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ORIGINAL ARTICLE

The effect of grapeseed oil on performance, rumen fermentation, antioxidant status and subcutaneous adipose fatty acid profile in lambs

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Summary

This experiment was carried out to examine the effect of grapeseed oil (GSO) on performance, rumen fermentation, antioxidant status and subcutaneous adipose fatty acid (FA) profile in lambs. Eighteen *Baluchi* lambs, 196 ± 14 days of age and 39.8 ± 1.7 kg body weight, were randomly assigned to three experimental diets: (i) diet without GSO (control), (ii) diet containing 2% of GSO (GSO2) and (iii) diet containing 4% of GSO (GSO4) for 42 days. Results showed that the experimental diets had no significant effect on dry matter intake and performance ($p > 0.05$). The supplemented diets with GSO had no effect on pH and NH_3-N of rumen fluid ($p > 0.05$), but GSO4 increased ($p = 0.003$) the concentration of propionic acid and reduced ($p = 0.002$) the concentration of acetate acid compared to the control. Inclusion 4% of GSO to the diet increased total antioxidant activity and decreased malondialdehyde in serum and muscle ($p < 0.001$). The level of blood glutathione peroxidase in diets containing GSO was higher than control diet ($p = 0.02$), but diets had no effect on superoxide dismutase in blood and muscle tissues ($p > 0.05$). The concentration of vaccenic acid, rumenic acid and linoleic acid and thus polyunsaturated fatty acid in subcutaneous fat was affected by experimental diets ($p < 0.001$), as control had the lowest amount of these FAs and GSO4 had the highest amount of them. Therefore, it can be concluded that the use of GSO up to 4% improves the antioxidant status and adipose fatty acid profile in lambs without effects on performance.

Keywords grapeseed oil, antioxidant status, subcutaneous fatty acid profile, rumen fermentation, lamb

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Introduction

Ruminant meat is characterized by high contents of saturated fatty acids (SFAs) and low levels of polyunsaturated fatty acids (PUFAs), which has been linked with a high cardiovascular diseases risk in humans (Givens, 2005). Diet is considered as very important factor to change the FA composition. Therefore, the use of plant oils having unsaturated fatty acid (UFA) such as soya bean, safflower, sunflower and linseed can change the FA profile of ruminant products (Nudda et al., 2015). Also, diet polyphenols can affect some bacterial strains involved in the biohydrogenation pathway of FA and change the composition of FA (Vasta et al., 2009, 2010). In particular, it has been shown that polyphenols can inhibit the proliferation and activity of *Butyrivibrio proteoclasticus*, which is involved in the last step of biohydrogenation of PUFA, which consists of the enzymatic reduction of vaccenic acid (VA) to stearic acid (Buccioni et al., 2015). The consequent ruminal accumulation of PUFA and their biohydrogenation intermediates (Khiaosa-Ard et al., 2009; Vasta et al., 2009) could enhance the extent of rumen escape of these FAs and, consequently, could increase their concentrations in adipose tissues (Moate et al., 2014; Buccioni et al., 2015). Increasing the content of unsaturated fatty acids in milk could also increase susceptibility to oxidation. Therefore, the addition of antioxidants to PUFA-supplemented diets to improve milk quality seems to be an advisable practice. (Manso et al., 2015). Many of the antioxidants used in the food industry are chemically synthesized (Singh et al., 2005). Natural antioxidants which derived from edible materials are in high demand for

food applications amidst concerns over safety and toxicity of the consumption of these synthetic antioxidants (Ito et al., 1985).

In 2013, Iran produced more than two million metric tons of grapes (FAOSTAT, 2013). As grapeseeds consist approximately 5% of the fruit weight (Choi and Lee, 2009), so around 100 kton of grapeseeds is discarded annually in Iran.

Grapeseed oil is produced from the seeds in the pomace left over from juice and wine production by cold pressing method and thus adds value to the industry. This agricultural by product contain 10–20% oil (Crews et al., 2006), so annual production of GSO is approximately 10–20 kton in Iran and its nutritive value can be similar to other oil sources such as sunflower, flax and fish oils. An important characteristic of GSO is having very high amount of linoleic acid and presence of tocopherols and tocotrienols which exhibit strong antioxidant activity (Beveridge et al., 2005; Choi and Lee, 2009). In addition, grapeseed has higher level of polyphenol compounds such as proanthocyanidins, mainly composed of catechin, epicatechin, gallic acid and polymeric and oligomeric procyanidins, which can be recovered by extraction (Monagas et al., 2003). So GSO can consider an alternative for other oil sources in the diet of ruminants to improve meat and milk quality. Jerónimo et al. (2012) found that adding grapeseed extract had no effect on lamb muscle FAs but reduces oxidation of meat lipids by lowering concentration of malondialdehyde (MDA). Therefore, it seems that GSO due to having high levels of linoleic acid, in addition to its capability to increase PUFA, it can also act as a natural antioxidant. According to our information, while a few studies have been conducted on grape by-products in ruminant, there is no study about using GSO in ruminant nutrition. This study was carried out to evaluate the effect of GSO on performance, rumen fermentation, antioxidant status and adipose FA profile in lambs.

