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Effect of Grape By-Products Inclusion on Ruminal Fermentation, Blood Metabolites, and Milk Fatty Acid Composition in Lactating Saanen Goats

Research Article

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ABSTRACT

Grape by-product is one of the distillery industries which could be used in animal nutrition. In this 60-d trial, 16 lactating Saanen goats were assigned to four homogeneous groups and fed as follows: (1) control (CON) diet, (2) diet supplemented with 50 g/kg dry matter (DM) of grape by-product (GPB5), (3) diet supplemented with 100 g/kg DM of grape by-product (GPB10), and (4) diet supplemented with 150 g/kg DM of grape by-product (GPB15). The dry matter intake and apparent total-tract digestibility of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were not affected (P>0.05) by grape by-products supplementation. Ruminal fermentation characteristics include pH and NH_3-N were not affected by GBP treatments (P>0.05). Supplementing with GBP reduced propionate (P=0.06) without effect on other volatile fatty acids (VFAs). Increasing the percentage of grape residues to 10% of the total diet had no significant effect on goat milk production (P>0.05), but milk fat and protein percentage decreased in diets containing GBP (P<0.05). Plasma concentrations of glucose, cholesterol, and total protein were not affected by dietary treatments, but plasma concentration of triglyceride increased in GPB15. Inclusion of grape by-products in lactating Saanen goats diets had no significant effects (P>0.05) on the concentration of major classes of milk fatty acid (FA) according to the degree of saturation (i.e., saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)). These findings indicated that the inclusion of GBP to 15% in replacement of beet pulp in the diet of dairy Saanen goats have no adverse effects on ruminal fermentation, blood metabolites and milk fatty acid.

KEY WORDS blood metabolites, grape by-products, milk fatty acid, ruminal fermentation, Saanen goat.

INTRODUCTION

Grape by-product is obtained during grape juice production process. This product makes up about 30% of the moist weight of the fruit. Use of this agro industrial by products reduce the pollution load from the environment on dumping sites. In recent years, researchers have paid attention to recycling useful products from grape by-products and improving its quality for animal feed (Santos, *et al.* 2014; Correddu *et al.* 2015). However, these residues contain relatively large amounts of sugar (mainly glucose, fructose and sucrose), tannin compounds (anthocyanins and flavonoids) that can be recovered and used (Santos *et al.* 2014). Grape by-products are a good source of anthocyanins, the strongest natural antioxidants found in fruits and vegetables (Santos *et al.* 2014). In addition, studies have shown the effects of supplementation of grape residual tannins on increasing microbial protein synthesis (Alipour and Rouzbehan, 2007) and 13-16% reduction in ruminal methanogenesis (Grainger *et al.* 2009).

On the other hand, the consumption of saturated fatty acids in the milk and meat products of ruminants is associated with an increased prevalence of cardiovascular disease (Jenkins *et al*. 2008). The World Health Organization recommends reducing the consumption of 12: 0, 14: 0 and 16: 0 fatty acids and trans fatty acids in human nutrition (FAO, 2010). With this approach, the interest in improving the nutritional value of ruminant products for human consumption by increasing polyunsaturated fatty acids (PUFA) (n-3), rumenic acid (RA), vaccenic acid (VA) and reducing the amount of saturated fatty acids (SFAs) and harmful trans acids are an unavoidable necessity for human consumption (Correddu *et al*. 2015).

Hence, the objective of this experiment was to study the effect of grape by-products to manipulate and to improve the fatty acid composition of ruminant milk.

MATERIALS AND METHODS

Experimental design, goats and treatments

Animal care

The experiment was carried out according to the care and use of agricultural animals in research and teaching (FASS, 2010) guidelines. All procedures and guidelines involving animals were approved by the Ethic Committee for Use of Animals in Experimentation at the Birjand University, Birjand, Iran.

Grape (residues) by-products

Siah Gohar grape variety were collected from the orchards of South of Khorasan Razavi and dried after dewatering which contained soft external hull, seeds and twinges.

Sampling procedures

The research was carried out the experimental farm of the Department of Animal Sciences, Faculty of Agriculture, Birjand University, (Iran).

Sixteen multiparous Saanen dairy goats in the first part of lactation (<50 days in milk, DIM) were assigned to four groups of four animals each, homogeneous for milk production, body weight, DIM, and lactation order. Groups were randomly assigned to one of the four experimental diets (Table 1): 1. control (CON) diet (without grape byproduct), 2. diet supplemented with 50 g/kg DM of grape by-product (GPB5), 3. diet supplemented with 100 g/kg DM of grape by-product (GPB10), and 4. diet supplemented with 150 g/kg DM of grape by-product (GPB15). On the basis of results from previous studies, this levels of grape by-product was considered safe for the animal and practical for farmers (Buccioni *et al*. 2015). Higher intake of grape residue also may have led ruminants to deposit less body fat due to greater consumption of phenolic compounds (Santos *et al*. 2014).

