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Effects of Dietary Grape Pomace Supplementation on Performance, Carcass Traits and Meat Quality of Lambs

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Abstract. *Background/Aim: A previous study revealed that the inclusion of grape pomace (GP) in the diet for growing lambs had beneficial effects on the redox status and fecal microbiota. Herein, we investigated the effect of GP inclusion on performance, carcass traits and fatty acid composition of meat. Materials and Methods: In the experimental trial of 55 days, lambs were fed with standard or diet supplemented with GP. Performance, carcass traits and fatty acid profile of quadriceps muscle were assessed. Results: GP inclusion in the diet improved growth performance, since the average daily gain was significantly increased by 2-fold in GP group. Regarding the fatty acid composition of meat, GP inclusion significantly increased the content of long chain n-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, and reduced the n-6/n-3 ratio compared to the control group. Conclusion: GP supplementation in lamb diet may improve performance and may have beneficial effects on meat quality.*

Apart from being an important source of protein, vitamins and dietary fiber, meat also provides fat, including saturated fatty acids (SFA), unsaturated fatty acids (UFA), cholesterol and triacylglycerol. Fatty acids in meat are vital components that contribute to the nutrient value of meat in terms of physiological and biochemical functions. Several studies have shown that the content of fatty acids can strongly be influenced by nutrition (1, 2).

Sheep meat and products are widely consumed worldwide. Ruminant edible fat contains high content of SFA, poor content of health-promoting polyunsaturated fatty acids (PUFA) and variable amounts of fatty acids derived from rumen metabolism such as *trans*-fatty acids and conjugated fatty acids. The high saturation of edible fat has raised concerns about its contribution to the increase risk of cardiovascular diseases and metabolic syndrome. Thus, manipulation of fatty acid composition of meat in order to reduce SFA content and the n-6/n-3 ratio, and increase the PUFA content is a major target in the field of ruminant research (3).

Recently, researchers have demonstrated the possibility of developing meat products with potential health benefits by introducing bioactive compounds with antioxidant properties into feedstuffs for animals (4, 5). Our research group has also performed *in vivo* studies showing that by-products rich in polyphenols, generated from olive oil and the wine industry, improved antioxidant capacity, meat quality and welfare of productive animals, such as chickens, pigs and lambs (6-13).

In the field of animal nutrition, plant extracts rich in polyphenols have been extensively studied as potential sources of natural antioxidants to replace their synthetic counterparts. The valorization of by-products, such as those of the wine industry, is of important interest since their use in animal nutrition may provide benefits in animal health, productivity and meat quality. At the same time, the extensive use of winery by-products in feedstuffs for productive animals may have beneficial effects on the environment by reducing the risk of phytotoxic phenomena from their deposition on soil (14).

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Grape pomace (GP) is a by-product from wine production and refers to the solid remains following pressing of grapes for juice. It contains high levels of polyphenols and dietary fiber (15). GP exhibits important antioxidant and antibacterial properties (16). Furthermore, the use of GP in animal feed not only prevents oxidation, but also improves meat quality (17).

It is well established that meat quality refers to the attractiveness of meat to consumers. As mentioned above, meat quality can be influenced principally by the animal's nutrition, through its effects on the amount and type of fat in meat. In recent years, interest in meat fatty acid composition has stemmed mainly from the need to find nutritional strategies to produce healthier meat, *i.e.* meat with a higher ratio of PUFA to SFA and a lower n-6/n-3 ratio. In particular, the n-6/n-3 ratio in meat has become of important significance in human nutrition, since consumption of meat enriched with long chain n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is beneficial. The main benefits associated with EPA and DHA are their anti-inflammatory properties (18), cardiovascular protective effects (19) and reduction of the risks for specific types of cancer (20).

In a previous study, we showed that GP inclusion in the diet of lambs had beneficial effects on the redox status and fecal microbiota of growing lambs (9, 11). In extension of this, the objective of this study was to evaluate GP supplementation in diets for growing lambs relative to growth performance, carcass traits and fatty acid composition of meat.

Materials and Methods

Winery by-product and silage preparation. GP from red grapes (*Vitis vinifera* L. var. Moschato) was obtained from a winery in Tyrnavos (Larissa, Greece). Immediately after its production, GP was stored and processed under the common practice of ensilage. Silage was prepared as described previously (9).

