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MEAT QUALITY, METABOLIC PROFILE AND ANTIOXIDANT STATUS OF LAMBS FED ON SEEDLESS GRAPE POMACE (*VITIS VINIFERA* L.)

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Abstract

This study aimed to research the effect of the seedless grape pomace (GP) added to feed mixture on meat quality, metabolic profile, and antioxidant status of Merinolandschaf lambs. The 90-day-old lambs in the control group (C, n=10) were fed feed mixture without GP while in experimental groups they were fed with 10% (GP10, n=10) or 20% (GP20, n=10) of GP in feed mixture for 30 days. There was a significant increase in L*, a* values and hue angle as well as a decrease in b* values of lamb carcasses in GP10 and GP20 compared to C group. When lambs were fed GP glucose concentrations decreased, while non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) increased on the 30th day in GP10 and GP20 compared with C group. A significant increase in SOD activity on day 30 and GPx activity on day 15 in lambs' blood of GP10 and GP20 compared with C group was determined. Also, an increase in DPPH in lamb GP10 and GP20 compared with C was determined. The obtained results of the meat quality, metabolic profile and antioxidant status parameters of lamb meat showed that the use of 10% GP in the diet is justified, while 20% of GP was questionable.

Key words: lambs, seedless grape pomace, blood parameters, meat

The production of grapes is among the highest produced fruits in the world (78 034 332 t), while 36% of world's production is in Europe in which the highest production is in Italy, Spain and France (FAO, 2022). When grapes are processed into wine, a pomace is produced. In the world 10.5–13.1 million tonnes of grape pomace is produced annually (Dávila et al., 2017), while in the Republic of Croatia approximately 40 600 t is produced (Bucić-Kojić et al., 2017). According to Beres et al. (2017), grape pomace consists of skins and pulp (around 51% DM), seeds (around 47% DM), and stems (around 2% DM). It has a low pH (3.5–5.4), high content of organic matter (873–947 g/kg DM), macronutrients (especially K and Ca: 24.2 and 9.4 g/kg DM), and polyphenols (0.9–13.6 g/kg DM), and low concentrations of heavy metals and micronutrients (Bustamante et al., 2007). Grape pomace has high fiber content, low pectin and hemicellulose content, and is a good source of antioxidants due to its high polyphenol content (Gonzalez-Centeno et al., 2010). The skin of grapes is rich in polyphenolic compounds. Polyphenols have anti-inflammatory and antioxidant effects that can have a beneficial effect on livestock health and performance as well as meat quality, including prevention of undesirable changes in color, odor, and flavor (Castillo et al., 2013). The content of anthocyanins (free anthocyanins and polymer pigments), flavan-3-ol monomers, oligomers, and polymers (tannins), flavonols, stilbenes, and phenolic acids, which are dominant in grape marc, strongly depends on the grape variety, climatic conditions of the place, and the technology used (Dragović-Uzelac et al., 2017). Anthocyanins are mainly found in the skin and flavanols in the seeds (56–65% of total flavanols). Grape pomace can be used fresh, ensiled, or dried. It is less commonly used in animal feed because of its high content of lignin and tannins (Spanghero et al., 2009), which reduces the digestibility of the diet and its energy value (Baumgärtel et al., 2007). However, the amount and physical form in which they are added to foods have a significant impact on their effects on animals. There are numerous studies investigating the use of different amounts of grape pomace in lamb diets (Bahrami et al., 2010; Zhao et al., 2017; Calderón-Cortés et al., 2018; Chikwanha et al., 2019). However, in the available literature, there are no studies on the use of seedless grape pomace in the feeding of ruminants, including lambs. In general, most studies indicate that increasing the amount of grape pomace in diets decreases digestion in the rumen as well as the ability to utilize nitrogen in sheep, negatively affecting their production traits (Abarghuei et al., 2010; Bahrami et al., 2010; Nistor et al., 2014). However, Tayengwa and Mapiye (2018) indicated that the use of up to 15% of

wine industry by-products (grape pomace) in ruminant diets has the potential to improve their production traits and meat quality. The advantage of using these wine industry by-products in the diet is shown by the positive effect of phenolic compounds on animals, especially in very demanding and stressful production stages (lactation, high pregnancy, weaning), when they enhance antioxidant activity and reduce the cost of their care and possibly unhindered disposal (Antunović et al., 2020). The aim of this study was to investigate the effects of adding seedless grape pomace to lambs' feed on meat quality, metabolic profile, and antioxidant status.