Materials and methods

Animals, diets and experimental design

Animals were cared according to the guidelines of the Iranian Council of Animal Care (1995). A total of eighteen male Baluchi lambs, with an initial average body weight (BW) of 39.8 ± 1.7 kg and at age of 196 ± 14 days, were randomly assigned to three experimental diets (n = 6 per group): (i) diet without GSO (control), (ii) diet containing 2% of GSO (GSO2) and (iii) diet containing 4% of GSO (GSO4) on DM basis. The animals were housed in individual pens. Cold-pressed GSO was obtained from the 'Zait Company-Kerman, Iran' factory. FA composition, phenolic compounds and antioxidant activity of cold-pressed GSO are presented in Table 1. Linoleic acid (66.43%) was the most abundant FA in cold-pressed GSO, followed by oleic acid (21.48%), palmitic acid (7.49%) and stearic acid (3.84%). Experimental diets were formulated to meet the requirements according to NRC (2007). Diets, isoenergetic and isoproteic, were fed as a total mixed ration (TMR) with 63:37 forage to concentrate ratio and were isocaloric and isonitrogenous (Table 1). The lambs were allowed a 14-days adjustment period and were then fed for 42 days. Diets were offered twice daily *ad libitum* (08:00 and 16:00 hours), and lambs had free access to water and orts were recorded daily. Individual DMI was calculated using daily feed offered and feed refuse averaged over the interval of the experimental period (days 0–42).

Table 1 Ingredients and chemical composition of experimental diets

	Diets†		
	Control	GSO2	GSO4
Ingredients (% DM)			
Alfalfa hay	44.5	44.5	44.5
Wheat straw	19.0	19.0	19.0
Barley grain	25.4	23.4	21.4
Grapeseed oil‡	0.0	2.0	4.0
Soya bean meal	2.0	2.0	2.0
Wheat bran	7.0	7.0	7.0
Calcium carbonate	0.8	0.8	0.8
Vitamin–mineral Mix§	0.7	0.7	0.7
Salt	0.3	0.3	0.3
Urea	0.3	0.3	0.3
Chemical composition, (% DM)			
ME¶ (Mcal/kg of DM)	2.19	2.29	2.39
CP	12.4	12.3	12.2
NDF	46.3	45.7	45.2
NFC††	33.1	32.0	31.0
Ether extract	2.7	4.7	6.6
Ca	0.9	0.9	0.9
P	0.5	0.5	0.5
Total phenolic compounds	0.81	0.87	0.95

†Control, GSO2 and GSO4 diets contained 0%, 2% and 4% of GSO (DM basis) respectively.

‡FA content (% of total FA): C14:0: 0.04; *cis*-9 C14:1: 0.01, C15:0: 0.01, C16:0:7.49, *cis*-9 C16:1: 0.11, C17:0: 0.06, *cis*-9 C17:1: 0.03, C18:0: 3.84, *cis*-9 C18:1: 21.48, C18:2 *n*−6: 66.43, C18:3 *n*−3: 0.29, C20:0:0.18 Total phenolic compounds (mg GAE/g): 0.742; relative DPPH scavenging capacity (mmol TE/g): 1.22.

§Contained (/kg of premix; DM basis): 330 000 IU of vitamin A, 60 000 IU of vitamin D, 1000 IU of vitamin E, 160 g Ca, 85 g P, 63 g Na, 45 g Mg, 2100 mg Zn, 1500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

¶ME calculated by NRC Software (2007).

††NFC calculated as 100 − (CP + Ash + NDF + EE).

Animals were weighed after a 16-h fast before the first feeding in the morning at the beginning and the end of the experimental period to determine the average daily gain (ADG). Feed conversion ratio (FCR) was calculated according to FCR = [DMI, (kg/d)/ADG, (kg/d)].

Sampling and slaughter procedures

Diets and refused feed were sampled daily during four consecutive days (days 38–41) in the experimental period. During the last 7 days of the experiment, faecal samples were collected every morning around the feeding time. Subsequently, samples of the diets, feed refusals and faeces were mixed and dried in an oven at 60 °C for 48 h (Behdad, Iran) and then ground to pass through a 2-mm screen and stored for later analysis.

On day 39 of experiment, 10 ml of blood samples was collected from the jugular vein of each lamb, before morning feeding. Blood samples were centrifuged at 3000 *g* for 10 min, followed by separation serum and finally frozen at −20 °C for assay of antioxidant factors [ferric reducing antioxidant power (FRAP) and MDA]. Another whole blood sample was collected in a tube containing potassium ethylene diamine tetra-acetic acid (K-EDTA) to determine glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities.

Rumen fluid samples were taken from animals using a stomach tube attached to a vacuum pump 2 h after the morning feeding on day 40. The pH values of the fluid samples were determined and recorded using a pH meter (Metrohm 691). Approximately 100 ml of ruminal content was strained through four layers of cheesecloth. A subsample of 10 ml was combined with 10 ml of HCl 0.2 N for NH_3-*N* analysis. Another sample (5 ml) was put into a plastic bottle containing 1 ml of 0.25 g/ml metaphosphoric acid for later determination of volatile fatty acid (VFA) analysis. Ruminal subsamples were frozen at −20 °C until laboratory analyses.

At the end of the experiment, lambs were weighed and transported to the experimental abattoir of the University of Mashhad where they were fasted for 16 h with free access to water. Lambs were slaughtered, by sectioning jugular veins and the carotid arteries. After the slaughter, non-carcass components were removed, carcass was chilled at 4 °C for 24 h, and then, adipose tissue samples were removed from the subcutaneous fat (from the back between the 10th and the 13th ribs) to determine of FA profile. Samples of muscle were taken from the *longissimus lumborum* (LL) muscle (between the 12th thoracic and 5th lumbar vertebrae) to determine the FRAP, MDA and GSH-Px and SOD activities. Samples were frozen at −20 °C until laboratory analyses.