Experimental design, animals, management and diets. Twenty-four male lambs of Chios breed were selected from the flock of the Research Institute of Animal Science/Hellenic Agricultural Organization – Demeter (Giannitsa, Greece). At the commencement of the study, lambs were 15 days old and weighed on average 7.99±1.80 kg and were divided into two homogeneous groups according to their body weight. For 27 days (15 to 42 days of age), the control group (n=12) was fed with standard ration, while the GP group (n=12) was fed with ration containing silage with polyphenolic additives from GP. Until the age of weaning (42 days of age), lambs remained in two separate stalls (one for each group) along with their mothers to allow suckling. Each stall was equipped with similar troughs for feeding and the lambs had free access to the rations (either to standard or to experimental ration), alfalfa hay and water for consumption *ad libitum*. The ewes were fed with standard ration without having access to the rations of lambs. After weaning, lambs were separated from their mothers and the two groups of lambs were fed with their respective diet and alfalfa hay for 28 days (*i.e.* from 42 to 70 days of age) until the end of the experiment. The diets for lambs were formulated according to the National Research Council (21) and the chemical composition was determined according to methods of the Association of Official Analytical Chemists (22) (Table I). The net energy (NE) content of the diets was estimated by the equations of Van Es (23). The experiment was reviewed and approved by the Institutional Review Board of the University of Thessaly (no. 89/10.12.2014). All lambs used in this study were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (24).

During the 55-day experimental trial, lambs were weighed individually weekly and average daily gain (ADG, g/day) was calculated. At 42 and at 70 days of age, six lambs of each group were fasted for 18 h (water was allowed *ad libitum*), weighed and slaughtered. Additionally, fasting body weight, cold carcass weight, carcass yield and carcass traits were assessed. At 70 days of age, the intramuscular fatty acid profile of quadriceps muscle was also assessed. All relevant procedures (*e.g.* slaughter, bleeding, skin removal, gutting, viscera separation and washing) were executed by specialized personnel. In a previous study, the effect of GP inclusion on lipid peroxidation and protein oxidation was also assessed. Specifically, at 42 and 70 days of age, oxidative stress biomarkers including thiobarbituric acid-reactive substances (TBARS) and protein carbonyls were measured in quadriceps muscle in order to evaluate the effect of GP inclusion on meat oxidation (9).

Carcass assessment, yield and traits. After dressing and storing for 24 h at 3˚C carcasses were weighed according to European Economic Community (EEC) guidelines (25). To assess carcass traits, a 10-point scale was used according to Christodoulou *et al.* (26). The following carcass traits were assessed: lean color ('1' being most red and '10' being most pink), fat color ('1' being most yellow and '10' being most white), fat firmness ('1' being most oily and '10' being most firm), carcass wetness ('1' being most wet and '10' being least wet) and overall acceptability ('1' being least acceptable and '10' being most acceptable).

Sample preparation and fatty acid methyl ester (FAME) synthesis. At 70 days of age, after slaughter, tissues of quadriceps muscle were quickly removed and snap-frozen in liquid nitrogen. The homogenization of tissues was performed as described previously (7). Briefly, one part of tissue powder was homogenized with two parts (weight/volume) of 0.01 M phosphate-buffered saline pH 7.4 (138 mM NaCl, 2.7 mM KCl, and 1 mM EDTA) and a protease inhibitor tablet (Complete™ mini protease inhibitors; Roche, Munich, Germany) was added. The homogenate was then vortexed, briefly sonicated on ice and then centrifuged at 12,000 × *g* for 30 min at 4˚C and the supernatant was collected. The homogenized tissues were then stored at –80˚C until fatty acid conversion to FAME for subsequent analysis.

The method of FAME synthesis was applied according to Gerasopoulos *et al.* (8). Briefly, to 0.5 ml of homogenized tissue, 1 ml methanolic solution of 600 μg/ml tridecanoid acid (13:0) was added as an internal standard. Subsequently, 0.4 ml of 10 N KOH and 2.7 ml of pure methanol were added. For proper hydrolysis of samples, the tubes were placed in a water-bath at 55˚C for 1.5 h with vigorous stirring every 20 min. Cooling with tap water was followed for 15 min. Then, 0.3 ml of 24 N H_2SO_4 was added and the tubes were placed in a water-bath at 55˚C for 1.5 h, with vigorous stirring every 20 min. Subsequently, the samples were

Table I. *Ingredient composition (% w/w) and nutrient content [g/kg dry matter (DM)] of the diets given before and after Iamb weaning.*