Material and methods

Experimental design

The study was carried out on a sheep farm in Gašinci in Croatia (at 45° 20' 8.33"N latitude and 18° 18' 40.95" E longitude). Weaned Merinolandschaf lambs aged 90 days were included in the study. The research was preceded by a selection of lambs in a flock of 250 sheep and the same number of lambs. Lambs of both sexes were selected in equal numbers and were in good health and satisfactory condition immediately after weaning. The study lasted 30 days. Depending on the feed, the lambs were set into three groups of 10 lambs each, and divided by sex (50% male and 50% female). The lambs were housed in boxes in the barn. To acclimate the lambs to the new diet, a 7-day preparation period was conducted, during which the lambs were fed on the 1st, 15th, and 30th day. Body condition score (BCS) was determined by using a 1- to 5-points scale according to Russel (1991) ranging from 1 (thin) to 5 (obese) with 0.25 intervals. All personnel who handled live lambs received appropriate training and education.

Preparation and feed analyses

The pomace of Cabernet franc black grapes (*Vitis vinifera* L.) was cleaned, the seeds were separated and the rest (skins, pulp, stems) was dried at 50°C for 10 h, ground and used as an additive in feed mixtures. After pressing in the winery, the pomace was brought to the production line. The machine S-800-seed cleaner (Berenyi, Mezőtúr, Hungary) is used to separate the grape seeds from the skin and stalks. The machine works on the principle of a fan that separates the seeds and the skin. The air flow can be adjusted using four latches. All parts that come into contact with food are made of stainless steel and meet all standards. The machine has 3 stainless steel sieves of different perforations through which the seeds are separated.

Table 1. Ingredients and chemical composition of feed mixtures, hay and grape pomace in feeding of fattening lambs

Ingredients (g/kg feed mixture)	Feed mixture			Hay	Grape pomace
	C	GP10	GP20		
Corn grain	561	493	427		
Grape pomace	–	100	200		
Oat grain	100	100	100		
Soybean meal (46% CP)	205	201	195		
Soybean hulls	100	50	–		
Sunflower oil	–	22	44		
Salt	4	4	4		
Mineral-vitamin premix[1]	30	30	30		
Chemical content (g/kg DM)					
DM (g/kg)	921	927	921	941	367
crude protein	162	159	161	111	50.0
crude fiber	44.1	45.6	43.2	300	77.0
crude ash	58.4	60.1	63.0	101	36.0
ether extract	32.0	43.0	45.0	14.0	21.2
neutral detergent fiber	220	266	295	564	173
acid detergent fiber	642	917	128	386	118
lignin	26	282	575	963	83.1
ME (MJ/kg DM)	11.53	11.44	11.35	7.10	5.00
polyphenols (total) (mg/kg)	231	244	288	–	3969
anthocyanins (total) (mg/kg)	0	232	343	–	2984

C – control; GP10 – grape pomace 10%, GP20 – grape pomace 20%.

[1]Mineral-vitamin premix for lambs: 8% Ca, 5% P, 9.5% Na, 2,00% Mg, 400 000 IU vitamin A, 40 000 IU vitamin D, 500 mg vitamin E, 4 000 mg Zn, 2 000 mg Mn, 60 mg I, 10 mg Co, 50 mg Se; ME – metabolizable energy.

Lambs were offered the feed mixture according to their requirements following NRC (2007). The feed mixture for the lambs of the control group (C) did not contain grape pomace, while in the experimental group I (GP10) grape pomace was included in an amount of 10% and in the experimental group II (GP20) grape pomace was included in an amount of 20%. A slight correction was also made in the composition of the feed mixtures of the experimental groups in order to obtain isoenergetic feed mixtures. Feed mixtures, meadow hay, and water were consumed by lambs *ad libitum*. Lambs were fed with feed mixture in the morning and evening and no refusals were observed. All feed samples (feed mixtures and hay) were ground to a fine particle by using an ultra-centrifugal mill (a heavy metal-free, Retsch ZM 200). Forage composition was determined using standard methods by the Association of Official Agricultural Chemists (2006). The ingredients and chemical compositions of the foods are listed in Table 1. Crude protein in feed was determined from concentration of nitrogen using Kjeldahl method according to ISO 5983-1:2005 (International Organization for Standardization, 2005). Feed samples were analyzed for ash according to ISO 5984:2002 (International Organization for Standardization, 2002) and ether extract of feed was analyzed by extraction system B-811 (Biichi, Flawil, Switzerland), while crude fiber in feed was determined by the Weende method (ISO 6865:2000). Amylase-treated NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by EN ISO 1647:2006 and EN ISO 13906 (2008), respectively, while metabolizable energy was estimated according to INRAE-CIRAD-AFZ (2022).

