samples. Similar observations were documented in beef [27] and chevon [1].

Diets can alter the synthesis and deposition of FA in muscles which can modulate the expression of lipogenic genes [13,14]. It is expected that a better knowledge of the genes and mechanisms involved would allow a better control of the content and composition of FA in ruminant meat [14]. In the current study, we limited our investigation on lipogenic gene expression to the LD and ST muscles because dietary supplements had no significant effect on the FA composition of SS muscles in Dorper lambs.

Dietary supplementation of medicinal plants did not affect the expression of *LPL* gene in LD and ST muscles in Dorper lambs. This observation could be attributed to the similar muscle IMF among the treatments. *LPL* is the rate limiting enzyme for the import of triglyceride (TAG) derived FAs by muscle, for utilization, and adipose tissue, for storage, and plays an important role in the differentiation and maturation of adipose cells [28]. Lipoprotein lipase serves as a central factor in hydrolysis of triacylglycerol and uptake of free FAs from the plasma [29]. Moreover, it controls the triglycerides partitioning between adipose tissue and muscles [30]. The current observation is consistent with those of Anderson et al [31] and Bonnet et al [32] who observed that IMF deposition had a positive relationship with *LPL* gene expression in sheep.

The expression of *SCD* is physiologically important in the synthesis and metabolism of fat and plays a vital role in energy homeostasis [14]. Dietary NS seeds, RO leaves and their combination did not affect the expression of *SCD* gene in LD and ST muscles in Dorper lambs. This observation suggests that the changes in the monounsaturated FAs content in the muscles are of dietary origin. In addition, the similarity in IMF among the diets could be responsible for the non-significant differences in *SCD* expression. Similarly, dietary alfalfa hay or concentrate did not affect the IMF and *SCD* expression despite changes in the FA profile of *longissimus dorsi* in lambs [14].

The *SREBF1* is a member of the basic helix-loop-helix-leucine zipper family of transcription factors involved in adipocyte differentiation, biosynthesis of FAs and cholesterol [33] and plays an important role in energy homeostasis [34]. The expression of sterol regulatory element-binding protein 1 (*SREBP1*) in LD muscle of Dorper lambs did not differ among dietary treatments. Nonetheless, dietary supplementation of medicinal plants influenced the expression of *SREBP1* in ST muscles in Dorper lambs. Dietary supplementation of NS seeds and RO leaves up regulated the expression of *SREBP1* gene in ST muscle in Dorper lambs compared with that of the control lambs. This observation could be attributed to the greater concentration of C18:1n-9 and C18:3n-3 in the T3 and T2 lambs compared to the control lambs. The relative expression of *SREBP1* in the ST muscles in Dorper lambs fed diet supplemented with blend of RO leaves and NS seeds did not differ from those fed other diets. This observation is consistent with

the concentration of C18:1n-9 and C18:3n-3 in the T4 lambs, which was not significantly different from those of lambs, fed other dietary treatments. The current observation is consistent with the report of Bhuiyan et al [34] who observed that the expression of *SREBP1* had a positive relationship with the C18:1n-9 and polyunsaturated fatty acids contents in different muscles in Hanwoo cattle.

The *PRKAA2* plays an important role in the regulation of FA and cholesterol [35]. Dietary supplementation of medicinal plants affected the expression of *PRKAA2* gene in LD and ST muscles in Dorper lambs. Lambs fed the T2 and T3 diets had greater expression of *PRKAA2* gene in LD muscle than the lambs fed the control and blend of RO leaves and NS seeds. Similar trend was observed in the ST muscle of Dorper lambs. The current observation could be attributed to the greater proportion of C18:1n-9 in the muscle of lambs fed T2 and T3 diets compared to those fed the control and T4 diets. This observation contradicts the findings of Dervishi et al [13] who observed that differences in feeding system and FA composition of muscles did not alter the expression of *PRKAA2* gene in lambs.

CONCLUSION

Dietary supplementation of NS seeds, RO leaves and their combination can be used to enhance water holding capacity, oxidative stability and tenderness of mutton. The muscle-dependent changes in FA composition in response to dietary supplements, induced changes in the expression of lipogenic genes. These results provide insight into the mechanisms involved in diet-induced changes in the muscle FA composition of Dorper lambs.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Higher Education, Malaysia through Fundamental Research Grant Scheme (Project code: 07-01-14-1436FR). The funder had no role in carrying out the experiment, writing of the manuscript and the decision to submit the manuscript for publication.

REFERENCES

1. Adeyemi KD, Shittu RM, Sabow AB, Ebrahimi M, Sazili AQ. Influence of diets and *postmortem* ageing on oxidative stability of lipids, myoglobin, and myofibrillar proteins and quality attributes of *gluteus medius* muscle in goats. Plos One 2016;11:

