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Source / Izvornik: **Agriculture, 2024, 14**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:151:903368

agriculture

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The Effect of Grape Seed Cake as a Dietary Supplement Rich in Polyphenols on the Quantity and Quality of Milk, Metabolic Profile of Blood, and Antioxidative Status of Lactating Dairy Goats

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Abstract: The objective of this study was to assess the impact that diets supplemented with grape seed cake rich in polyphenols had on lactating goats. The study investigated the quantity and quality of goat milk, the metabolic profile of blood, and the antioxidative status. The study involved 24 French Alpine dairy goats throughout their lactation period. The goats were, on average, 5 years old (±three months) and in the fourth lactation. The experiment lasted for 58 days. The control group (CON) had a diet without grape seed cake (GSC). The experimental groups were given a diet containing 5% and 10% GSC on a dry matter basis (GSC5 and GSC10, respectively). A slightly higher milk production, as well as protein and fat milk content, were found in GSC5 and GSC10, but the differences were not significant. Goat milk in the GSC10 group exhibited significantly higher activity of superoxide dismutase and glutathione reductase, as well as decreased concentrations of GUK and SCC. The feeding treatments did not affect significant differences in hematological and biochemical indicators, except for the BHB content, which can be associated with a higher energy value of feed containing GSC. There was an observed elevation in the activity of SOD within the blood of GSC5, and GSC10 was measured as well. The determined changes justify the supplementation of GSC rich in polyphenols to goat feed, especially in the amount of 10%, as it can reduce stress caused by lactation, which is known as a very stressful production period for animals.

Keywords: grape seed cake; goat; milk; blood; antioxidative status; blood metabolic profile

Citation: Antunović, Z.; Novoselec, J.; Klir Šalavardić, Ž.; Steiner, Z.; Drenjančević, M.; Pavić, V.; Đidara, M.; Ronta, M.; Jakobek Barron, L.; Mioč, B. The Effect of Grape Seed Cake as a Dietary Supplement Rich in Polyphenols on the Quantity and Quality of Milk, Metabolic Profile of Blood, and Antioxidative Status of Lactating Dairy Goats. *Agriculture* **2024**, *14*, 479. https://doi.org/10.3390/agriculture14030479

Academic Editors: Tatiana Dumitra Panaite and Mihaela Hăbeanu

Received: 10 February 2024
Revised: 9 March 2024
Accepted: 14 March 2024
Published: 15 March 2024

1. Introduction

Lactating dairy goats play a crucial role in providing milk, a valuable source of nutrition for human consumption. However, the physiological demands of lactation can often lead to stress and metabolic challenges for these animals. Investigating nutritional strategies to improve the production and well-being of lactating goats has drawn more attention in recent years [1]. Polyphenols found abundantly in various plant sources have been recognized for their antioxidant properties and potential health-promoting effects [2]. Grape seed cake, a by-product of the winemaking industry, is rich in polyphenols and has been increasingly considered as a potential feed supplement for livestock [3,4].

Grapevine (*Vitis vinifera* L.) ranks among the most widely cultivated crops globally, primarily renowned for its nutritional benefits, largely attributed to its abundant polyphenol content [5]. Global wine production reached 260 million hectoliters in 2021 [6], causing millions of tonnes of by-products to be produced each year. Mediterranean countries such as Italy, France, and Spain are prominent contributors to this production. In Croatia, wine production amounted to approximately 726,000 hectoliters in 2022. The processing of grapes results in various by-products, one of which is grape seeds. The wine industry's by-products frequently contain beneficial nutrients and chemicals that can be used as additives for livestock feed, thereby reducing waste and promoting resource efficiency [7,8].