Animals fed diets containing less than 4% phenols on a DM basis resulted in higher retention of nitrogen and lower plasma urea because of the ability of tannin to protect feed protein from rumen microbial degradation (Frutos *et al*. 2004).

Table 1 Chemical composition and fatty acid composition of grape by-products

Item[1]	Grape by-products
DM (%)	53.60
CP (% of dry matter)	9.43
EE (% of dry matter)	6.42
ADF (% of dry matter)	21.90
NDF (% of dry matter)	29.95
Ash (% of dry matter)	4.26
Ca (% of dry matter)	1.16
P (% of dry matter)	0.226
Mg (% of dry matter)	0.149
Fe (ppm)	116.7
WSC (% of dry matter)	27.05
TP (% of dry matter)	18.072
TT (% of dry matter)	11.616
Fatty acid (g/100 g of total fatty acids)	
C12:0	0.03
C14:0	0.08
C16:0	7.84
cis9-C16:1	0.56
C17:0	0.10
C18:0	2.27
cis9-18:1	17.38
cis6-18:2	69.89
cis3-18:3	0.26
C20:0	0.43
C20:1	0.16
SFA	10.75
MUFA	18.10
PUFA	71.15

DM: dry matter; CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber; WSC: water soluble carbohydrates; TP: total phenolic; TT: total tannin; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Moreover, the levels of grape by-product was chosen to obtain a tannin concentration in the diet of almost 0.5-2% of expected DMI that was considered safe for the animal and practical for farmers. Considering that the total phenolic content of grape by-product was 18.072% of dry matter (DM; mean), diet containing 50 g/kg DM of grape by-product provide approximately 1.36 g/kg DM of diet of total polyphenols (Table 2).

All animals were offered the same basal diet consisting of concentrate and forages (alfalfa hay, wheat straw and corn silage). Diets were formulated to meet the goat energy and protein requirements using the small ruminant nutrition system (SRNS) (Cannas *et al*. 2010). In addition, they received a mixed diet in different proportions depending on the dietary treatments, to obtain isoenergetic and isonitrogenous diets.

Table 2 Ingredient composition of experimental diets

Item	Treatments (diets containing different levels of grape by-product, (GBP))			
	CON	5% GBP	10% GBP	15% GBP
Ingredients, %				
Alfalfa hay	12	12	12	12
Wheat straw	12	12	12	12
Corn silage	20	20	20	20
Barley	11.5	11.5	11.5	11.5
Corn	9	9	9	9
Soybean meal	7	7	7	7
Canola meal	3	3	3	3
Wheat bran	8	8	8	8
Beet pulp	15	10	5	0
GBP	0	5	10	15
Salt	0.3	0.3	0.3	0.3
Calcium carbonate	0.7	0.7	0.7	0.7
Bicarbonate Sodium	0.5	0.5	0.5	0.5
Vitamin-mineral mix[1]	1	1	1	1
Chemical composition, % of DM				
DM	75	75	75	75
NE_L (Mcal/kg)	1.49	1.49	1.49	1.49
CP	14.9	14.9	14.9	14.9
Calcium	0.95	1	1	1
Phosphorus	0.42	0.45	0.45	0.45
NDF	34.7	32.4	32.4	32.4
ADF	21.3	19.8	19.8	19.8
EE	4.55	4.85	5.20	5.55
Total phenols	0.46	1.36	2.27	3.17
Total tannins	0.16	0.74	1.32	1.90

[1] Contained (/kg of premix; DM basis): vitamin A: 330000 IU; vitamin D: 60000 IU; vitamin E: 1000 IU; Ca: 160 g; P: 85 g; Na: 63 g; Mg: 45 g; Zn: 2100 mg; Mn: 1500 mg; Cu: 535 mg; Se: 12 mg and I: 45 mg.

DM: dry matter; NE_L: Net energy lactation; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract.

The animals received total mixed ration at the fixed amount in two equal meals daily after daily milking (7:30 and 17:30). Clean water was always available. The experiment lasted 10 weeks, with 2 weeks of adaptation period and 8 weeks of data collection. For determine of dry matter intake and apparent total-tract DM, organic matter (OM), CP, ash-free neutral detergent fiber (NDFom) and ash-free acid detergent fiber (ADFom) digestibility, before experiment period, the experimental diets were offered *ad libitum* and the maximum levels of feed consumed by the investigational goats determined. Then the amount of each diet was slightly decreased to eliminate orts. During the last week of the experiment, samples of feeds and feces from each goat on each treatment were weighed and 10% representative sample was frozen for later analysis. Total apparent digestibility of DM, organic matter (OM), CP, ash-free neutral detergent fiber (NDFom) and ash-free acid detergent fiber (ADFom) were measured using the total fecal collection method described by (Givens *et al.* 2000).

On day 60 of the experiment, rumen fluid was collected from each goat at 3 h after the morning feeding, using a stomach tube and checked to confirm that it did not contain saliva.

