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

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**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*

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**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 ×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 | 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.

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

| Fatty acid | Dietary treatment |
|-----------|-------------------|
|           | T1 | 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 FA1)             |    |    |    |    |
| 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.

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 epimysial 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 epimysial 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 iTQMSYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Each 20 μL PCR reaction contained 10 μL 2× 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°C/min, and the fluorescence was measured at every 5 s interval to construct the melting curve. The comparative CT method (ΔΔCT) 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 ΔΔCT 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 (°C) | Accession No. |
|----------|---------------|---------|---------------|-----------------------------|---------------|
| 1        | LPL           | F-5′aatgaagagaagtgaacaggaagc-3′  
R-5′gacctcccacacaggtgt-3′ | 119 | 60 | NM_001009394 |
| 2        | SCD           | F- 5′cccaagtctagagaaaagg-3′ 
R- 5′gaaagacaaacagcagga-3′ | 115 | 60 | AJ001048 |
| 3        | SREBF1        | F-5′ctgctatgcaggcagc-3′  
R-5′ggttgatgggcagc-3′ | 99 | 60 | GU206528 |
| 4        | YWHAZ         | F-5′ttaggaggccggctgccctgct-3′  
R-5′ttctctttgattctggctccact-3′ | 102 | 60 | AY970970 |
| 5        | PRKAA2        | F-5′acctcccccttgagatgta-3′  
R-5′ggcaacagcaggtga-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.
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 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 4. Carcass traits in Dorper lambs fed diets containing Nigella sativa seeds, Rosmarinus officinalis leaves and their combination

| Parameter                        | T1 | T2 | T3 | T4 | SEM | p value |
|----------------------------------|----|----|----|----|-----|---------|
| Slaughter weight (kg)            | 31.97<sup>b</sup> | 34.80<sup>a</sup> | 31.85<sup>b</sup> | 33.00<sup>b</sup> | 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<sup>a</sup> | 14.10<sup>a</sup> | 13.17<sup>a</sup> | 13.95<sup>a</sup> | 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<sup>b</sup> | 68.36<sup>b</sup> | 72.15<sup>a</sup> | 68.99<sup>b</sup> | 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.

<sup>1</sup>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.

<sup>2</sup>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 and ST muscles 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.

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 shear 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.
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.

| Parameter | Treatment | SEM | p value |
|-----------|-----------|-----|---------|
| IMF (g/100 g) | T1<sup>1</sup> | 5.34 | 0.32 | 0.32 | 0.32 |
| | T2 | 5.32 | 0.32 | 0.32 |
| | T3 | 5.23 | 0.32 | 0.32 |
| | T4 | 5.23 | 0.32 | 0.32 |

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

<sup>1</sup>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.

<sup>2</sup>ΣSFA = C14:0+C16:0+C18:0; ΣMUFA = C16:1+C18:1+C18:2-trans 11; ΣPUFA = C16:1+C18:1+3Σn-3+Σn-6; ΣSFA = 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).

### 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 |
|-----------|-----------|-----|---------|
| C14:0     | T1<sup>1</sup> | 3.71 | 0.22 | 0.558 | 0.090 |
| | T2 | 2.72 | 0.22 | 0.558 |
| | T3 | 3.00 | 0.22 | 0.558 |
| | T4 | 4.05 | 0.22 | 0.558 |
| C16:0     | T1<sup>1</sup> | 26.03 | 0.18 | 2.100 | 0.176 |
| | T2 | 24.29 | 0.18 | 2.100 |
| | T3 | 24.03 | 0.18 | 2.100 |
| | T4 | 25.64 | 0.18 | 2.100 |
| C16:1     | T1<sup>1</sup> | 0.28 | 0.24 | 0.027 | 0.227 |
| | T2 | 0.28 | 0.24 | 0.027 |
| | T3 | 0.25 | 0.24 | 0.027 |
| | T4 | 0.25 | 0.24 | 0.027 |

### 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 PKAA2 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 PKAA2 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 PKAA2 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 PKAA2 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
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-

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  |
|--------------------|--------------|--------------------|------|--------------|-----|----------|
|                    |              |                    |      | 0            | 1   |          |
| pH (unit)          | LD           | T1<sup>B</sup>     | 5.92 | 6.11         | 0.03| 0.228    |
|                    |              | T2<sup>A</sup>     | 5.99 | 6.00         | 0.02| 0.223    |
| Drip loss (%)      | LD           | T1<sup>B</sup>     | 3.28 | 3.50         | 0.03| 0.462    |
|                    |              | T2<sup>A</sup>     | 3.27 | 3.50         | 0.04| 0.462    |
| Cooking loss (%)   | LD           | T1<sup>B</sup>     | 29.95| 27.69        | 0.08| 0.002    |
|                    |              | T2<sup>A</sup>     | 30.44| 27.69        | 0.05| 0.002    |
| Shear force (kg)   | LD           | T1<sup>B</sup>     | 1.03 | 1.06         | 0.06| <0.0001  |
|                    |              | T2<sup>A</sup>     | 1.15 | 1.06         | 0.05| <0.0001  |
| Yellowness (b*)    | LD           | T1<sup>B</sup>     | 11.46| 11.05        | 0.22| 0.035    |
|                    |              | T2<sup>A</sup>     | 11.32| 11.05        | 0.23| 0.035    |
| Lightness (L*)     | LD           | T1<sup>B</sup>     | 35.44| 36.29        | 0.12| 0.03     |
|                    |              | T2<sup>A</sup>     | 34.11| 36.29        | 0.23| 0.03     |
| Redness (a*)       | LD           | T1<sup>B</sup>     | 38.32| 40.18        | 0.04| 0.04     |
|                    |              | T2<sup>A</sup>     | 39.97| 40.18        | 0.48| 0.04     |
| TBARS (mg MDA/kg)  | LD           | T1<sup>B</sup>     | 0.42 | 0.49         | 0.18| 0.23     |
|                    |              | T2<sup>A</sup>     | 0.39 | 0.49         | 0.23| 0.23     |

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

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 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.
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].

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

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**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.

**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. Means with different superscript are significantly different (p<0.05).

**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. 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. 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 Doper 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 isoelectric 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].
Dietary supplementation of medicinal plants did not affect the expression of SREBP1 in the ST muscles in Dorper lambs. This 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 Bhuiany 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.

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.

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