The share of seeds in grape pomace amounts to 15–52% of DM [9]. Grape seeds are complex in structure; their chemical composition usually depends on ecological conditions (growing and harvesting of grapes, etc.). According to Bucić-Kojić et al. [10], grape seeds contain around 40% fibre (with a significant share of cellulose), 16% essential oils, 11% protein, 7% polyphenols (flavonols, flavanols, anthocyanins, phenolic acids, and resveratrol) and other compounds (minerals, sugars, and neo-phenolic antioxidant-β-carotene). The most common minerals in grape seeds are iron (Fe) and copper (Cu), but their use in food is limited because of the high levels of lignin, acid detergent fibre (ADF), and neutral detergent fibre (NDF). In addition to the above-mentioned ingredients, grape seeds are rich in vitamin E, which contributes to their significant antioxidant activity [11]. The food, cosmetic, and pharmaceutical industries regularly utilize grape seeds [12]. In recent years, grape seeds have also been processed for oil extraction. After crushing the grape seeds for oil extraction, the resulting by-product, known as grape seed cake (GSC), can be utilized as a nutritional supplement in human nutrition. More recently, GSC as a by-product has been used in animal feeding as well. Although the potentials of GSC supplementation to animal feed are still not fully recognized, farmers are slowly starting to consider GSC as a dietary supplement because of its nutritional value and rich source of natural antioxidants. Lutterodt et al. [13] determined that total phenol content was 100 times lower in the oils than in grape seed flour, thus confirming it to be a valuable source of total phenols. Incorporating large quantities of grape by-products into the diet of monogastric animals may negatively impact growth features since anti-nutritional substances have been present [3,14]. There are few studies referring to the use of grape seed cake in the feeding of lactating ruminants, yet none of them are conducted on lactating goats. Alba et al. [15] investigated the impact of supplementing sheep diet with grape residue flour (GRF) at levels of 0%, 1%, and 2%. They found no effect on the produced quantities and composition of milk despite the fact that the experimental sheep groups produced a greater amount of milk than the control. However, the indicators of antioxidative status were better in the experimental groups. Correddu et al. [16] pointed out that feeding ruminants with agro-industrial by-products, including grape by-products, has many advantages. These include reducing storage and disposal costs, adding value to dairy products by improving their quality, enhancing animal health by enriching animal's diets with polyphenols, and contributing to the preservation of environmental biodiversity.

Lactation is a highly demanding and stressful period for animals, often disrupting homeostasis maintenance and leading to the occurrence of various diseases. In order to prevent such conditions during highly demanding production phases, various antioxidants are often added to animal feed. The purpose of this study is to establish the feasibility and efficacy of using grape seed cake, rich in polyphenols, as a dietary supplement for lactating goats and to assess its effects on the quantity and quality of milk, metabolic profile and antioxidative status of goats. Utilizing industry by-products, such as grape seed cake, in animal nutrition presents a promising avenue for advancing both animal welfare and sustainable agricultural practices. Furthermore, integrating industry by-products into animal nutrition strategies can contribute to the development of circular economies within the agricultural sector [17].

2. Materials and Methods

2.1. Experimental Design and Bioethics Standard

This study involved 24 French Alpine lactating goats at a small-scale family-operated farm in Osijek-Baranja County (Croatia). The selected goats were approximately 5 years of age (±three months), in the fourth lactation. Every goat was in good health and physical condition, had a starting body weight of 49.4 kg, and morning milk production was 1.53 kg. A herd of fifty goats was used to select the goats, and all goats had given birth within one week. The trial started on day 40 after kidding, with an adaptation period of eight days for experimental nutrition. According to the dietary treatment, three groups were formed, with eight goats in each. Goats were kept in a barn, and milking was completed in a separate parlour. The trial lasted for 58 days, during which goats were weighed at the beginning of the experiment (the preparatory period lasted for 7 days) on days 1, 29, and 58. On the same days, goats' body measures were recorded, and milk and blood samples were collected. According to Santucci and Maestrini [18], the body condition score (BCS) was measured on a 1-to-5-point scale, with the intervals between 1 (thin) and 5 (obesity) being 0.25. Every employee handling live goats had the necessary training and education. The Faculty of Agrobiotechnical Sciences Osijek's Committee for Animal Welfare gave the study their approval (644-01/22-01/03 from 30 June 2022). The declaration of Helsinki and the legislative guidelines established by the Animal Protection Act (the Republic of Croatia Official Gazette Nos. 133 (2006), No. 37 (2013) and No. 125 (2013)) were followed throughout.

2.2. Feed and Analysis of Feedstuffs

The goats were fed with a feed mixture offered twice a day (during machine milking), individually (1.5 kg per day) in separate feeding troughs, according to NRC [19]. In addition, goats were fed with alfalfa hay ad libitum. The ratio of the voluminous and concentrated parts of the diet was 60:40. The diet was isonitrogenous and isoenergetic. Kids were removed from the mothers 24 h prior to each milk sample collection day (1st, 29th, and 58th day of trial). The ratio was formulated according to body weight before the experiment started, 49.4 kg, and morning milk production of 1.53 kg. Diets differed in the amount of grape seed cake (GSC) supplemented to the feed mixture. The pomace of Cabernet franc (*Vitis vinifera* L.) grapes was purified after maceration lasting for 5 days. Seeds were separated in a separating machine manufactured by Crystal-Mezőtúr Kft., model S800. After separation, seeds were washed of impurities and dried for 10 h at a temperature of 50 °C. After drying, seeds were pressed in the Komet oil press, model CA59G, at a screw conveyor temperature of 80 °C, screw conveyor diameter of 10 mm, and frequency of 60°/min, with the aim of separating the oil. The residue (grape seed cake) was ground in the Albrigi Luigi hammer mill. A sieve with holes of 1.5 mm diameter was used to grind the cake. The ground grape seed cake was used as a supplement in the feed mixture for goats.