The rumen fluid samples were filtered through four layers of cheesecloth and immediately used to measure pH, using a glass electrode pH-meter (691 Metrohm, Herisau, Switzerland). The ruminal fluid was subsequently acidified with 5.0 mL was collected into 1 mL of HCl 0.2 N to stop fermentation, transported to the laboratory and frozen -20 ˚C for ammonia analysis. For analysis of volatile fatty acids (VFA), 0.25 ml of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mM 2-ethyl-butyric acid was added to 1 mL of rumen fluid and then frozen at -20 ˚C. Blood samples from all the goats were obtained from the jugular vein 3 h after the morning feeding (10 mL into sterile tubes containing EDTA solution) on day 60 of the experiment.

The blood samples were then centrifuged at 3000 rpm for 15 minutes to obtain plasma which was separated, frozen, and stored at -18 ˚C until further analysis. The plasma concentrations of glucose, cholesterol, triglyceride, blood urea nitrogen (BUN), total protein, albumin, and liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)] were determined using an automated biochemestery analyzer (Midrary BS800M, Shenzhen, China) according to the manufacturer's instructions.

Goats were milked 2 times daily (7:00 and 17:00) and the amount of milk produced for each goat at each milking was recorded using special graduated jars. Before each milking, goats were monitored for udder inflammation and presence of milk clots in the teats to ensure that milk yield and composition were not affected by mastitis. During the last week of experimental period, individual milk samples were taken from each goat at each milking (morning and evening milking) were allotted into 2 aliquots for analysis: the first aliquot immediately analyzed to assess fat, protein, lactose and SNF contents in milk by using a Milk-O-Scan 4000 infrared analyzer (Foss Electric, Hillerød, Denmark). The second aliquot of milk samples was stored at -80 ˚C until analysis for FA extraction and composition by gas chromatography according to (Buccioni *et al*. 2010).

Laboratory analysis

For chemical analyses, the samples were milled to pass through a 1 mm screen by a CyclotecTM 1093 Sample Mill (Foss Companies, Hillerød, Denmark). Samples of feed were analyzed for DM (method 934.01; AOAC, 1990), organic matter (method 920.39; AOAC, 1990), ether extract (method 920.39; AOAC, 1990) and CP (method 988.05; AOAC, 1990) standard procedures. Concentrations of acid detergent fiber (ADF) inclusive of residual ash (method 973.18c; AOAC, 1990) and neutral detergent fiber (NDF) inclusive of residual ash were determined sequentially without the use of sodium sulphite and with the inclusion of α-amylase (Van Soest *et al*. 1991).

The rumen fluid samples were collected by stomach tube from each goat on day 60 of the collection period just 3 h after morning feeding. The pH was determined immediately after sampling. Then, the samples were strained through four layers of cheesecloth and acidified with 5.0 mL was collected into 1 mL of HCl 0.2 N to stop fermentation, transported to the laboratory and frozen (-20 ˚C) for ammonia analysis. Strained rumen fluid was analyzed for ammonia-N according to (Broderick and Kang, 1980). For analysis of volatile fatty acids (VFA), 0.25 mL of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mM 2-ethyl-butyric acid was added to 1 ml of rumen fluid and then frozen at -20 ˚C. After thawing, strained rumen fluid samples were centrifuged (14,000 rpm, -5 ˚C, 15 min) and VFA were determined by gas chromatography using ethyl-butyric acid as the internal standard according to the procedure of (Stewart and Duncan, 1985).

For tannin assay, samples of grape by-product were dried at 40 ˚C to constant weight to minimize changes in tannin content and activity, and dried samples were ground through a 0.5-mm screen before analysis (Makkar, 2000). Phenolic compounds were extracted using 200 mg of dried samples.

The extraction process involved the sample being made up to 10 mL with aqueous acetone water (700:300, v/v), and the extraction was left at 4 ˚C overnight. The extracts were centrifuged at 3000 g at 4 ˚C for 15 min, and the supernatant was obtained and used in the following assay. The concentration of total phenolic compounds (TP) was determined using the Folin–Ciocalteu procedure (Singleton and Rossi, 1965) and the regression equation of tannic acid (Merck GmbH, Darmstadt, Germany) standard. Total tannins (TT) were estimated indirectly after being absorbed to insoluble polyvinylpolypyrrolidone (PVPP). Concentration of TT was calculated by subtracting the TP remaining after the PVPP treatment in the assay mixture (Makkar, 2000). The concentrations of glucose, cholesterol, triglyceride, blood urea nitrogen (BUN), total protein, albumin, and liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)] were determined using an automated biochemistery analyzer (Midrary BS800M, Shenzhen, China) according to the manufacturer's instructions.