	Before weaning		After weaning	
Ingredient	Control	GP	Control	GP
Corn silage[a]	45.0	45.0	45.0	45.0
Wheat bran	9.0	9.0	15.0	15.0
Wheat grain	0	0	13.0	13.0
Sunflower meal	0	0	4.0	4.0
Soybean meal 44%	21.0	21.0	18.0	18.0
Milk replacer	20.0	20.0	0	0
Vitamin and mineral premix (2.5%)	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5
Limestone	1.2	1.2	1.2	1.2
Monocalcium phosphate	0.8	0.8	0.8	0.8
Chemical composition[b] (g/kg DM)				
Net energy for gain[c] (MJ/kg DM)	4.85	5.30	5.01	5.45
Dry matter	902	936	883	917
Crude protein	183	184	168	169
Crude fat	57.7	60.0	29.9	32.1
Crude fiber	35.7	62.3	50.1	76.6
Ash	43.3	46.0	30.1	32.8
Calcium	12.9	13.8	13.1	14.0
Phosphorus	6.27	6.04	7.46	7.24

Control: Standard diet; GP: diet supplemented with grape pomace. [a]Silage contained 60% corn solids and 40% water in the control diet, and 51% corn solids, 9% grape pomace and 40% water in the GP diet. [b]Diets were analyzed according to the Association of Official Analytical Chemists (22). [c]Calculated from equations of Van Es (23).

cooled with water for 15 min. Finally, 3 ml of hexane was added as solvent and the samples were vortexed for 3 min and centrifuged at 6,000 × *g*, for 15 min at room temperature. The supernatant was placed in gas chromatography (GC) vials of 2 ml and stored at –20˚C until GC/mass spectrometric (MS) analysis.

Quantification of FAME. The intramuscular fatty acid profile in quadriceps muscle of lambs was determined according to Gerasopoulos *et al*. (8). Fatty acids were identified using FAME standards supplied by Supelco (Bellefonte, PA, USA): 37 Component FAME Mix (product number 47885-U) and PUFA 2 (product number 47015-U). To identify the various esters of lipid oxides, MS recognition/identification of peaks in conjunction with spectral data available from the National Institute of Standards and Technology was used. Varian CP-3800 GC-MS chromatography apparatus (Varian Inc, Palo Alto, CA, USA) and an Agilent J&W 112-88A7:100 m×0.25 mm×0.25 μm silica column (Agilent, Frankfurt, Germany) were used. The data on fatty acid composition were processed to compute the sum of SFA, monounsaturated fatty acids, and PUFA. Fatty acids were expressed as g/100 g of total FAME.

Statistical analysis. Growth performance, carcass traits and intramuscular fatty acid composition were analyzed by one-way ANOVA. The level of statistical significance was set at $p<0.05$. All results are expressed as mean±SEM. Data were analyzed using SPSS, version 22.0 (IBM Corp., Armonk, NY, USA).

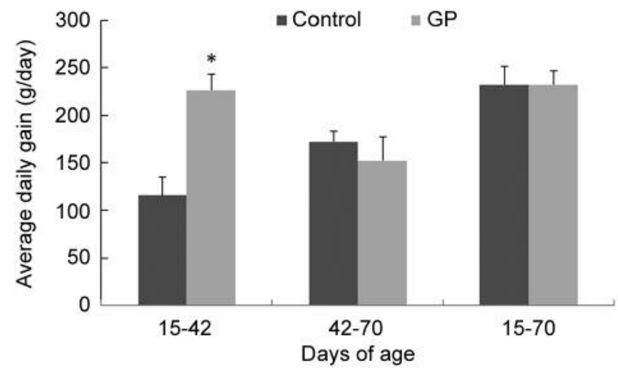

Figure 1. *Average daily gain (ADG) in growing lambs fed with diet supplemented with grape pomace (GP). *Significantly different from the control group (p<0.05). Data are expressed as the mean±SEM.*

Results

Growth performance. Inclusion of GP in lamb diet improved performance during the experimental trial. In particular, before the weaning period (*i.e*. from 15 to 42 days of age), ADG was increased significantly by 2-fold in the GP group compared to the control group. However, after weaning and considering overall period, ADG was not affected by GP inclusion in the diet (Figure 1).

Carcass yield and traits. Fasting body weight, cold carcass weight, carcass yield and carcass traits are presented in Table II. At 42 days of age, fasted body weight and cold carcass weight were increased significantly in the GP group compared with the control group. Furthermore, carcasses traits, such as fat firmness and overall acceptability were affected by the experimental diet supplemented with GP. At 70 days of age, lean color, fat firmness, carcass wetness and overall acceptability were not affected by GP inclusion in the diet. In contrast, fat color of the carcasses was significantly lower in the GP group compared to the control.