Laboratory analysis

The total phenolic compounds (TPC) and relative DPPH scavenging capacity (RDSC) assay of GSO were determined, in triplicate, according to Parry et al. (2006) and Cheng et al. (2006) methods, respectively, and also, the TPC of experimental diet were determined according to Makkar (2000) methods.

The samples of diets and faeces were analysed for crude protein (CP), ash, natural detergent fibre (NDF), organic matter (OM) and acid-insoluble ash (AIA) in three replicates. Ash (AOAC, 2005, method 942.05) and crude protein (CP) (Kjeldahl $N \times 6.25$) were determined by the block digestion method using copper catalyst and steam distillation into boric acid on 2100 Kjeltec distillation unit according to AOAC (2005). Concentration of natural detergent fibre (NDF) inclusive of residual ash was determined without sodium sulphite and with the inclusion of heat-stable α-amylase (100 ml/0.5 *g* of sample) by method described by Van Soest et al. (1991). Acid-insoluble ash (AIA) content was used as an internal marker to determine the apparent digestibility of DM, organic matter (OM), CP and NDF, as reported by Van Keulen and Young (1977).

Ruminal fluid samples were thawed, centrifuged at 1200 *g* for 10 min, where the supernatant fluid was analysed for VFA by gas chromatography (Hewlett-Packard, model 5890, Avondale, PA). The NH_3-*N* concentration of rumen fluid samples was analysed by the procedure developed by Weatherburn (1967).

GSH-Px and SOD in blood and muscle were measured using a Randox kit (Randox Laboratories, London, UK) according to instruction of the kit and expressed as U/g of haemoglobin (Hb) and U/mg protein respectively. Haemoglobin content was determined by a commercial colorimetric kit (Sigma Diagnostic, Milan, Italy).

Total antioxidant activity in serum and LL muscle was determined by FRAP method based on the method described by Benzie and Strain (1999). The MDA contents of serum and LL muscle were assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Placer et al. (1966) and Esterbauer and Cheeseman (1990) respectively. Results were expressed as mg of MDA/kg for the LL muscle and nmol MDA/ml for serum. The FA composition of subcutaneous fat was determined

after extraction of total lipids in accordance with the method described by Folch et al. (1957). Briefly, a sample of each (0.5 g, two replicates) was placed in 5-ml screw-top test tube, 2 ml of methanolic potassium hydroxide (2N) was added, and tube cap was tightened and vigorously shaken for 2 s. Then, 2 ml of hexane was added into the tube and was shaken for 5 s. Tube was put on the ultrasonic bath for 15 min at 35 °C. The upper layer was separated and passed through the filter (0.45 m) containing sodium sulphate anhydrous, and then, 1 μl of filtrate was injected to gas chromatograph (GC, Youngling 6100, Korea). The GC was equipped with a J&W CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, 0.20 m film thickness, Agilent Technologies, Santa Clara, CA, USA). The temperatures of injector and detector ports were set at 270 °C and 300 °C respectively. The FA composition was analysed by isotherm program. The column temperature was held at 175 °C for 60 min. The identification of individual FAME was based on a standard mixture of 37 Component FAME Mix (Sigma–Aldrich, Supelco-18919-1AMP, F.A.M.E. Mix, C4-C24, St. Louis, MO, USA) and 60 individual FAME standards (Sigma–Aldrich). FAs were expressed as g/100 g of total FAME.

Statistical analysis

A completely randomized design with three treatments (diets) and six replicates (lambs) was used for the study. Data of apparent digestibility of nutrients, ruminal fermentation parameters, antioxidant activity and FA profile of subcutaneous fat were analysed using the general linear model procedure of SAS (2002). Data of DMI and performance were analysed by a mixed model for repeated measurements. Least-square means were computed and tested for differences by the Tukey's test. Differences of least-square means were considered to be significant at $p \leq 0.05$, and that of $p \leq 0.10$ was considered as a tendency.

Results

Animal performance

The results of DMI, growth performance and nutrient digestibility are presented in Table 2. Supplementation of GSO had no effect on DMI and growth performance of the lambs ($p > 0.05$). The apparent digestibility of DM, OM and CP was not affected by dietary GSO ($p > 0.05$), but NDF digestibility was reduced when animals fed GSO4 diet ($p < 0.001$).

Rumen fermentation

The mean of ruminal fermentation parameters is presented in Table 3. The average pH and NH_3-N were similar ($p > 0.05$) for all the diets. The molar proportion of propionate in the rumen was increased by addition of GSO to the diet and was minimum in control ($p = 0.002$). The molar proportion of acetate was lower in GSO4 diet compared to the control and GSO2 diets ($p = 0.003$) which in turn decreased acetate/propionate ratio ($p = 0.001$). Diets did not affect total VFA, butyrate acid, valerate acid and isovalerate acid ($p > 0.05$).

Table 2 Effect of treatment on dry matter intake, performance and diet digestibility

	Treatment†				
Item	Control	GSO2	GSO4	SEM	p value
DMI (g/d)	1326	1331	1343	25	0.88
Performance					
Initial BW (kg)	40.3	39.7	39.4	1.05	0.80
Final BW (kg)	46.3	45.7	45.5	1.06	0.87
ADG (g/day)	141	143	145	2.69	0.48
FCR	9.40	9.27	9.21	0.21	0.83
Diet digestibility (%)					
DM	68.2	67.4	66.1	1.54	0.57
OM	63.8	62.2	61.8	1.62	0.52
CP	63.3	64.37	63.6	1.94	0.92
NDF	48.5[a]	46.1[a]	40.9[b]	1.12	<0.001

BW, body weight; ADG, average daily gain; FCR, feed conversion ratio. Different letters in the same line indicate significant statistical differences ($p \leq 0.05$).