The control diet did not contain GSC. In the first experimental group (GSC5), grape seed cake was added to the feed mixture in the amount of 5%/DM. In the second experimental group (GSC10), grape seed cake was added to the feed mixture in the amount of 10%/DM. Grape seed cake was added to the feed mixture of group GSC5 in the amount of 75 g/day and to the feed mixture of GSC10 in the amount of 150 g/day. Feed mixture, hay, and GSC were dehydrated and pulverized by using an ultra-centrifugal mill (heavy-metal-free). To ascertain feed composition, AOAC [20] standard procedures were applied. The raw material and chemical composition of the feed are presented in Table 1.

Table 1. Ingredients and chemical composition of feed mixture, grape seed cake, and hay.

Ingredient (g/kg Feed Mixture)	Feed Mixture			GSC	Hay
	CON	GSC5	GSC10		
Corn	487	569	553		
Oat	100	100	100		
Wheat flour	100	-	-		
Soybean meal (46% CP)	179	197	199		
Soybean hulls	100	50	-		
Grape seed cake	-	50	100		
Fat	-	-	14		
Salt	4	4	4		
Mineral vitamin premix [1]	30	30	30		
Chemical content (g/kg DM)					
DM	889	888	889	909	900
Crude protein	165	161	159	119	143
Crude fibre	54	51	53	422	293
Crude ash	54	54	51	32	61
EE	28	29	38	46	10
NDF	386	273	367	614	686
ADF	85	79	88	501	395
ADL	21	8.6	37	422	68
ME (MJ/kg DM)	11.36	11.20	11.21	3.50	7.21
Polyphenols (total), mg/kg	56.06	418.22	812.06	5065.36	-
Anthocyanins (total), mg/kg	144.23	155.80	240.46	105.53	-

CON control group; GSC5: grape seed cake 5%, GSC10: grape seed cake 10%; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; ME: metabolic energy; [1] The mineral vitamin premix1 is composed of 21% calcium, 5% phosphorus, 6% sodium, 5% magnesium, 1,200,000 IU/kg vitamin A, 140,000 IU/kg vitamin D3, 3500 mg/kg vitamin E, 600 mg/kg iron sulphate monohydrate, 490 mg/kg copper sulphate pentahydrate, 500 mg/kg copper (in the form of chelates), 5 mg/kg manganese sulphate pentahydrate, 6500 mg/kg zinc oxide, 1500 mg/kg zinc (in the form of chelates), 60 mg/kg iodine in the form of anhydrous calcium iodate, 40 mg/kg cobalt carbonate monohydrate, and 50 mg/kg selenium selenite.

All feed samples (grape seed cake, alfalfa hay, and feed mixture) were dried before being processed into a powder in a knife mill (GM 200, Retsch GmbH, Haan, Germany) or heavy metal-free ultra-centrifugal mill (ZM 200, Retsch GmbH, Haan, Germany). The Association of Official Analytical Chemists'-established procedures were used to determine the feed composition [20]. Table 1 displays the ingredients and chemical compositions of the diets. The Kjeldahl method was used to assess the crude protein content of the feed using a Kjeldahl steam distillation apparatus (Behr, Stuttgart, Germany). The universal extraction system B-811 (Buchi, Flawil, Switzerland) was used to estimate the ether extract. The Weende technique was used to calculate the crude fibre content, and INRAE-CIRAD-AFZ was used to determine the ME [21]. Using the instruments and methods outlined by Jakobek et al. [22], the total polyphenols in the feed were extracted and determined. The same procedure was repeated to obtain three extracts of feed. The total polyphenol concentration was presented as mg of gallic acid equivalents (GAE) per kg of sample weight. Total anthocyanins and polyphenols analyses were carried out by using a Shimadzu UV-1280 spectrophotometer (Shimadzu Europe GmbH) following the method by Jakobek et al. [22].