Feed samples were weighed (0.2 g) and placed into a plastic tube, methanol in water 80% (v/v) was added (1.5 ml), the sample was shaken and placed in ultrasonic water bath (15 min; RK 100, Berlin, Germany), and centrifuged at 10 000 rpm (10 min; Eppendorf, Hamburg, Germany) to obtain the extraction of polyphenols. The residue was extracted a second time using this procedure by using 0.5 ml of methanol (80% (v/v)). These two extracts were combined to give a final measured volume of extract (2 ml). The same procedure was repeated to obtain a second and third parallel extract from the feed. Total polyphenols of the feed were determined by the Folin-Ciocalteu micro method. An aliquot (20 µl) of the diluted extract was mixed with distilled water (1580 µl) and Folin-Ciocalteu reagent (100 µl). The 300 µl of sodium carbonate solution (200 g/l) was added, shaken, and after incubation (40°C for 30 min) in a water bath, the absorbance was measured at 765 nm versus blank. Total polyphenols were expressed as mg gallic acid equivalents (GAE)/kg sample weight. Results were estimated as mean ± standard deviation of three measurements, each measured twice. Total anthocyanins and polyphenols were determined according to the method described in Jakobek et al. (2021) by using a Shimadzu UV-1280 spectrophotometer (Shimadzu Europe GmbH).

Blood sampling and analysis

The samples of blood were collected from each lamb after morning feeding, at the beginning (1st day), middle (15th day), and end (30th day) of the study from the jugular vein (10 ml) into the vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) with ethylenediaminetetraacetic acid (EDTA) as anticoagulant for hematological analysis. After collection, the samples were transferred to ice and under refrigerated conditions (–60°C) to the Department of Animal Production and Biotechnology, Small Ruminant and Non-Ruminant Laboratory, and Animal Nutrition and Physiology at the Faculty of Agrobiotechnical Sciences Osijek, Croatia. The EDTA tubes were gently mixed several times. The Sysmex PocH-100iV automated three-difference hematology analyzer (Sysmex Europe GmbH, Hamburg, Germany) was used to determine the hematological parameters: number of red (RBC) and white blood cells (WBC), hemoglobin content (HGB), hematocrit (HCT), mean hemoglobin content in red blood cells (MCH), mean corpuscular volume (MCV), and mean hemoglobin concentration in red blood cells (MCHC) in whole blood of lambs. Then, blood samples collected in sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) were centrifuged at 1609.92 g within 10 minutes and serum samples were set in the Olympus AU400. The concentrations of biochemical parameters (calcium, inorganic phosphorus, iron, magnesium, urea, glucose-GUK, total proteins-PROT, albumin-ALB, cholesterol-CHOL, LDL cholesterol-LDL, HDL cholesterol-HDL, triglycerides-TGC, β-hydroxybutyrate-BHB, and non-esterified fatty acids-NEFA) were determined in serum. The activities of the enzymes were detected in serum such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), γ-glutamyltransferase (GGT), and glutathione reductase (GR). Globulin content (GLOB) was estimated by subtracting albumin from total protein. Serum glutathione peroxidase (GPx) activity and total superoxide dismutase (SOD) activity were determined by using a Ransel® and Ransod® kit (Randox, UK). All biochemical parameters were analyzed by Olympus AU 400 automated analyzer (Olympus, Japan) with Olympus system reagents (Olympus Diagnostic GmbH, Lismeehan, Ireland).

Analysis of carcass and meat quality

Before slaughter, the lambs were weighed with a digital animal scale. After lambs were slaughtered and after bleeding, the skin was removed from the carcasses, and then the organs of the abdominal cavity (forestomach, stomach, spleen, intestines and liver) and the thoracic cavity (trachea with lungs and heart) were removed. Immediately after, the organs, skin, lower parts of the legs and carcasses were weighed. Standard carcass developmental measurements (linear measurements) were taken: carcass length (os pubis–atlas) and carcass girth (at the scapula). Carcass parameters and samples of lamb meat (*m. semimembranosus*) were taken from all lambs in each

group after slaughter and bleeding immediately, while pH_1 values and color were determined 45 minutes post mortem and pH_2 24 h after slaughter and chilling. The pH was measured with the contact pH meter Mettler Toledo, while the color of the meat was measured with a portable instrument Minolta Chromameter CR-410 (Minolta Camera Co. Ltd., Japan) according to the standard CIE L*a*b* color system (1976). Then, the carcass dressing was calculated (weight before slaughter - weight of carcass/100). Water holding capacity (WHC) was measured according to Sierra (1973). Hue angle (H*) was estimated by formula (1), while chroma (C*) was estimated by formula (2):