†Control, GSO2 and GSO4 diets contained 0%, 2% and 4% of grapeseed oil (DM basis) respectively.

Table 3 Effect of treatment on ruminal fermentation parameters

	Treatment†				
Item	Control	GSO2	GSO4	SEM	p value
pH	6.56	6.46	6.59	0.07	0.83
NH_3-N (mg/dl)	19.9	21.9	22.2	1.2	0.42
Total VFA (mM/l)	71.8	73.6	72.7	1.0	0.54
Individual VFA (% total VFA)					
Acetate	62.6[a]	61.8[a]	60.2[b]	0.40	0.003
Propionate	19.5[b]	20.4[a]	21.8[a]	0.32	0.002
Butyrate	15.8	16.2	16.4	0.40	0.55
Valerate	1.40	1.04	1.04	0.18	0.33
Isovalerate	0.52	0.43	0.41	0.04	0.26
Acetate/propionate	3.20[a]	3.01[b]	2.76[c]	0.05	0.001

Different letters in the same line indicate significant statistical differences ($p \leq 0.05$).

†Control, GSO2 and GSO4 diets contained 0%, 2% and 4% of grapeseed oil (DM basis) respectively.

Antioxidant status of blood and LL muscle

The effects of the diets on antioxidant status of blood and LL muscle are presented in Table 4. The supplementation of the diet with 4% GSO increased TAC in blood and LL muscle ($p < 0.001$). The level of MDA in both blood and LL muscle was significantly decreased by adding GSO to the diet and was greatest in lamb fed GSO4 diet ($p < 0.001$). The level of blood GSH-Px was higher in GSO2 and GSO4 compare to the control ($p = 0.02$), but the value of SOD in blood and LL muscle was not affected by diets ($p > 0.05$).

Fatty acid compositions of subcutaneous lipids

Fatty acid profiles of subcutaneous fat in lambs are shown in Tables 5. The concentration of palmitic acid decreased in subcutaneous fat of lambs fed GSO4 diet compared with lambs fed control diet ($p = 0.006$). Increasing of GSO to the diet increased ($p < 0.001$) the concentration of *trans*-9 C18:1, VA, *cis*-9 *trans*-11 C18:2 CLA (rumenic acid, RA), total CLA and linoleic acid as the GSO4 had the highest and control had the lowest concentration of these FA, GSO2 was intermediate. The results showed that the increased ($p < 0.001$) proportion of GSO in the diet increased the concentrations of PUFA and PUFA/SFA ratio.

Table 4 Effect of treatment on antioxidant activity in blood and LL muscle

	Treatment†				
Item	Control	GSO2	GSO4	SEM	p value
Blood					
TAC (mmol/l)	0.83[b]	1.21[b]	1.83[a]	0.11	<0.001
MDA (nmol/ml)	2.37[a]	2.00[b]	1.62[c]	0.07	<0.001
SOD (U/g Haemoglobin)	1473	1480	1454	24.83	0.75
GSH-Px (U/g Haemoglobin)	42.62[b]	56.90[a]	58.85[a]	3.74	0.02
LL muscle					
TAC (U/mg protein)	0.42[c]	0.65[b]	1.03[a]	0.05	<0.001
MDA (g/kg)	0.33[a]	0.27[b]	0.19[c]	0.01	<0.001
SOD (U/mg protein)	1.11	1.14	1.13	0.03	0.80
GSH-Px (U/mg protein)	0.82	0.91	0.83	0.03	0.24

TAC, total antioxidant capacity and measured by FRAP; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. Different letters in the same line indicate significant statistical differences ($p \leq 0.05$).

†Control, GSO2 and GSO4 diets contained 0%, 2% and 4% of GSO (DM basis) respectively.

Discussion

Characteristics of GSO

Fatty acids profile of GSO has been investigated in several studies, and it has been shown that linoleic acid is prevalent in GSO (Kamel et al., 1985; Abou-Rayan et al., 1998; Crews et al., 2006). Beveridge et al. (2005) reported that 66.8–73.6% linoleic acid can be seen in the GSO of seeds from seven different varieties of grapes, which is consistent with the results of other studies. Lutterodt et al. (2011) reported that 0.16–0.80 mg GAE/g TPC and 0.07–2.22 mmol TE/g RDSC can be found in the GSO in various species.

Performance and apparent total-tract digestibility of nutrients

The effects of oils on the ruminants DMI and digestion depend on oil type (i.e. saturated vs. unsaturated) and level (Doreau and Chilliard, 1997). It has been shown that the micro-organisms of the rumen are able to endure up to 5% fatty acids depend on the nature of the fatty acids (Hatch et al., 1972). However, it has also been indicated that adding 6% soya bean oil (8.2% diet) to the diet of lambs did not affect the final BW and ADG (van Cleef et al., 2016). Castro et al. (2005) showed that adding 4.1% (6.56% diet) palm oil did not affect the performance characteristics of the lambs, which is consistent with the results obtained in the current study.