Fat, protein, lactose and SNF proportions in milk were measured using a Milk-O-Scan 4000 infrared analyzer (Foss Electric, Hillerød, Denmark). For milk FA measurement, milk lipids were extracted Folch *et al*. (1957); this method consists of homogenizing the tissue with a 2:1 (v/v) chloroform/methanol mixture and washing the extract by addition to it of 0.2 its volume of either water or an appropriate salt solution (KCL). The resulting mixture separates into two phases. The lower phase is the total pure lipid extract. Fatty acid methyl esters (FAME) were prepared according to the method described by (Van Wijngaarden, 1967). Fatty acid profile was determined by gas chromatography. A fused silica capillary column (WCOT Fused Silica Capillary, DANI, Model 1000, Rome, Italy) with 120 m length, 0.32 mm internal diameter and 0.2 µM film thickness on an HP 6890 GC equipped with flame ionization detector was used to qualify and quantify FAMEs. The initial column temperature was set at 180 ˚C for 20 min, which increased to 225 ˚C by increments of 5 ˚C/min, then to 250 ˚C by 10 ˚C/min and held for 12 min. Hydrogen was used as carrier gas with a flow of 1.7 mL/min for the first 10 min. Then, the flow was decreased to 1.3 mL/min which was kept until the end of the analysis. The detector temperature was set at 300 ˚C. Identification of FA was performed by comparison with the retention times of FAMEs standards (Sigma–Aldrich, Catalog #18919). The trans-18:1 isomers were identified by order of elution as described by (Precht *et al*. 2001). Separations of all FA were obtained with a single chromatographic run.

Statistical analysis

The data was analyzed as a completely randomized design using the PROC GLM procedure of SAS 9.4 (SAS, 2013),

with the animal as the experimental based on the statistical model:

$Y_{ij}= \mu + A_i + e_{ij}$

Where:
Y_{ij}: measured value.
μ: mean of the population.
A_i: treatment.
e_{ij}: error term.

Comparisons of means of the different treatments were made using Duncan Multi-Range Test.

RESULTS AND DISCUSSION

The dry matter intake and apparent total-tract digestibility of DM, OM, CP, NDF and ADF were not affected (P>0.05) by grape by- products supplementation (Table 3), which can be explained by the optimum ruminal pH values (Table 4) in all treatments. Similar to our result, Santos *et al.* (2014) and Bahrami *et al.* (2010) observed no difference in DM intake when grape residue was added at 100 g/kg DM to the diet of Holstein cows and lambs, respectively. in agreement with Bahrami *et al.* (2010), digestibility of crude protein at the level of 15% grape residue were not affected by diets, but Santos *et al.* (2014) reported that the increased proportion of grape residue silage in the diets linearly decreased the digestibility of dietary CP and DM. The high contents of polyphenols and lignin of grape residue may be responsible, respectively, for decreased digestibility of crude protein and DM. Other studies showed that adding sources of polyphenols, such as grape residues, reduced protein degradability (Abarghuei *et al.* 2010; Dschaak *et al.* 2011), because of their ability to bind proteins and reduce the activity of microbial enzymes by decreasing the growth of proteolytic bacteria (Molan *et al.* 2001; Correddu *et al.* 2015). Dschaak *et al.* (2011) observed no effects on digestibility of DM, OM, CP, and ADFom with the addition of quebracho condensed tannin extract at 3% of DM. The lack of a treatment effect on digestibility of DM, OM and ADF is consistent with the results of Gholizadeh *et al.* (2010) who observed that supplementing Pistachio by-products at 100 g/kg DM (TP; 9.6 g tannic acid equivalent/100 g of DM) in the diets of dairy cows had no effect on apparent digestibility of DM, NDF and ADF, this could possibly be attributed to the nature of tannin and its concentration. Conversely, Carulla *et al.* (2005) reported that supplementing sheep diet with CT at 25 g/kg DM from *Acacia mearnsii* extract (black wattle tree) decreased digestibilities of OM, CP, NDF and ADF.

Furthermore, Sedighi-Vesagh *et al.* (2015) reported that although Pistachio by-products replacement for alfalfa hay decreased the apparent digestibility of CP, there were no effects on apparent digestibility of DM, OM and ADF.

In the present study, DMI was not influenced by inclusion of grape by-products in the diets which was probably due to lack of differences in nutrient digestibility (Table 3). These results indicate that the effects of secondary metabolites on nutrients digestibility vary with the concentration of these metabolites, chemical structure and with the source of the plant used (Abarghuei *et al.* 2010).

As shown in Table 4, pH and NH_3-N were not affected by GBP treatments (P>0.05). Values of pH in all of treatments were ranged between 5.5 to 7 which were within the normal range for rumen liquor (Dziuk, 1984). During the trial, none of the animals recorded pH values below 5.5 this indicated that consumption of sufficient amounts of forage in all treatments prevented a decrease in ruminal pH. These results were consistent with other studies Abarghuei *et al.* (2010) and Yildiz *et al.* (2005) which reported that rumen pH was not significantly affected by supplementation with grape residues as a polyphenolic source. The results of this study were consistent with the findings of Correddu *et al.* (2015) which showed that addition of 300 g of grape seed daily to lactating ewes did not significantly change the pH parameters.