$$H^* = \tan^{-1}(b^*/a^*) \quad (1)$$

$$C^* = \sqrt{(a^{*2}+b^{*2})} \quad (2)$$

Meat and blood lipid peroxidation and antioxidative activity

Lamb's meat samples were prepared (10% w/v) in 0.05 M phosphate buffer (pH 7) using an Ultra Turrax (IKA T18 Basic, Labortechnik, Staufen, Germany) homogenizer and centrifuged at 12 000 ×*g* (60 min at 4°C). The supernatant fluids were kept in the dark place at –20°C until further analysis for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and thiobarbituric acid reactive substances (TBARS) was carried out. Extraction was obtained in triplicate.

Total antioxidant activities in serum and extracts of muscles were determined using the DPPH radical scavenging assay described earlier by Qwele et al. (2013) with some modifications, while TBARS were measured according to the guidelines described by Liu et al. (2010) with slight modifications. These modified methods were described in Antunović et al. (2021 a).

Statistical analyses

Shapiro-Wilk test (PROC UNIVARIATE) was used to verify the normality of distributed data. Results were estimated by using PROC MEANS and effects of dietary treatment (C group; GP10 and GP20) were analyzed by using PROC GLM. Tukey's test was used to estimate significant differences between the groups at $P<0.05$, while $P\leq0.10$ was considered as trend of data. All data were analyzed by SAS 9.4.

Table 2. Production traits and body condition scores of lambs

Indicators	Day	Groups (mean)			SEM	P-value		
		C	GP10	GP20		D	P	D×P
Body weight (kg)	1st	23.5	23.5	23.6	0.31			
	15th	28.8	28.0	27.7	0.39	0.682	<0.001	0.435
	30th	33.5	32.3	32.6	0.44			
Daily weight gain (kg/day)	1st–15th	0.358	0.300	0.271	0.01			
	15th–30th	0.310	0.289	0.326	0.02	0.415	0.997	0.367
	1st–30th	0.334	0.294	0.299	0.01			
Average daily intake (kg/day)	1st–15th	0.939	0.823	0.931				
	15th–30th	1.060	1.137	1.028				
	1st–30th	1.000	0.980	0.980				
Feed conversion (kg/kg)	1st–15th	2.62	2.75	3.43				
	15th–30th	3.42	3.94	3.15				
	1st–30th	2.99	3.33	3.28				
Body condition score	1st	3.66	3.72	3.70	0.05			
	15th	3.75	3.52	3.50	0.06	0.447	<0.001	0.082
	30th	3.95	3.79	3.83	0.04			

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; D: diet; P: period.

Table 3. Slaughtering characteristics of lamb carcasses and carcasses measures in lambs fed on grape pomace

Indicators (kg)	Groups (mean)			SEM	P-value
	C	GP10	GP20		
Slaughter characteristics					
live body weight	33.3	33.0	32.6	0.38	0.743
carcass weight	16.8	16.2	16.0	0.22	0.337
entrails weight*	1.48	1.41	1.39	0.03	0.486
skin and legs weight	4.32	4.67	4.28	0.09	0.051
forestomach and intestines weight	7.59	8.01	7.50	0.12	0.052
dressing percentage	50.3	48.9	50.0	0.13	0.125
Measures of lamb carcasses (cm)					
carcass length	75.89	74.72	73.78	0.52	0.256
carcass circumference	64.33	65.11	65.83	0.47	0.436

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; *lungs, trachea, heart, liver.

Table 4. Physical properties of carcasses in lambs fed on grape pomace

Indicators	Groups (mean)			SEM	P-value
	C	GP10	GP20		
pH_1	6.66	6.69	6.59	0.04	0.591
pH_2	5.66	5.66	5.71	0.02	0.623
WHC (%)	25.3	25.5	28.4	1.18	0.493
L*	43.6 A	52.5 B	49.6 B	0.91	<0.001
a*	18.7 Aa	21.6 B	20.4 b	0.34	<0.001
b*	2.29 a	2.65 ab	2.71 b	0.08	0.041
Hue angle	0.10 A	0.11 A	0.13 B	0.24	<0.001
Chroma	18.9 Aa	21.7 B	20.6 b	0.34	<0.001

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; a, b – values in rows with different letters differ significantly (P<0.05); A, B – values in rows with different letters differ significantly (P<0.01); WHC: water holding capacity.