In the present study, increasing the level of GSO in diet from 2 to 4% significantly decreased NDF digestibility. Benchaar et al.(2015) reported that adding 3% linseed oil can decrease the ruminal NDF digestion. Adding 6% oil can reduce the NDF digestibility, but the lower levels have no effect on the NDF digestion (Lunsin et al., 2012). The difference between studies might be partially due to composition of the basal diet (i.e. forage type), feeding strategy (i.e. maintenance vs. *ad libitum*), fat level and type. These findings suggest that the amount of added oil is a major determinative factor for the negative effect of linseed FA on nutrient digestibility (Doreau and Ferlay, 1995).

Rumen fermentation

Oil supplementation can cause a change in VFA profile through increasing the proportion of propionate and decreasing acetate (Ivan et al., 2013). Dietary strategies such as use of PUFA to increase propionate production in the rumen can also reduce

Table 5 Effect of treatment on total fat (mg/g subcutaneous fat) and fatty acid profile (% of total FAME) subcutaneous fat

	Treatment†				
Fatty acid	Control	GSO2	GSO4	SEM	p value
Total FA (mg/g)	685.4	693.5	691.2	21.4	0.91
FA composition, g/100 g of total FAME					
C12:0 (Lauric)	0.48	0.53	0.40	0.07	0.53
C14:0 (Myristic)	4.15	4.07	3.80	0.17	0.38
cis-9 C14:1 (Myristoleic)	0.45	0.30	0.36	0.07	0.40
C15:0 (Pentadecanoic)	0.76	0.68	0.69	0.05	0.64
C16:0 (Palmitic)	29.93^{a}	28.31^{ab}	26.69^{b}	0.52	0.006
cis-9 C16:1 (Palmitoleic)	0.72	0.72	0.68	0.03	0.68
C17:0 (Heptadecanoic)	0.89	0.89	0.97	0.04	0.40
cis-9 C17:1 (Heptadecenoic)	0.60	0.68	0.70	0.05	0.39
C18:0 (Stearic)	17.81	18.43	19.23	0.61	0.31
trans-9 C18:1 (Elaidic)	0.14^{c}	0.26^{b}	0.28^{a}	0.01	< 0.001
trans-11 C18:1 (Vaccenic)	0.65^{c}	1.22^{b}	2.18^{a}	0.04	< 0.001
cis-9 C18:1 (Oleic)	36.27	35.13	34.32	0.86	0.32
C18:2 *n*−6 (Linoleic)	3.11^{c}	4.17^{b}	5.35^{a}	0.17	<0.001
cis- 9 *trans*-11 C18:2 Rumenic acid (CLA)	0.62^{c}	0.77^{b}	0.94^{a}	0.01	<0.001
C18:3 *n*−3 (Linolenic)	0.50	0.67	0.52	0.10	0.45
C20:0 (Arachidic)	0.57	0.48	0.48	0.08	0.74
C20:1 (Eicosenoic)	0.12	0.13	0.13	0.01	0.91
C20:5 *n*−3 (Eicosapentaenoic)	0.02	0.04	0.03	0.006	0.87
C22:0 (Behenic)	0.07	0.09	0.07	0.01	0.48
C22:5 *n*−3 (Docosapentaenoic)	0.03	0.04	0.04	0.006	0.52
SFA	54.68	53.51	52.37	0.76	0.19
MUFA	38.97	38.46	38.87	0.87	0.35
PUFA	4.38^{c}	5.81^{b}	7.02^{a}	0.22	<0.001
MUFA/SFA	0.71	0.71	0.74	0.02	0.87
PUFA/SFA	0.07^{c}	0.10^{b}	0.13^{a}	0.004	<0.001
Total CLA	0.70^{c}	0.87^{b}	1.06^{a}	0.01	0.001

FAME, fatty acid methyl esters; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, polyunsaturated fatty acid.
Different letters in the same line indicate significant statistical differences ($p \leq 0.05$).
†Control, GSO2 and GSO4 diets contained 0%, 2% and 4% of GSO (DM basis) respectively.

methanogenesis through making the proper conditions for lower hydrogen availability for methane production (Martin et al., 2010). In agreement with results of the current study, Benchaar et al. (2015) and Lunsin et al. (2012) reported that supplementing the diet with linseed or rice bran oil increased propionic acid and decreased acetate acids in rumen fluid respectively. Mapato et al. (2010) reported that 6% sunflower oil compared with the 3% sunflower oil can decrease molar proportion of ruminal acetate and can also increase propionate leading to the decreased acetate: propionate ratio. Oil supplementation can reduce the fibre degradation in the rumen, as fibres play an essential role in acetate production, and a reduction in the fibre digestibility reduces the acetate (Doreau and Ferlay, 1995). As it was explained before, adding GSO to the diet decreased the NDF digestibility and consequently decreased in the acetate proportion and acetate: propionate ratio.

Antioxidant status of blood and LL muscle

Lipid oxidation is considered as one of the main reasons to reduce the quality of meat and meat products. A large number of compounds are generated during the oxidation processes which adversely affect texture, colour, flavour, nutritive value and safety of meat products (Lahucky et al., 2010) which can limit the shelf-life of the meat (Karakaya et al., 2011). Antioxidants can be used to prevent or delay these oxidation processes. Antioxidants can consider as a defence mechanisms against free radical species for the protection of both intracellular and extracellular components (Jiang and Xiong, 2016). In line with the results of our study, Liu et al. (2016) found that inclusion of polyphenols from chestnut tannins increased TAC, GSH-Px and decreased MDA content in serum and liver of the lamb which can be due to the selectively induction of antioxidant enzyme gene expression by polyphenols (condensed tannin). Emami et al. (2015)

reported that adding pomegranate seed as a source of natural antioxidant to the kids diet decreased MDA and increased TAC in plasma, liver and LL muscle (Emami et al., 2015). Dong et al. (2015) reported that polyphenolic extracts of *Castanea mollissima Blume* increased the contents of GSH-Px and TAC and decreased MDA in intestinal crypt-like epithelial cell *in vitro*. In contrast with our study, Sgorlon et al. (2006) found that grape skin extract as a supplementation in the sheep diet, which is rich in polyphenols and condensed tannins increased the expression of SOD in plasma. Sun et al. (2015) reported that TAC, GSH-Px, MDA and catalase content of muscle lambs fed with *Suaeda glauca* seed (a source of natural antioxidant) did not change through the treatments.