Diet containing 5% of GBP had a tendency to the highest ruminal concentration of propionate (Table 4, P=0.06). Which was probably due to the depressive effect of condensed tannins on both carbohydrate and protein degradation, that decreased propionate (Table 4, P=0.06) without effect on other VFA's, which is probably due to the lack of significant effect on DMI (Abarghuei *et al.* 2013). Previous studies reported controversial data concerning the effect of tannins on total VFA or on their molar proportion in rumen liquor.

Hervás *et al.* (2003), Abarghuei *et al.* (2010) Dschaak *et al.* (2011) and Toral *et al.* (2011), found that tannins did not affect total VFA concentration or their molar proportions in rumen liquor from ewes. Abarghuei *et al.* (2013), reported that the concentrations of total VFA and molar proportions of individual VFA were not affected by inclusion of pomegranate peel extract in dairy cows.

Buccioni *et al.* (2015) observed several differences in the molar proportion of rumen VFA among treatments, because the chestnut tannin diet increased the concentrations of acetic and butyric acids, whereas the quebracho tannin decreased all VFA with the exception of butyric acid. Whereas Bhatta *et al.* (2009) found that condensed tannins from mimosa reduced total VFA and increased production of propionate.

Table 3 Dry matter intake and apparent nutrient digestibility of dairy goats fed different levels of grape by-products supplementation

Parameter	Treatments				SEM	P-value
	CON	GBP5	GBP10	GBP15		
DM intake (kg/day)	2.54	2.37	2.30	2.43	0.34	0.49
Total tract apparent digestibility (%)						
DM	0.58	0.59	0.61	0.55	0.10	0.63
OM	0.74	0.72	0.74	0.66	0.12	0.45
CP	0.47	0.52	0.52	0.49	0.12	0.73
NDF	0.85	0.86	0.87	0.86	0.08	0.91
ADF	0.75	0.73	0.82	0.77	0.12	0.41

CON: control and GBP: grape by-product.
DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber and ADF: acid detergent fiber.
SEM: standard error of the means.

Table 4 Effect of diets including different levels of grape by-products on rumen fermentation parameters of lactating goats

Item	Treatments				SEM	P-value
	CON	GBP5	GBP10	GBP15		
Ruminal parameters						
Ruminal fluid pH	5.77	5.64	5.78	5.84	0.06	0.80
Ruminal NH_3-N (mg/dL)	15.65	17.08	16.19	14.93	3.84	0.68
Individual volatile fatty acids (mol/100 mol)						
Acetate (mol/100 mol)	63.29	66.11	63.08	66.47	4.88	0.23
Propionate (mol/100 mol)	21.57^{ab}	23.06^{a}	20.92^{ab}	19.56^{b}	1.31	0.06
Butyrate (mol/100 mol)	13.70	12.14	11.25	13.71	1.61	0.14
Iso butyrate (mol/100 mol)	0.66	0.65	0.70	0.77	0.05	0.93
Valerate (mol/100 mol)	1.06	1.20	1.13	1.19	0.09	0.95
Iso valerate (mol/100 mol)	0.80	0.78	0.97	0.99	0.12	0.84

CON: control and GBP: grape by-product.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Other researchers have shown that polyphenol levels, greater than 5 g/kg of dietary DM had a significant effect on the rumen bacterial population and VFA concentration (Hervás *et al*. 2003; Vasta *et al*. 2010; Anantasook *et al*. 2014). These controversial results may arise from the use of different dosages or different kind of tannins and of associative effects between tannins and other ingredients (carbohydrate source, forage: concentrate ratio, amount and type of oil or lipid (linoleic acid (LA) or linolenic acid (LNA)) of the basal diet (Buccioni *et al*. 2015).

The effect of diet containing different levels of GBP on the plasma concentrations of metabolites are shown in Table 5. Plasma concentrations of metabolites did not affected by GBP supplementation, except for triglycerides concentration and diet supplemented with 150 g/kg DM had the greatest concentration. Increased grape by-product increased serum triglycerides due to increased phenolic compounds and interfere with luminal emulsification, hydrolysis and micellar solubilization of lipids by reducing the gastric and pancreatic lipase activity (Ahmed *et al*. 2015). Plasma concentrations of creatinine (P=0.06) and blood urea nitrogen (BUN) (P=0.07) tended to have greatest concentration in diets supplemented with 50 g/kg DM of GBP.

Reducing concentration of BUN may be due to decreasing the proteolysis in rumen and decline in ammonia production, consequently reduced the ammonia absorption of rumen. This result are in accordance with the results of Sedighi-Vesagh *et al*. (2015) who reported lower BUN concentrations for Saanen dairy goats fed 320 g/kg DM pistachio by-products (3.18% DM, total phenols; 2.12 total tannins). Similarly, Abarghuei *et al*. (2013) documented that blood urea nitrogen, ruminal ammonia, and urinary N loss were lower when cows were fed pomegranate-peel extract that contained tannins (4.56% DM, total phenols; 1.92 total tannins).