Assessment of fatty acids in lamb meat. The effect of GP inclusion in the diet on the fatty acid composition of lamb meat is summarized in Table III. Dietary GP supplementation led to significantly higher content of arachidic acid (20:0), eicosadienoic acid (cis-11, 14, 20:2n-6), EPA (20:5n-3) and DHA (22:6n-3), compared to the control group. Moreover, the meat from the GP group exhibited significantly higher content of n-3 fatty acids and significantly lower n-6/n-3 ratio compared to the control group.

Discussion

Growth performance. In livestock production, numerous factors can induce oxidative stress damage to cellular

Table II. *Fasting body weight (BW), cold carcass weight, carcass yield and traits of growing lambs.*

	Group		
	Control	GP	*p*-Value
Before weaning (15 to 42 days)			
Fasted BW (kg)	10.16±1.06	14.08±1.20*	**0.034**
Cold carcass weight[a] (kg)	5.08±0.52	7.25±0.62*	**0.023**
Carcass yield (kg/100 kg of BW)	50.03±0.99	51.46±0.65	0.261
Carcass traits			
Lean color	8.21±0.08	8.43±0.07	0.091
Fat color	8.30±0.05	8.50±0.09	0.155
Fat firmness	8.23±0.04	8.43±0.06*	**0.034**
Carcass wetness	8.41±0.06	8.51±0.06	0.443
Overall acceptability	8.65±0.04	8.83±0.05*	**0.022**
After weaning (42 to 70 days)			
Fasted BW (kg)	19.16±1.24	19.58±0.75	0.785
Cold carcass weight (kg)	9.96±0.66	10.33±0.41	0.662
Carcass yield (kg/100 kg of BW)	52.03±0.58	52.78±0.46	0.347
Carcass traits*			
Lean color	7.81±0.16	7.50±0.34	0.401
Fat color	8.10±0.16	7.33±0.21*	**0.011**
Fat firmness	7.63±0.21	8.12±0.16	0.095
Carcass wetness	7.91±0.06	7.94±0.05	0.601
Overall acceptability	7.94±0.04	7.91±0.06	0.344

Control group: Standard diet; GP group: diet supplemented with grape pomace. Lambs/group=12. At 42 and 70 days of age, six lambs were slaughter from each group. [a]According to (25). *Score of 1-10 according to Christodoulou *et al*. (26). Bold values indicate statistically significant differences compared to the control group ($p<0.05$).

Table III. *Intramuscular fatty acid composition in quadriceps of lambs.*

Fatty acid (g/100 g of total fatty acids)	Group		
	Control	GP	*p*-Value
10:0	0.23±0.03	0.23±0.04	0.951
12:0	0.60±0.10	0.66±0.11	0.683
14:0	4.12±0.60	5.21±0.73	0.276
14:1 *cis*-9	0.21±0.02	0.28±0.05	0.292
15:0	0.46±0.04	0.44±0.04	0.691
16:0	19.48±0.97	21.00±1.09	0.324
16:1 *trans*-9	0.45±0.02	0.48±0.03	0.415
16:1 *cis*-9	1.64±0.14	1.93±0.22	0.292
17:0	0.81±0.04	0.72±0.03	0.100
17:1 *cis*-10	0.53±0.05	0.55±0.05	0.761
18:0	13.08±0.41	11.93±0.79	0.222
18:1 *trans*-9	0.03±0.01	0.02±0.01	0.862
18:1 *cis*-9	29.85±1.73	32.33±1.56	0.313
18:1	1.67±0.08	1.39±0.14	0.126
18:2 *trans*-9,12 n-6	0.24±0.03	0.24±0.02	0.931
18:2 *cis*-9,12 n-6	10.93±1.03	9.47±0.95	0.320
18:3 *cis*-6,9,12 n-6	0.12±0.02	0.12±0.01	0.924
18:3 n-3 (ALA)	0.87±0.06	0.72±0.07	0.135
20:0	0.04±0.02	0.09±0.01*	**0.038**
20:1 *cis*-11	0.11±0.02	0.16±0.01	0.097
20:2 *cis*-11,14 n-6	0.03±0.02	0.11±0.02*	**0.009**
21:0	0.25±0.04	0.22±0.05	0.682
20:4 *cis*-5,8,11,14 n-6	5.10±0.71	3.77±0.69	0.212
20:5 n-3 (EPA)	0.45±0.04	0.67±0.05*	**0.007**
22:6 n-3 (DHA)	0.42±0.07	0.79±0.05*	**0.001**
24:1 *cis*-15	0.17±0.03	0.19±0.03	0.663
Other[a]	8.14±1.13	6.31±1.20	0.291
Sum of n-6 PUFA	16.41±1.71	13.71±1.56	0.272
Sum of n-3 PUFA	1.74±0.16	2.17±0.08*	**0.036**
n-6/n-3 ratio	9.43±0.54	6.28±0.64*	**0.004**
SFA	39.06±1.50	40.49±1.18	0.476
MUFA	34.93±1.84	37.50±1.71	0.334
PUFA	18.73±1.79	16.39±1.55	0.371
PUFA/SFA	0.49±0.07	0.41±0.05	0.387
(MUFA+PUFA)/SFA	1.39±0.07	1.34±0.05	0.603