The strong antioxidant potential of grapeseed can be attributed to the extremely high concentrations of polyphenols, such as gallic acid, gallocatechin and epigallocatechin of the grapeseed (Shi et al., 2003). Also, grapeseed can provide more significant protection against free radicals and free radical-induced lipid peroxidation than vitamins C, E and β-carotene (Bagchi et al., 1997; Balogh et al., 2000). Colindres and Susan Brewer (2011) compared the effect of three natural (grapeseed extract, oleoresin rosemary and oregano extract) with three synthetic [butylated hydroxyanisole, butylated (BHA), hydroxytoluene (BHT), propyl gallate (PG)] antioxidants on oxidative stability of ground beef patties and results showed that effective antioxidants were more in PG > grapeseed extract > oleoresin rosemary > BHA > oregano extract > BHT > control group respectively. Generally, a higher intake of natural antioxidants can results in transferring these molecules to animal tissues with a consequent increase of total antioxidant capacity (Descalzo and Sancho, 2008). In this study, it seems that the higher intake of antioxidants from GSO such as polyphenols or tocotrienols cease transfer this compounds to blood and meat and increased antioxidant parameters in this tissues.

Adipose fatty acid

UFAs supplementation usually decrease the palmitic concentration, which is favourable as this FA is the major hypercholesterolaemic FA (Givens, 2005). Jerónimo et al. (2012) reported that adding 6% vegetable oil to the lamb diet containing grapeseed extract causes a reduction in the palmitic concentration in muscle. Mapiye et al. (2013) stated that flaxseed supplementation decreased the palmitic concentration in the adipose tissue of steers.

As expected, adding GSO which contains a high concentration of linoleic acid can increase this FA concentration in the adipose tissue which is consistent with previous results on lambs fed with high-linoleic diets (Boles et al., 2005; Bas et al., 2007). Bessa et al. (2007) reported that adding sunflower oil and linseed oil increased the linoleic and linolenic acids concentrations in the lambs' muscular tissue respectively.

Several studies also demonstrated that oil supplementation with high levels of linoleic and linolenic acids can increase CLA, VA and PUFA and decrease SFA in ruminant adipose tissues, which is consistent with our results. Boles et al. (2005) reported that PUFA and its isomers particularly CLA in the muscle can be increased by elevating safflower oil levels to the diet due to its high linoleic acid content. In the past decade, it was believed that the CLA produced in the rumen was the most important source of CLA in milk and meat, but recently it is strongly suggested that endogenous synthesis of VA by Δ^9-desaturase in the mammary gland (Griinari et al., 2000) and subcutaneous or intramuscular fat (Raes et al., 2004) can be the predominant production pathway Thus, in our study, increasing the concentration of RA and total CLA in subcutaneous fat by adding GSO to the diet can be attributed to the increase in VA concentration in the rumen. The use of GSO in ruminant nutrition could improve the quality of the derived foods, by the increase of the content of fatty acids, such as CLA, VA and PUFA, which are considered beneficial to human health.

Conclusion

According to our results, dietary supplementation of GSO had no effect on lamb performance and nutrient digestibility, except NDF which was decreased by addition of oil to the diet. Diets with GSO had the higher TAC content and lower MDA content in serum and meat of lambs. Dietary inclusion of GSO improved adipose FA composition by increasing the proportion of VA, CLA, PUFA and PUFA/SFA ratio. Thus, our results suggest that use of GSO up to 4% in the lamb diet might be a good approach to increase meat antioxidant activity and improvement of adipose FA composition from the point of view of the human consumer, without effects on animal performance.

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References

Abou-Rayan, M.; Abd-el-Naby, A.; Abou Samaha, O.; Mohamed, M., 1998: Characteristics and composition of grape seed oil. *Alexandria Journal of Agricultural Research* **43**, 67–79.

Association of Official Analytical Chemists (AOAC), 2005: *Official Methods of Analysis*, 18th edn. AOAC international, Gaithersburg, MD, USA.

Bagchi, D.; Garg, A.; Krohn, R.; Bagchi, M.; Tran, M.; Stohs, S., 1997: Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. *Research Communications in Molecular Pathology and Pharmacology* **95**, 179–189.

Balogh, Z.; Gray, J.; Gomaa, E.; Booren, A., 2000: Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. *Food and Chemical Toxicology* **38**, 395–401.

Bas, P.; Berthelot, V.; Pottier, E.; Normand, J., 2007: Effect of level of linseed on fatty acid composition of muscles and adipose tissues of lambs with emphasis on trans fatty acids. *Meat Science* **77**, 678–688.

Benchaar, C.; Hassanat, F.; Martineau, R.; Gervais, R., 2015: Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. *Journal of Dairy Science* **98**, 7993–8008.

Benzie, I. F.; Strain, J., 1999: Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**, 15–27.