Plasma glucose agrees with those reported by Ghaffari *et al*. (2014) who reported that inclusion of 30% pistachio by-products (3.31% DM, total phenols; 1.81 total tannins) in the diet of early lactation Saanen dairy goats had no effects on their blood glucose. Similar results were reported by Bohloli *et al*. (2009) and Rezaeenia *et al*. (2012) (5.5% DM, tannins) for their blood glucose in the diet of early lactation dairy cows, may be tannins in diets were not enough to combine with cell wall structure and inhibition enzymes action could not reduce soluble carbohydrate (Correddu *et al*. 2015).

Table 5 Effect of diets including different levels of grape by-products treatments on plasma concentrations of metabolites of lactating goats

Item	Treatments				SEM	P-value
	CON	GBP5	GBP10	GBP15		
Blood parameters						
Glucose (mg/dL)	63	57	60.75	61.33	7.82	0.40
Triglyceride (mg/dL)	12.67^{b}	11.25^{b}	16.25^{ab}	19.33^{a}	5.61	0.04
Cholesterol (mg/dL)	78.33	84.75	80	81.33	23.45	0.93
Albumin (g/dL)	3.40	3.65	3.30	3.00	0.56	0.14
Creatinine (mg/dL)	0.83	0.79	0.72	0.70	0.10	0.06
Total protein (g/dL)	7.33	7.62	7.35	7.77	0.80	0.59
BUN (mg/dL)	46.33	53	49	46.50	5.81	0.07
AST (U/L)	82.00	77.25	83.25	88.00	17.78	0.62
ALT (U/L)	17.67	17.00	21.25	22.67	6.59	0.23

CON: control; GBP: grape by-product; BUN: blood urea nitrogen; AST: aspartate aminotransferase and ALT: alanine aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The lack of differences in blood concentrations of glucose with GPB was consistent with its lack of effect on DM intake (Abarghuei *et al*. 2014). Concentration of cholesterol in this present study were not affected (P>0.05) by the treatments, this is consistent with the results of Ghaffari *et al*. (2014) and Sedighi-Vesagh *et al*. (2015). Normal levels of total protein and albumin were within the ranges of 56-96 g/L and 18.9-44.5 g/L for total protein and albumin, respectively, for healthy goats (Žubčić, 2001). Similarly, Abarghuei *et al*. (2014); Ghaffari *et al*. (2014), and Sedighi-Vesagh *et al*. (2015) reported that administration by-products containing tannins had no effect on albumin and blood total protein concentration. Blood total protein concentration is an indicator of the long-term protein status of dairy cows (Topps and Thompson, 1984) and no effect on total protein content of blood in our study represents the normal protein.

Alanine aminotransferase is a liver specific hepatocellular enzyme released by hepatocellular damage that increases in serum when liver cells are damaged (Mahgoub *et al*. 2008).

The normal ranges for ALT and AST are 7-24 IU/L and 43-132 IU/L (Sirois, 1995; Daramola *et al*. 2005). The fact that these blood metabolic profiles in the present study were within the normal ranges for goats suggest that no damage to the liver occurred. The result, is in agreement with that of Silanikove *et al*. (1996), contradicts the report that the tannins in pistachio by-products diets caused tissue damage in goats (Sedighi-Vesagh *et al*. 2015).

Increasing the percentage of grape residues to 10% of the total diet had no significant effect on goat milk production (P>0.05) (Table 6), that according to the findings of Santos *et al*. (2014), but level above this amount (150 g/kg DM) reduced milk production. The reasons for this reduction can be a decrease in propionate production in the rumen. Milk fat and protein percentage decreased in diets containing grape residues (P<0.05).

In previous experiments (Toral *et al*. 2011; Santos *et al*. 2014; Buccioni *et al*. 2015), milk fat contents were not influenced by the addition of tannins at low or moderate doses, but numerical trends were observed, where higher level of tannins in present study reduced milk fat content. The reduction in milk fat percentage was consistent with milk fat depression theory as reported by Bauman and Griinari, (2001), when a source of plant oil rich in linoleic acid such as grape seed oil reaches the rumen, biohydrogenation of PUFA produces trans 10, 18:1 and results in milk fat depression. Diets containing grape by-product tend to decrease the percentage of milk protein as observed in this study, an effect that has not always been observed (Toral *et al*. 2011; Liu *et al*. 2013; Buccioni *et al*. 2015). In these studies, milk protein composition numerically increased with tannins supplementation in the diet of ewes. Furthermore, Aguerre *et al*. (2016) noted that milk true protein concentration of dairy cows was increased with tannins addition at a rate of 0.45% of DM. This study showed that adding tannins to a higher-level diet could potentially adversely affect milk protein content. An inconsistency is observed among the results reported in the literature on the impacts of tannin consumption on milk yield and composition, such as a decrease in Cabiddu *et al*. (2009), increase in Abbeddou *et al*. (2011) or no effect (Santos *et al*. 2014; Ghaffari *et al*. 2014; Buccioni *et al*. 2015; Sedighi-Vesagh *et al*. 2015), which may be attributed to differences in dose and type of tannins and basal diet composition (Vasta *et al*. 2009). Reports on the impact of plants containing tannins on milk fatty acid (FA) composition are inconsistent. The results obtained in the present study on milk FA composition (Table 7) are similar to those of Ghaffari *et al*. (2014), who reported that the inclusion of 30% pistachio by-products in the diet supplemented with sunflower oil had no effects (P>0.05) on the concentration of major classes of milk fatty acid (FA) according to the degree of saturation (i.e., SFA, MUFA, and PUFA) in Saanen dairy goats.