Table 5. Hematological parameters of lambs fed on grape pomace

Indicators	Day	Groups (mean)			SEM	P-value		
		C	GP10	GP20		D	P	D × P
White blood cells ($\times10^9$/L blood)	1	12.8	13.4	12.4	0.98			
	15	9.40	12.5	11.7	0.46	0.519	0.121	0.633
	30	11.2	12.3	11.6	0.72			
Red blood cells ($\times10^{12}$/L blood)	1	9.47	9.77	9.70	0.20			
	15	9.20 A	11.8 B	11.7 B	0.34	0.011	<0.001	<0.001
	30	9.10	9.83	9.44	0.19			
Hemoglobin (g/L blood)	1	116	115	120	2.63			
	15	106	112	121	2.48	0.243	0.031	0.516
	30	117	123	124	2.57			
Hematocrit (g/L blood)	1	0.36	0.37	0.37	0.01			
	15	0.35	0.41	0.42	0.01	0.089	0.011	0.030
	30	0.35	0.38	0.36	0.008			
Mean corpuscular volume (fL)	1	38.1	37.9	37.7	0.25			
	15	37.5	36.5	35.7	0.42	0.290	<0.001	0.306
	30	39.2	38.4	37.5	0.39			
Mean corpuscular hemoglobin (pg)	1	13.0	11.8	12.4	0.20			
	15	11.7	9.94	10.4	0.22	0.018	<0.001	0.064
	30	12.9	12.5	13.2	0.18			
Mean corpuscular hemoglobin concentration (g/L blood)	1	325	311	329	6.30			
	15	312	273	290	5.62	0.119	<0.001	0.105
	30	329	327	352	6.30			
Platelet blood count ($\times10^9$/L blood)	1	842	727	892	47.90			
	15	670	544	661	32.41	0.291	<0.001	0.774
	30	687	617	645	40.50			

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; D: diet; P: period; A, B – values in rows with different letters differ significantly (P<0.01).

Results

The analysis of lambs' production traits did not reveal any significant differences depending on the dietary treatment (Table 2). No significant difference was found depending on the dietary treatment for slaughter characteristics and carcass measures of lambs as well (Table 3). A significant period effect for body weight and BCS was found as expected. Analysis of the physical properties of lamb carcasses revealed a significant increase in L*, a* and huge angle values as well as a decrease in b* values of lamb carcasses in GP10 and GP20 compared to C group (Table 4). There were no significant differences in most hematological parameters in lambs fed on GP (Table 5). A significant influence of the period and diet interaction on the number of RBC and HCT content and the influence of the period on the content of HGB, HCT, MCV, MCH, MCHC, and the number of PLT was determined. There were no significant differences in most of biochemical parameters and mineral concentrations in blood of lambs as affected by different feeding treatment (Tables 6 and 7). The lowest glucose concentration was determined in GP10 followed by GP20, and then in control group in which the highest concentration was determined at day 30. The NEFA concentration increased in GP20 compared to GP10 at day 15, while at day 30 it increased compared to GP10 and C group. The BHB concentration increased in both GP10 and GP20 compared to C group at day 30. The period significantly affected the concentration of Mg and Fe and most biochemical indicators, except TP, ALB, GLOB and TGC. A significant effect of the D×P interaction on the concentrations of Mg, P, glucose, TP, ALB, GLOB, TGC, and NEFA in the blood of lambs was also detected. By analyzing the enzymes activities in lambs' blood it is evident that the diet treatment did

not reveal any significant effect, except the significantly increased SOD at day 30 in G10 lambs compared to C group and increased GPx in G20 compared to C on day 15 (Table 8). The period effect was evident in the activity of AST, ALP, GGT, SOD, GPx, while effect of diet and period interaction was determined only in AST and CK activity in lambs' blood. Dietary treatment did not affect antioxidative status in blood and meat of lambs, although there was a slight decrease of TBARS in blood and meat, altogether with DPPH which significantly increased in meat of lambs fed GP10 and GP20 compared to C group at day 30 (Table 9).

Table 6. Mineral concentration in the serum of lambs fed on grape pomace

Indicators (mmol/L)	Day	Groups (mean)			SEM	P-value		
		C	GP10	GP20		D	P	D×P
Mg	1	1.67	1.68	1.42	0.03			
	15	1.39	1.38	1.59	0.02	0.159	<0.001	<0.001
	30	1.49	1.36	1.31	0.03			
Ca	1	2.62	2.71	2.65	0.03			
	15	2.63	2.73	2.76	0.02	0.095	0.219	0.702
	30	2.59	2.71	2.67	0.03			
Fe (µmol/L)	1	35.2	31.5	25.9	1.63			
	15	27.3	24.6	35.9	2.06	0.539	0.040	0.058
	30	27.6	23.8	22.1	2.33			
P	1	2.96	2.91	2.91	0.05			
	15	3.03	3.14	3.00	0.04	0.113	0.116	0.017
	30	3.18	2.70	2.88	0.06			

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; D: diet; P: period.