Bessa, R. J.; Alves, S. P.; Jerónimo, E.; Alfaia, C. M.; Prates, J. A.; Santos-Silva, J., 2007: Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lambs. *European Journal of Lipid Science and Technology* **109**, 868–878.

Beveridge, T. H.; Girard, B.; Kopp, T.; Drover, J. C., 2005: Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects. *Journal of Agricultural and Food Chemistry* **53**, 1799–1804.

Boles, J.; Kott, R.; Hatfield, P.; Bergman, J.; Flynn, C., 2005: Supplemental safflower oil affects the fatty acid profile, including conjugated linoleic acid, of lamb. *Journal of Animal Science* **83**, 2175–2181.

Buccioni, A.; Pauselli, M.; Viti, C.; Minieri, S.; Pallara, G.; Roscini, V.; Rapaccini, S.; Marinucci, M. T.; Lupi, P.; Conte, G., 2015: Milk fatty acid composition, rumen microbial population, and animal performances in response to diets rich in linoleic acid supplemented with chestnut or quebracho tannins in dairy ewes. *Journal of Dairy Science* **98**, 1145–1156.

Castro, T.; Manso, T.; Mantecón, A.; Guirao, J.; Jimeno, V., 2005: Fatty acid composition and carcass characteristics of growing lambs fed diets containing palm oil supplements. *Meat Science* **69**, 757–764.

Cheng, Z.; Moore, J.; Yu, L., 2006: High-throughput relative DPPH radical scavenging capacity assay. *Journal of Agricultural and Food Chemistry* **54**, 7429–7436.

Choi, Y.; Lee, J., 2009: Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds. *Food Chemistry* **114**, 1386–1390.

van Cleef, F.d. O. S.; Ezequiel, J. M. B.; D'Aurea, A. P.; Almeida, M. T. C.; Perez, H. L.; Vancleef, E. H. C. B., 2016: Feeding behavior, nutrient digestibility, feedlot performance, carcass traits, and meat characteristics of crossbred lambs fed high levels of yellow grease or soybean oil. *Small Ruminant Research* **137**, 151–156.

Colindres, P.; Susan Brewer, M., 2011: Oxidative stability of cooked, frozen, reheated beef patties: effect of antioxidants. *Journal of the Science of Food and Agriculture* **91**, 963–968.

Crews, C.; Hough, P.; Godward, J.; Brereton, P.; Lees, M.; Guiet, S.; Winkelmann, W., 2006: Quantitation of the main constituents of some authentic grape-seed oils of different origin. *Journal of Agricultural and Food Chemistry* **54**, 6261–6265.

Descalzo, A.; Sancho, A., 2008: A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science* **79**, 423–436.

Dong, S.; Li, H.; Gasco, L.; Xiong, Y.; Guo, K.; Zoccarato, I., 2015: Antioxidative activity of the polyphenols from the involucres of Castanea mollissima Blume and their mitigating effects on heat stress. *Poultry Science* **94**, 1096–1104.

Doreau, M.; Chilliard, Y., 1997: Digestion and metabolism of dietary fat in farm animals. *British Journal of Nutrition* **78**, S15–S35.

Doreau, M.; Ferlay, A., 1995: Effect of dietary lipids on nitrogen metabolism in the rumen: a review. *Livestock Production Science* **43**, 97–110.

Emami, A.; Ganjkhanlou, M.; Nasri, M. F.; Zali, A.; Rashidi, L., 2015: Pomegranate seed pulp as a novel replacement of dietary cereal grains for kids. *Small Ruminant Research* **123**, 238–245.

Esterbauer, H.; Cheeseman, K. H., 1990: Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology* **186**, 407–421.

FAOSTAT, 2013: Statistical databases. http://faostat.fao.org (Accessed 13 Dec 2016).

Folch, J.; Lees, M.; Sloane-Stanley, G., 1957: A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry* **226**, 497–509.

Givens, D., 2005: The role of animal nutrition in improving the nutritive value of animal-derived foods in relation to chronic disease. *Proceedings of the Nutrition Society* **64**, 395–402.

Griinari, J.; Corl, B.; Lacy, S.; Chouinard, P.; Nurmela, K.; Bauman, D., 2000: Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. *The Journal of Nutrition* **130**, 2285–2291.

Hatch, C.; Perry, T.; Mohler, M.; Beeson, W., 1972: Effect of added fat with graded levels of calcium to urea-containing rations for beef cattle. *Journal of Animal Science* **34**, 483–487.

Iranian Council of Animal Care, 1995: *Guide to the Care and Use of Experimental Animals*, Vol **1**. Isfahan University of Technology, Isfahan, Iran.

Ito, N.; Fukushima, S.; Tsuda, H., 1985: Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *CRC Critical Reviews in Toxicology* **15**, 109–150.

Ivan, M.; Petit, H.; Chiquette, J.; Wright, A.-D., 2013: Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids. *British Journal of Nutrition* **109**, 1211–1218.