Table 6 Effect of diets including different levels of grape by-products treatments on milk yield and composition of lactating goats

Item	Treatments				SEM	P-value
	CON	GBP5	GBP10	GBP15		
Milk production (g/day)	3200.4^{a}	3324.4^{a}	3032.5^{ab}	2815.4^{b}	167.19	0.04
4% FCM[2]	3405.9^{a}	3242.4^{ab}	2836.2^{ab}	2615.3^{b}	0.60	0.03
Milk composition (%)						
Fat	4.69^{a}	4.48^{ab}	4.01^{b}	4.27^{ab}	0.36	0.04
Protein	2.87^{a}	2.77^{b}	2.81^{b}	2.78^{b}	0.00	0.04
Lactose	4.34	4.25	4.28	4.20	0.03	0.25
Solids- nonfat	7.87^{a}	7.67^{b}	7.69^{b}	7.62^{b}	0.03	0.03
Milk yield (g/day)						
Fat	143.28^{a}	135.97^{ab}	114.04^{ab}	106.83^{b}	30.01	0.02
Protein	86.95^{a}	83.57^{a}	76.69^{ab}	70.12^{b}	12.75	0.15
Lactose	131.43^{a}	126.19^{a}	118.64^{ab}	106.06^{b}	18.68	0.20
Solids-nonfat	246.7^{a}	229.2^{a}	216.4^{ab}	192.4^{b}	30.49	0.04

CON: control; GBP: grape by-product and FCM: fat corrected milk yield.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Fatty acid composition in the milk of lactating goats fed different levels of grape by-products supplementation

Fatty acid (FA), g/100 g FA	Treatments[1]				SEM	P-value
	CON	GBP5	GBP10	GBP15		
C4:0	1.21^{b}	1.52^{a}	0.98^{c}	1.17^{bc}	0.22	0.01
C6:0	1.85^{ab}	2.24^{a}	1.51^{b}	1.70^{b}	0.44	0.04
C8:0	2.70	3.12	2.36	2.47	0.92	0.22
C10:0	10.78	10.71	9.73	10.02	1.71	0.28
C11:0	0.08	0.08	0.09	0.10	0.12	0.87
C12:0	4.27	4.39	4.80	4.65	1.25	0.56
C13:0	0.09	0.08	0.06	0.11	0.17	0.71
C14:0	11.32	11.15	11.63	11.60	2.88	0.94
C14:1	0.13	0.11	0.12	0.18	0.14	0.41
C15:0	0.11^{b}	0.93^{a}	1.12^{a}	1.16^{a}	0.60	0.04
C16:0	36.07	33.70	33.07	34.41	11.50	0.85
C16:1	0.58^{b}	0.62^{b}	0.62^{b}	0.73^{a}	0.08	0.02
C17:0	0.77	0.71	0.81	0.71	0.11	0.10
C17:1	0.00	0.00	0.00	0.12	0.39	0.61
C18:0 (SA)	7.65	8.86	10.75	8.44	5.78	0.37
C18:1 trans-9	0.53	0.56	0.63	0.41	0.59	0.60
C18:1 cis-9 (OA)	15.55	15.78	17.65	18.11	6.70	0.60
C18:2 cis-6 (LA)	3.73	2.86	3.10	2.77	1.15	0.25
C20:0	0.26^{a}	0.21^{b}	0.26^{ab}	0.23^{ab}	0.05	0.14
C20:1 n-9	0.73	0.69	0.61	0.51	0.29	0.26
C18:3 n-3 (LNA)	0.08	0.07	0.07	0.05	0.05	0.54
C21:0	0.05	0.06	0.07	0.06	0.03	0.33
C22:0	0.07	0.10	0.08	0.08	0.06	0.65
C20:3 n-3	0.10	0.13	0.20	0.21	0.12	0.15
C20:4 n-6	0.03^{c}	0.05^{a}	0.04^{b}	0.04^{b}	0.00	0.00
C23:0	0.17^{ab}	0.17^{ab}	0.13^{b}	0.20^{a}	0.06	0.06
C18:3 n-6	0.00^{b}	0.00^{b}	0.02^{a}	0.03^{a}	0.02	0.02
C18:2 trans-6	0.07^{b}	0.08^{a}	0.00^{c}	0.00^{c}	0.00	0.00
C20:2	0.04^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00	0.00
C20:3 n-6	0.04^{a}	0.02^{b}	0.00^{c}	0.00^{c}	0.00	0.00
C24:0	0.00^{b}	0.06^{a}	0.00^{b}	0.00^{b}	0.00	0.00
C20:5 n-3 (EPA)	0.00^{b}	0.04^{a}	0.00^{b}	0.00^{b}	0.00	0.00
Fatty acid, g/100 g of FA[2]						
SFA (g/100 g of FA)	77.45	78.09	77.10	77.14	5.02	0.92
MUFA (g/100 g of FA)	17.52	17.76	19.62	20.07	5.65	0.49
PUFA (g/100 g of FA)	4.09	3.25	3.43	3.11	1.16	0.25
TFA (g/100 g of FA)	0.60	0.64	0.63	0.41	0.59	0.55
SCFA (g/100 g of FA)	16.62^{ab}	17.67^{a}	14.67^{b}	15.47^{ab}	2.98	0.13
MCFA (g/100 g of FA)	52.57	50.98	51.08	52.85	11.45	0.90
LCFA (g/100 g of FA)	29.87	30.45	34.41	32.00	9.21	0.42