2.3. Milk Sampling and Analysis

During routine milking on experiment days 1, 29, and 58, three samples of milk were obtained from each goat at 7:00 a.m. None of the goats had mastitis throughout the experiment, as observed by forestripping before each milk sampling. One of these two milk samples obtained from each goat was transferred into bottles (30 mL) and cooled to 4 °C. The milkoScan FT 6000 analyzer (Foss Electric, Hillerød, Denmark) was used to obtain the

chemical composition of milk following HRN ISO 9622:2017. The following equation was used to calculate fat-corrected milk at 3.5% (FCM, kg/day; [23]):

$$\text{FCM} = \text{milk yield} \times (0.634 + 0.1046 \times \text{fat}) \quad (1)$$

A Fossomatic 5000 Analyser (Foss Electric, Hillerød, Denmark) was used to determine somatic cell count (SCC) with fluoro-opto-electronic method (HRN ISO 13366-2/Ispr.1:2007). According to Wiggans and Shook [24], the results were transformed to log values via the following equation:

$$\text{SCC} = 3 + \log2(\text{SCC}/100{,}000) \quad (2)$$

In order to determine biochemical indicators, fresh milk was centrifuged at 5000× *g* for 30 min to separate the fat, and until analysis, the milk plasma was stored at −80 °C. Biochemical markers, such as ALT (alanine aminotransferase), GGT (γ-glutamyl transferase) and AST (aspartate aminotransferase), GR (glutathione reductase), urea, albumin, glucose, and TP (total protein)) were determined using a biochemical analyser Olympus AU 400 (Olympus, Tokyo, Japan). Glutathione peroxidase (GPx) activity in milk was determined by Ransel® kit (Randox, Crumlin, UK) and SOD activity by Ransod® kit (Randox Laboratories, Crumlin, UK).

Milk antioxidant compounds were extracted in three replicates using a slightly modified method, according to Alyaqouba et al. [25]. Briefly, 10 parts of extraction solvent (1 N HCl/95% ethanol (*v*/*v*, 15/85)) were used for 1 part of milk and shaken for 1 h using a rotary shaker (Brunswick™ Innova® 43R Console Incubator Shaker, Eppendorf AG, Hamburg, Germany) at 30 °C and 300 rpm, while protected from light. The supernatants separated after centrifugation at 4400× *g* and 5 °C for 40 min (Hermle Z 326 K, Hermle Labortechnik GmbH, Wehingen, Germany) and were then stored at −20 °C until further analysis. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the milk extracts was assessed in three replicates using a modified method [26]. Briefly, 9 parts of methanolic solution of DPPH radical at 0.2 mM were mixed with 1 part of milk extract and left at room temperature for 30 min in the dark after being shaken vigorously. In brief, 9 parts of a 0.2 mM methanol DPPH radical solution and 1 part of milk extracts were combined and, after being vigorously shaken, were allowed to sit at room temperature for 30 min in the dark. Utilizing a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA), absorbance was measured at 517 nm. Equation (3) was used in order to compute the DPPH scavenging activity:

$$\text{DPPH scavenginming activity (\%)} = ((\text{Ab} + \text{As}) - \text{Am})/\text{Ab} \times 100)) \quad (3)$$

where Ab is the negative control absorbance, As is the milk sample control absorbance, and Am is the tested milk extracts and DPPH radical absorbance.

The method used for Thiobarbituric Acid Reactive Substances measurement was adapted from Oancea et al. [27] and Sun et al. [28]. To remove proteins, a mixture of 1 part of 0.1% trichloroacetic acid (TCA) and milk samples (3 parts) was prepared. The supernatants were collected and incubated with TBA/TCA reagent (0.5% thiobarbituric acid—TBA in 20% TCA) in a 2:1 ratio for 90 min at 80 °C, after centrifugation at 3000× *g* for 5 min at 4 °C (Hermle Z 326 K, Hermle Labortechnik GmbH, Wehingen, Germany). The incubation was followed by cooling on ice. Absorbance readings were taken at different wavelengths: 450 nm for saturated aldehydes, 532 nm for malondialdehyde (MDA), and 600 nm for non-specific absorption. Results were then calculated by subtracting non-specific absorbance at 600 nm and expressed as absorbance values at 450 nm ($TBARS_{450}$).

2.4. Blood Sampling and Analysis

Samples of blood (10 mL) were drawn from each goat's jugular vein for hematological analysis, and they were placed into sterile Venoject® (Sterile Terumo Europe, Leuven, Belgium) vacuum tubes that contained EDTA (ethylenediamine tetra-acetic acid). After

collection, blood samples were set on ice and stored in chilled conditions (0–6 °C). To ascertain the hematological parameters in goat whole blood (WBC (number of leukocytes), RBC (erythrocytes), HGB (the content of hemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (average hemoglobin content in erythrocytes), and MCHC (mean hemoglobin concentration in erythrocytes)), the three-part differential veterinary hematology analyzer (Sysmex PocH-100iV, Sysmex Europe GmbH, Norderstedt, Germany) was utilized.