Jerónimo, E.; Alfaia, C. M.; Alves, S. P.; Dentinho, M. T.; Prates, J. A.; Vasta, V.; Santos-Silva, J.; Bessa, R. J., 2012: Effect of dietary grape seed extract and Cistus ladanifer L. in combination with

vegetable oil supplementation on lamb meat quality. *Meat Science* **92**, 841–847.
Jiang, J.; Xiong, Y. L., 2016: Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: a review. *Meat Science* **120**, 117–120.
Kamel, B. S.; Dawson, H.; Kakuda, Y., 1985: Characteristics and composition of melon and grape seed oils and cakes. *Journal of the American Oil Chemists' Society* **62**, 881–883.
Karakaya, M.; Bayrak, E.; Ulusoy, K., 2011: Use of natural antioxidants in meat and meat products. *Journal of Food Science and Engineering* **1**, 1–10.
Khiaosa-Ard, R.; Bryner, S.; Scheeder, M.; Wettstein, H.-R.; Leiber, F.; Kreuzer, M.; Soliva, C., 2009: Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. *Journal of Dairy Science* **92**, 177–188.
Lahucky, R.; Nuernberg, K.; Kovac, L.; Bucko, O.; Nuernberg, G., 2010: Assessment of the antioxidant potential of selected plant extracts–in vitro and in vivo experiments on pork. *Meat Science* **85**, 779–784.
Liu, H.; Li, K.; Mingbin, L.; Zhao, J.; Xiong, B., 2016: Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs. *Meat Science* **116**, 236–242.
Lunsin, R.; Wanapat, M.; Yuangklang, C.; Rowlinson, P., 2012: Effect of rice bran oil supplementation on rumen fermentation, milk yield and milk composition in lactating dairy cows. *Livestock Science* **145**, 167–173.
Lutterodt, H.; Slavin, M.; Whent, M.; Turner, E.; Yu, L. L., 2011: Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chemistry* **128**, 391–399.
Makkar, H. P. S., 2000: Quantification of Tannins in Tree Foliage. A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage. Joint FAO/IAEA, FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-program. FAO/IAEA Working Document. IAEA, Vienna, Austria.
Manso, T.; Gallardo, B.; Salvá, A.; Guerra-Rivas, C.; Mantecón, A.; Lavín, P.; de la Fuente, M., 2015: Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk composition. *Journal of Dairy Science* **99**, 1111–11120.
Mapato, C.; Wanapat, M.; Cherdthong, A., 2010: Effects of urea treatment of straw and dietary level of vegetable oil on lactating dairy cows. *Tropical Animal Health and Production* **42**, 1635–1642.
Mapiye, C.; Turner, T.; Rolland, D.; Basarab, J.; Baron, V.; McAllister, T.; Block, H.; Uttaro, B.; Aalhus, J.; Dugan, M., 2013: Adipose tissue and muscle fatty acid profiles of steers fed red clover silage with and without flaxseed. *Livestock Science* **151**, 11–20.
Martin, C.; Morgavi, D.; Doreau, M., 2010: Methane mitigation in ruminants: from microbe to the farm scale. *Animal* **4**, 351–365.
Moate, P.; Williams, S.; Torok, V.; Hannah, M.; Ribaux, B.; Tavendale, M.; Eckard, R.; Jacobs, J.; Auldist, M.; Wales, W., 2014: Grape marc reduces methane emissions when fed to dairy cows. *Journal of Dairy Science* **97**, 5073–5087.
Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo da Silva, J. M., 2003: Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* **51**, 6475–6481.
NRC, 2007: *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. The National Academies Press, Washington, DC.
Nudda, A.; Correddu, F.; Marzano, A.; Battacone, G.; Nicolussi, P.; Bonelli, P.; Pulina, G., 2015: Effects of diets containing grape seed, linseed, or both on milk production traits, liver and kidney activities, and immunity of lactating dairy ewes. *Journal of Dairy Science* **98**, 1157–1166.
Parry, J.; Hao, Z.; Luther, M.; Su, L.; Zhou, K.; Yu, L. L., 2006: Characterization of cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils. *Journal of the American Oil Chemists' Society* **83**, 847–854.
Placer, Z. A.; Cushman, L. L.; Johnson, B. C., 1966: Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry* **16**, 359–364.
Raes, K.; Haak, L.; Balcaen, A.; Claeys, E.; Demeyer, D.; De Smet, S., 2004: Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. *Meat Science* **66**, 307–315.
SAS Institute, 2002: *SAS/STAT User's Guide: Version 9.1*. SAS Institute, Cary, NC.
Sgorlon, S.; Stradaioli, G.; Zanin, D.; Stefanon, B., 2006: Biochemical and molecular responses to antioxidant supplementation in sheep. *Small Ruminant Research* **64**, 143–151.
Shi, J.; Yu, J.; Pohorly, J.; Young, J. C.; Bryan, M.; Wu, Y., 2003: Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *Food Agriculture & Environment* **1**, 42–47.
Singh, G.; Marimuthu, P.; de Heluani, C. S.; Catalan, C., 2005: Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of Nigella sativa seeds. *Journal of the Science of Food and Agriculture* **85**, 2297–2306.
Sun, H.; Zhong, R.; Liu, H.; Wang, M.; Sun, J.; Zhou, D., 2015: Meat quality, fatty acid composition of tissue and gastrointestinal content, and antioxidant status of lamb fed seed of a halophyte (*Suaeda glauca*). *Meat Science* **100**, 10–16.
Van Keulen, J.; Young, B., 1977: Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science* **44**, 282–287.
Van Soest, P. V.; Robertson, J.; Lewis, B., 1991: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.
Vasta, V.; Mele, M.; Serra, A.; Scerra, M.; Luciano, G.; Lanza, M.; Priolo, A., 2009: Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *Journal of Animal Science* **87**, 2674.
Vasta, V.; Yáñez-Ruiz, D. R.; Mele, M.; Serra, A.; Luciano, G.; Lanza, M.; Biondi, L.; Priolo, A., 2010: Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. *Applied and Environmental Microbiology* **76**, 2549–2555.
Weatherburn, M., 1967: Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry* **39**, 971–974.