ALA: α-Linolenic acid; DHA: docohexaenoic acid; EPA: eicosapentaenoic acid; GP: grape pomace; MUFA: total monounsaturated acids; PUFA: total polyunsaturated fatty acids; SFA: total saturated fatty acids. Control group: Standard diet; GP group: diet supplemented with grape pomace. Lambs/group=6. Meat samples were collected at 70 days of age. [a]Represents other known and unidentified fatty acids. Bold values indicate statistically significant differences compared to the control group ($p<0.05$).

antioxidant defense. Oxidative stress causes increased production of reactive oxygen species (ROS) and can result in suboptimal health conditions of livestock and a reduction in production efficiency (27). In the present study, growth performance was increased by GP inclusion in lamb diet. ROS induce detrimental oxidative modifications of biomolecules, causing numerous impairments to intestinal membrane integrity (28). Thus, the beneficial effects of GP on growth performance may be due to its antioxidant properties by ROS scavenging, by reducing intestinal membrane damage, and consequently by improving gut functionality. Interestingly, a previous study of our group showed that GP supplementation of the diet reduced pathogenic microorganisms and increased probiotic bacteria in lambs (9). The inclusion of GP silage as a fermented product may be also an important factor in increased growth performance, since fermentable fiber in the diet stimulates microbial fermentation in the gut, and lactic acid- and fermentation-derived volatile fatty acids, are capable of inhibiting some intestinal pathogens *e.g*. *Escherichia coli* and *Salmonella* spp. (29). Other studies have shown that winery by-products such as grape seed extract and GP concentrate did not affect performance in chickens (30. 31). The present results suggest that GP could be included in the diet of growing lambs with considerable economic effects for animal breeders and agricultural economy.

Fatty acid composition and meat quality. It is well established that meat fatty acid composition has a great impact on meat quality and human health (32).

Consequently, in recent years, there has been an increased interest in strategies to manipulate the fatty acid composition of meat in productive animals through nutrition. Fatty acid profile analysis of both groups in this study revealed that among all fatty acids detected, oleic (*cis*-9, 18:1) and palmitic (16:0) contents were the highest. Palmitic and oleic acids are the predominant fatty acids in subcutaneous fat and in muscles (33). Furthermore, in the present study, GP supplementation affected the long-chain n-3 fatty acids and consequently the n-6/n-3 ratio. In particular, the inclusion of GP in the diet significantly increased the content of EPA and DHA. Since PUFAs are important for human nutrition and health (34), their increase in lamb meat reveals the beneficial effect of inclusion of GP in the animals' diet on meat quality. The long-chain docosapentaenoic acid (22:5n-3) was not detected in meat samples, although it was included in the standard FAME mixture that was used for FAME analysis. Regarding the n-6/n-3 ratio, the supplementation of winery by-product had beneficial effect on meat quality since n-6/n-3 ratio was significantly lower in GP group, compared to control group. This result may be mainly due to the increased content of EPA and DHA n-3 fatty acids. The fact that GP supplementation significantly reduced the n-6/n-3 ratio is of special interest since several studies have stressed the beneficial effects of consumption of meat characterized by a lower n-6/n-3 ratio on human health (35-37).

Our group previously demonstrated that GP inclusion in the diets of lambs did not significantly affect protein oxidation and lipid peroxidation in quadriceps muscle (9). However, at 42 and 70 days of age, a slight decrease in TBARS and protein carbonyls was observed. In another study by our research group, we also reported that diet enriched in polyphenols from GP reduced the n-6/n-3 ratio and improved the meat fatty acid profile in growing piglets (13). In a recent study, Muíño *et al*. (38) showed that inclusion of another winery by-product (red wine extract) in the diet also reduced the n-6/n-3 ratio of lamb meat.

In conclusion, from the present study it is evident that the valorization of winery by-product by its inclusion in the diet of growing lambs improved growth performance by increasing body weight and showed a significantly beneficial effect on meat quality by enriching meat with n-3 PUFA. Consequently, GP could be included in feedstuffs with no adverse effects on animal welfare and the use of GP in animal nutrition could be an alternative farm to fork strategy that would provide a considerable beneficial impact for animal breeders, consumers and the agricultural economy.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

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