Table 7. Biochemical parameters in serum of lambs fed on grape pomace

Blood metabolites	Day	Groups (mean)			SEM	P-value		
		C	GP10	GP20		D	P	D×P
Glucose (mmol/L)	1	6.14	6.00	5.99	0.09			
	15	6.67	6.80	5.36	0.08	<0.001	<0.001	0.022
	30	6.28 A	5.40 B	5.56 C	0.11			
Urea (mmol/L)	1	5.59	4.86	4.60	0.21			
	15	6.85	5.26	6.36	0.31	0.096	<0.001	0.273
	30	7.00	5.64	5.86	0.32			
Total protein (g/L)	1	64.2	67.3	64.3	0.84			
	15	63.4	65.8	64.4	0.86	0.802	0.907	<0.001
	30	68.0	64.2	64.5	0.70			
Albumin (g/L)	1	28.7	29.7	28.5	0.35			
	15	29.2	29.4	29.7	0.27	0.821	0.144	0.017
	30	30.7	29.2	29.2	0.33			
Globulin (g/L)	1	35.6	37.6	35.8	0.67			
	15	34.2	36.4	36.7	0.90	0.86	0.615	0.034
	30	37.4	35.0	35.3	0.70			
Cholesterol (mmol/L)	1	1.56	1.45	1.63	0.10			
	15	1.12	1.16	1.26	0.05	0.632	<0.001	0.743
	30	1.35	1.38	1.38	0.05			
Triglycerides (mmol/L)	1	0.28	0.25	0.26	0.01			
	15	0.26	0.26	0.33	0.02	0.668	0.092	<0.001
	30	0.22	0.29	0.23	0.01			
High-density lipoprotein (mmol/L)	1	0.87	0.83	0.89	0.04			
	15	0.65	0.71	0.81	0.03	0.507	<0.001	0.164
	30	0.72	0.74	0.71	0.02			
Low-density lipoprotein (mmol/L)	1	0.57	0.48	0.54	0.05			
	15	0.35	0.33	0.30	0.02	0.695	<0.001	0.853
	30	0.53	0.51	0.57	0.03			
Non-esterified fatty acids (mmol/L)	1	0.25	0.31	0.40	0.04			
	15	0.10 ab	0.06 a	0.12 b	0.04	<0.001	<0.001	0.046
	30	0.10 a	0.14 a	0.41 b	0.01			
β-hydroxybutyric acid (mmol/L)	1	0.26	0.32	0.27	0.02			
	15	0.32	0.38	0.37	0.02	<0.001	<0.001	0.071
	30	0.25 a	0.45 b	0.35 b	0.03			

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; D: diet; P: period; a, b – values in rows with different letters differ significantly (P<0.05); A, B – values in rows with different letters differ significantly (P<0.01).

Table 8. Activity of enzymes in serum of lambs fed on grape pomace

Enzymes	Day	Groups (mean)			SEM	P-value		
		C	GP10	GP20		D	P	D×P
Aspartate aminotransferase (AST, U/L)	1	114	100	106	3.465	0.519	<0.001	0.034
	15	121	124	121	4.45			
	30	138	111	109	5.56			
Alanine aminotransferase (ALT, U/L)	1	15.4	10.8	12.8	0.86	0.118	0.490	0.402
	15	14.6	11.5	16.6	1.02			
	30	15.5	11.5	13.0	1.02			
Alkaline phosphatase (ALP, U/L)	1	387	325	327	22.48	0.475	<0.001	0.905
	15	511	461	459	27.39			
	30	492	457	401	28.11			
Gamma-glutamyl transferase (GGT, U/L)	1	82.5	85.8	83.1	3.12	0.907	<0.001	0.519
	15	72.9	78.6	78.6	2.35			
	30	66.7	65.3	63.7	2.22			
Creatine kinase (CK, U/L)	1	227	129	136	13.12	0.348	0.278	0.015
	15	194	201	171	16.03			
	30	149	194	164	11.99			
Superoxide dismutase (SOD, U/mL)	1	0.39	0.47	0.55	0.03	0.049	<0.001	0.580
	15	0.56	0.67	0.64	0.03			
	30	0.46 a	0.57 a	0.55 ab	0.02			
Glutathione peroxidase (GPx, U/L)	1	1096	1236	1215	86.53	0.002	0.044	0.192
	15	1046 B	1199 AB	1437 A	56.51			
	30	1307	1353	1553	61.16			
Glutathione reductase (GR, U/L)	1	78.5	72.4	68.7	3.07	0.230	0.081	0.213
	15	80.7	85.8	73.5	3.06			
	30	71.6	81.6	66.4	3.15			

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10%; GP20: grape pomace 20%; D: diet; P: period; a, b – values in rows with different letters differ significantly (P<0.05); A, B – values in rows with different letters differ significantly (P<0.01).