CON: control; GBP: grape by-product; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. TFA: trans fatty acids; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids and LCFA: long-chain fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The variation in the concentration of short-chain FAs in milk fat suggests that the dietary treatments had effect on the synthesis of FAs *de novo* in the mammary gland. Adding GBP to diets decreased concentrations of short-chain, which may be attributed to the lower *de novo* synthesis of short-chain FA in the mammary gland with diets rich in polyunsaturated FA (Palmquist *et al*. 1993; Chilliard and Ferlay, 2004; Walker *et al*. 2004; Sedighi-Vesagh *et al*. 2015). Consistent with some studies where dairy goats were fed hydrolysable tannins (Ghaffari *et al*. 2014), dairy cows (Benchaar and Chouinard, 2009) and ewes were fed condensed tannins (Toral *et al*. 2011), our results may suggest that the low content of polyphenols in the grape by-product used in this trial was probably not high enough to markedly modify ruminal biohydrogenation, which was reflected in insignificant increased LCFA proportions in milk fat. The sum of total trans fatty acids (TFA) was not influenced (P>0.05) by diet, but being higher in both groups (GBP5 and GBP10) than in the CON. The results of Ghaffari *et al*. (2014) and Sedighi-Vesagh *et al*. (2015) studies have also shown an increase in the amount of trans fatty acids. Consistent with our findings, Toral *et al*. (2013) reported that addition of 20 g/kg DM of quebracho tannins extract (QUE) increased the C18:1 trans-9 content in the milk fat of dairy ewes in the diets containing fish oil. Buccioni *et al*. (2015) showed about the microbiologic characterization of rumen liquor that the presence of tannins resulted in an increase in relative abundance of *B. fibrisolvens*, whereas the *B. proteoclasticus* population was strongly depressed, particularly with the QUE diet. These data are in accordance with previous *in vivo* and *in vitro* studies (Vasta *et al*. 2010; Buccioni *et al*. 2011) that reported a significant effect of chestnut tannin extract (CHT) and QUE on rumen BH, favoring the accumulation of VA and negatively affecting the growth of *B. proteoclasticus*. Hence, the effect of tannin extracts on the milk FA profile observed in the present experiment could be due to the modulation of rumen BH because of changes in the microbial ecosystem.

The increase in trans fatty acid (TFA), although with a different pattern among diets, reflected the observed reduction in its precursor linoleic acid (LA) during the trial. The highest concentration of TFA in GBP5 was likely associated mainly with the intake of its precursor (LA) and, to a lesser extent, with the presence of polyphenols in grape by-product. Some authors found that the accumulation of rumenic acid (RA) and vaccenic acid (VA) in the rumen was increased by tannins (Vasta *et al*. 2010; Buccioni *et al*. 2011), which can inhibit the last step of the biohydrogenation (BH) process of VA to stearic acid (SA) (Khiaosa-Ard *et al*. 2009; Vasta *et al*. 2009; Rana *et al*. 2012).

The low content of polyphenols in the grape by-product used in this trial was probably not high enough to markedly influence this BH process, as supported by the high rumen accumulation of SA and by the decrease in LA supplemented with grape by-product. The higher content of SA in milk fatty acids of all supplemented groups compared with CON could be also a consequence of the high extent of oleic acid (OA) biohydrogenation, as evidenced by the low accumulation of OA in the milk fatty acids, despite its high intake.

CONCLUSION

The present study revealed that GBP could be used in the diet of dairy goats without interfering with either milk yield or milk composition. It was also found that replacement of GBP for beet pulp was no effects on apparent digestibility of DM, OM, CP, NDF and ADF. Using the experimental diets in this study, the concentrations of the major classes of FAs (i.e., saturated, monounsaturated, and polyunsaturated, medium, and long-chain FAs) remained in the same range with decrease in short-chain FAs in milk. However, inclusion of GBP in the diet of dairy goats increased the concentration of C18:3 n-6 (gamma-linolenic acid) FAs in milk fat. Though, it is necessary to apply higher levels of GBP, especially on animal responses to affirm the nutritional characteristics reported in this study.

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