- e0154603.
2. Bouvard V, Loomis D, Guyton KZ, et al. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* 2015; 16:1599-600.
 3. Jeong SH, Kang D, Lim MW, Kang CS, Sung HJ. Risk assessment of growth hormones and antimicrobial residues in meat. *Toxicol Res* 2010;26:301-13.
 4. Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Domínguez-Díaz D, González-Ríos H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. *J Anim Sci Technol* 2017;59:8.
 5. Karami M, Alimon AR, Sazili AQ, Goh YM, Ivan M. Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of longissimus muscle in Kacang goat with ageing time. *Meat Sci* 2011;88:102-8.
 6. Nieto G, Bañón S, Garrido MD. Incorporation of thyme leaves in the diet of pregnant and lactating ewes: effect on the fatty acid profile of lamb. *Small Rum Res* 2012;105:140-7.
 7. Qwele K, Hugo A, Oyedemi SO, et al. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Sci* 2013;93:455-62.
 8. Ahmad A, Husain A, Mujeeb M, et al. Review on therapeutic potential of *Nigella sativa*: a miracle herb. *Asian Pac J Trop Biomed* 2013;3:337-52.
 9. Aruoma OI, Spencer JP, Rossi R, et al. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal herbs. *Food Chem Toxicol* 1996;34:449-56.
 10. Hassan AS, Hassan MK, Al-Rubeii A. Carcass yield and characteristics of Karadi lambs as affected by dietary supplement of rumen undegradable nitrogen fed with *Nigella sativa*. *Afr J Biotech* 2013;10:1491-5.
 11. Allam SM, Abou-Ammou FF, Farghaly MS, Othman AA. Effect of some natural antioxidants on lamb performance. 1. Carcass characteristics of lambs fed partial full fat soybean with natural additives. *Egyptian J Nutr Feed* 2005;8:275-9.
 12. Yusuf AL. Evaluation of dietary supplementation of *Andrographis paniculata* on growth performance and meat quality of Boer goats [PhD thesis]. Serdang, Malaysia: Universiti Putra Malaysia; 2014.
 13. Dervishi E, Serrano C, Joy M, et al. The effect of feeding system in the expression of genes related with fat metabolism in semi-tendinosus muscle in sheep. *Meat Sci* 2011;89:91-7.
 14. González-Calvo L, Joy M, Blanco M, et al. Effect of vitamin E supplementation or alfalfa grazing on fatty acid composition and expression of genes related to lipid metabolism in lambs. *J Anim Sci* 2015;93:3044-54.
 15. National Research Council. Nutrient requirements of small ruminant. 6th edition. Washington, DC, USA: National Academy Press; 2007.
 16. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 18th ed. Washington DC, USA: AOAC International; 2007.
 17. Van Soest PV, Robertson J, Lewis B. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991;74:3583-97.
 18. Makkar HP, Blümmel M, Borowy NK, Becker K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J Sci Food Agric* 1993;61: 161-5.
 19. MS1500: 2009. (2nd revision) Halal food production, preparation, handling and storage-general guideline. Putrajaya, Malaysia: Department of Standards Malaysia; 2009. pp. 1-13.
 20. Adeyemi KD, Ebrahimi M, Samsudin AA, Sabow AB, Sazili, AQ. Carcass traits, meat yield and fatty acid composition of adipose tissues and *Supraspinatus* muscle in goats fed blend of canola oil and palm oil. *J Anim Sci Technol* 2015;5:1-17.
 21. Lokman NS, Sabow AB, Abubakar AA, Adeyemi KD, Sazili AQ. Comparison of carcass and meat quality in goats subjected to pre-slaughter head-only electrical stunning or slaughtered without stunning. *CyTA J Food* 2017;15:99-104.
 22. SAS, Statistical Analysis System package (SAS) Version 9.2 software. Cary, NC, USA: SAS Institute Inc.; 2003.
 23. Adeyemi KD, Sabow AB, Shittu RM, et al. Impact of chill storage on antioxidant status, lipid and protein oxidation, color, drip loss and fatty acids of semimembranosus muscle in goats. *CyTA J Food* 2016;14:405-14.
 24. Kang MG, Kim HJ, Jang AR, et al. Effect of dietary supplementation of quercetin on antioxidant activity and meat quality of beef cattle. *CNU J Agric Sci* 2012;39:61-8.
 25. Lawrie R, Ledward D. Lawrie's meat science. 7th ed. Cambridge, UK: Woodhead Publishing Ltd; 2006.
 26. Adeyemi KD, Shittu RM, Sabow AB, et al. Comparison of myofibrillar protein degradation, antioxidant profile, fatty acids, metmyoglobin reducing activity, physicochemical properties and sensory attributes of *gluteus medius* and *infraspinatus* muscles in goats. *J Anim Sci Technol* 2016;58:23.
 27. Popova T, Marinova P, Vasileva V, Gorinov Y, Lidji, K. Oxidative changes in lipids and proteins in beef during storage. *Arch Zootech* 2009;3:30-8.
 28. Weinstock PH, Levak-Frank S, Hudgins LC, et al. Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. In: Proceedings of National Academy of Science, 1997; 1997 September 16; National Academy of Science; 1997;94:10261-6.
 29. Zhao W, Hu S, Yu K, et al. Lipoprotein lipase, tissue expression and effects on genes related to fatty acid synthesis in goat mammary epithelial cells. *Int J Mol Sci* 2014;15:22757-71.
 30. Sorisky A. From preadipocyte to adipocyte: differentiation-directed signals of insulin from the cell surface to the nucleus. *Crit Rev Clin Lab Sci* 1999;36:1-34.
 31. Andersen MK, Bailey JW, Wilken C, Rule DC. Lipoprotein lipase and glycerophosphate acyltransferase in ovine tissues

- are influenced by growth and energy intake regimen. *J Nutr Biochem* 1996;7:610-6.
32. Bonnet M, Leroux C, Faulconnier Y, Martin P, Chilliard Y. Lipoprotein lipase activity and mRNA are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. *J Nutr* 2000;130:749-57.
33. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997;89:331-40.
34. Bhuiyan MS, Yu SL, Jeon JT, et al. DNA polymorphisms in SREBF1 and FASN genes affect fatty acid composition in Korean cattle (Hanwoo). *Asian-Australas J Anim Sci* 2009;22:765-73.
35. Lee, HY, Choi BH, Lee JS, et al. Molecular characterization and chromosomal mapping of the porcine AMP-activated protein kinase α 2 (PRKAA 2) Gene. *Asian-Australas J Anim Sci* 2007;20:615-21.