Table 9. Antioxidative status of blood and meat in lambs fed on grape pomace

Indicators	Day	Groups (mean)			SEM	P-value
		C	GP10	GP20		
TBARS (nmol MDA/mL serum)	1st	0.57	0.56	0.58	0.02	0.289
	30th	0.59	0.53	0.50	0.02	
DPPH blood (%)*	1st	56.6	57.1	61.7	5.17	0.841
	30th	40.6	42.6	45.7	3.61	
TBARS (mg MDAeq/kg muscle)	30th	3.55	3.35	3.44	0.04	0.106
DPPH muscle (%)**	30th	52.1 A	58.8 B	58.9 B	0.94	<0.001

Mean: mean value; SEM: standard error of mean; C: control; GP10: grape pomace 10; GP20: grape pomace 20%; TBARS – thiobarbituric acid reactive substances; *% DPPH radical scavenging activity at final serum concentration 25 µL/ml; **% DPPH radical scavenging activity at final muscle concentration 0.01 g/ml; D: diet; P: period; A, B – values in rows with different letters differ significantly (P<0.01).

Discussion

The intake of a diet which contains a moderate level of polyphenols may improve animal performance primarily due to better utilization of feed proteins (Frutos et al., 2004). As pointed out by Toral et al. (2011), polyphenols can bind feed proteins and reduce their degradation in the rumen, consequently increasing the flow of amino acids to small intestine. In the present study, no significant changes in the production traits and slaughter characteristics of lambs as affected by dietary treatment were found. However, there were opposite results in the literature presented. In the study by Calderón-Cortés et al. (2018) lambs were fed with diet containing grape pomace (300 g/kg DM), and no differences were determined in the production traits and slaughtering characteristics. Similarly, Chikwanha et al. (2019) found no differences in the production traits of lambs fed GP added in diets. Authors concluded that GP included in diets for lambs may be limited due to high NDF (306–693 g/kg DM) and proanthocyanidins (21–202 g/kg DM; Abarghuei et al., 2010). Therefore, suggestions were to include GP in lambs' diets in amounts up to 12.2%, since this content positively affected production traits and decreased costs of feed. With increasing the content of GP in lambs' diets 5, 10, 15, or 20% Bahrami et al. (2010) determined lowering of average daily weight gain and daily intake, as well as increasing feed conversion. Determined meta-

bolic profile revealed no significant differences in most of hematological and biochemical parameters, while most of them were within reference physiological values (Kramer, 2000; Kaneko et al., 2008; Lepherd et al., 2009; Antunović et al., 2017, 2021 b). In the present study, on 15th day, higher RBC was determined. Similar to present study, Mahdi and Yousif (2019) determined a higher RBC in lambs fed with GP compared to control group, explaining that this feeding boosted RBC, since it provides extra iron and polyphenols' concentration. Besides, effect of diet and period interaction was expected. The lowest glucose concentration was determined in GP10 followed by GP20, and then in C group in which the highest concentration was determined on day 30. The NEFA concentration increased in GP20 compared to GP10 on day 15, while on day 30 it increased compared to GP10 and C group. The BHB concentration increased in both GP10 and GP20 compared to C group on day 30. These changes indicate negative changes in energy metabolism. As it is presented in Table 2, GP20 lambs had lower feed intake and lower average daily weight gain and feed conversion, especially up to the 15th day of the study, which indicates the need to reduce the content of GP in lambs' feed at that age. This is in line with the conclusions made by Bahrami et al. (2010) and Makkar (2003) who pointed out that the intake of tannin-rich plants can reduce the intake (consumption) of feed. In a study by Molosse et al. (2021) carried out with suckling lambs fed with the addition of grape residue flour (2%), an increase in concentration of glucose was observed in the blood. The authors linked this to grape flour supplementation which possibly altered a microbe's composition and increased the propionate production and availability of glucose precursors in the liver. When feeding lambs with the addition of plant bioactive compounds, like grape, olive, or pomegranate, Poli et al. (2021) did not determine any decrease in the daily weight gain of lambs (275:258 g/day) nor the changes in hematological parameters and TBARS in the blood. Although, the authors determined slightly higher non-significant values of MDA in lamb meat which is in accordance with the present study. In the present study an increase in SOD activity on day 30 and GPx activity on day 15 in the blood was found, as well as a smaller decrease in TBARS in blood and meat and an increase in DPPH in lamb meat GP10 and GP20 compared to C. This may be related with a positive effect of GP on the antioxidant status of lambs. Even more pronounced improvement in antioxidant status in lambs and the absence of changes in lipid oxidation, indicated by MDA in the blood and meat of lambs, may be associated with lower availability of polyphenols in diet (Teng et al., 2012). Dietary polyphenols can be metabolized and inactivated by microbes in the rumen during fermentation (Welch et al., 2012). The addition of 0, 5, and 10% grape pomace to lambs at 4 months of age improved overall antioxidant capacity and, as in the present study as well, led to increased GPx and SOD activity in lambs' blood in the study by Zhao et al. (2017, 2018). Therefore, these authors recommend the use of GP feed lambs in order to alleviate induced oxidative stress. In the study by Zhang et al. (2019) the addition of grape pomace in the lamb diet reduced the concentration of reactive oxygen species, while significantly increased the activity of catalase, GPx, SOD and total antioxidant potential which led to alleviation of apoptosis of jejunum epithelium (jejunum cell death). Kerasioti et al. (2017) found that the addition of grape pomace increased glutathione transferase activity and protein expression in the liver, as well as glutathione transferase activity in the spleen of lambs. The authors emphasize the possibility of using grape pomace in stressful situations for lambs such as weaning. Jerónimo et al. (2012) pointed out that the application of wine industry by-products is effective for antioxidant activity in lamb meat and sheep plasma. The positive effects of dietary polyphenols, such as GP, lead to improved antioxidant status of ruminants (López-Andrés et al., 2013) and better ruminant health status (Athanasiadou et al., 2006; Gessner et al., 2017). In the present study, a significant increase in L* and a* values as well as hue angle and decrease in b* values of lamb carcasses were found in GP10 and GP20 compared to C. Besides, a non-significant increase in WHC was determined in GP20. Meat color is an important indicator that significantly influences consumers' choice when buying red meat (Morrissey et al., 1994). Gómez-Cortés et al. (2018) found a greater ability in meats' water holding capacity by including grape pomace in the feed of fattening lambs. The reason for this is the significant concentration of polyphenols in GP and its influence on the rate of oxidation in meat. Zhao et al. (2018) and Luciano et al. (2009) found an increase in meat redness (a*) when lambs were fed with the addition of GP. Tayengwa and Mapiye (2018) pointed out that there is an unclear effect on the redness of meat which can be linked to ability of polyphenols to chelate iron in myoglobin, which is consequently not available for oxidation (Guerra-Rivas et al., 2016), and/or resistance of myoglobin to oxidation (Kumar et al., 2015). By feeding lambs with antioxidant compounds Nieto et al. (2010) determined higher redness of meat which possibly protected meat pigments from oxidation. In the study by Mu et al. (2020) with using grape seeds rich in proanthocyanidins as supplementation in lambs' diets there was a significant decrease of MDA in *m. longissimus dorsi* (from 0.33 to 0.26) with increased content of grape seed (proanthocyanidins) in the diet from 10 up to 40 mg/kg of body weight per day. Besides, in this study redness of the *longissimus dorsi* muscle at 24 h increased linearly. However, Zhao et al. (2018) and Jerónimo et al. (2012) reported that color of lamb's meat did not change as affected by dietary GP or extract of grape seed. The difference may be due to different concentration of proanthocyanidins in dietary GP consumed by lambs. Giller et al. (2021) pointed out that grape pomace as by-product from production of wine and juice can be an alternative feedstuff for animal feeding which also would be helpful in order to reduce waste disposal problems. This is a very

good solution which should be applied in further research when using a GP in lambs' feeding.

Conclusion

The obtained results indicate the justification of the use of GP in feeding lambs, given the preservation of productivity and metabolic profile of blood. Although, attention should be paid to its amount, thus we recommend using 10% of GP instead of 20% to avoid possible negative effects on production traits. For future research, it is necessary to include several groups of lambs with different amounts of GP in the mixture, extend the duration of the investigation, and determine the fatty acid profile of lambs' meat.

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Declaration of interest statement

No potential conflict of interest was reported by the authors.

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