Following that, blood samples that had been obtained in sterile vacuum tubes devoid of EDTA were centrifuged using a centrifuge ROTOFIX 32A (Hettich GmbH & Co. KG, Tuttlingen, Germany) at 1609.92× *g* for ten minutes. The obtained serum samples were placed into the Olympus AU400. ALB (albumin), CHOL (cholesterol), BHB (β-hydroxybutyrate), TGC (triglycerides), GUK (glucose), PROT (total proteins), ALB (low-density lipoprotein), HDL (high-density lipoprotein), NEFA (non-esterified fatty acids), and minerals (calcium, magnesium, phosphorus, and iron) were among the biochemical parameters determined in the serum. Enzyme activities were measured for GGT (γ-glutamyl transferase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and GR (glutathione reductase) by using Olympus System reagents (Olympus Diagnostic GmbH, Ballymount, Ireland). The difference between total protein and albumin was expressed as globulin (GLOB) content. The GPx in the serum was determined using a Ransel® kit (Randox, Crumlin, UK), and SOD in serum was determined using a Ransod® kit (Randox, Crumlin, UK) on an Olympus AU 400 analyzer (Olympus, Tokyo, Japan).

2.5. Statistical Analyses

Mean values for milk performance, as well as hematological and antioxidative status, were estimated for each goat and for each time of sampling. These values were then subjected to a repeated-measure analysis using PROC MIXED (SAS 9.4; [29]), with the following model: $Y_{ijk} = \mu + d_i + h_{ij} + w_k + dw_{ik} + e_{ijk}$, where μ means overall mean, d_i means fixed effect of diet (i = C, GSC5, GSC10), h_{ij} means animal within diet as subject (j = C, GSC5, GSC10), w_k means fixed effect of sampling time in lactation (k = 1–3), d_{wik} means interaction between diet and sampling time (diet × sampling), and e_{ijk} means residual error. Diet × sampling time interactions were considered fixed effects. Mean values were compared with the Tukey significant difference test, where $p < 0.05$ indicated significant differences.

3. Results

Data on recorded body weight, body condition score, milk production, and composition of lactating dairy goats fed a diet enriched with varying amounts of grape seed cake are shown in Table 2.

Analysis of data presented in Table 2 confirmed a slightly higher amount of milk, as well as protein and fat content, in milk produced by goats that were fed diets supplemented with GSC; however, those differences were not significant. When comparing the milk of GSC10 goats to the control (CON) and GSC5 groups, the SCC was significantly reduced. Furthermore, when compared to the CON group, GSC5 and GSC10 milk had a significantly lower concentration of GUK. A significant influence on the periods of milk sampling was also determined for the concentration of ALB, and there was also a significant interaction of D × P for concentrations of TP, ALB, and ALT and AST activities in goat milk.

Table 3 provides an overview of the hematological parameters of dairy goats fed diets with different amounts of GSC.

The content of hematological indicators was not affected by the supplementation of GSC to goats' diets.

Analysis of the data presented in Table 4 confirmed a significant decrease only in the concentrations of BHB in experimental GSC10 groups as well as a non-significant increase in the concentrations of ALB, TGC, and CHOL, HDL, and LDL cholesterol, and a decrease

in the concentration of Fe in blood in GSC groups. A significant influence of the lactation stage was determined for concentrations of Ca, TP, TGC, and NEFA in goats' blood.

Table 2. Milk yield and composition and production traits of goats fed diets supplemented with GSC.

	Diets			SEM	*p* Value		
	CON	GSC5	GSC10		D	S	D × S
Milk yield (kg)	1.34	1.63	1.59	0.075	0.214	0.094	0.843
Body weight (kg)	50.02	49.57	47.74	0.881	0.588	0.982	0.986
BCS (point)	2.61	2.53	2.45	0.053	0.518	0.094	0.534
Milk composition (g/100 g)							
Fat	3.00	3.14	3.27	0.105	0.572	0.420	0.901
Protein	2.77	2.98	3.02	0.052	0.157	0.796	0.946
Lactose	4.29	4.40	4.39	0.023	0.088	0.100	0.174
Non-fat dry matter	8.25	8.31	8.49	0.050	0.146	0.470	0.763
AST (U/L)	18.70	21.56	26.22	1.674	0.083	0.095	0.024
ALT (U/L)	2.87	4.14	7.40	4.109	0.051	0.076	<0.001
GGT (U/L)	286.86	334.85	329.55	14.134	0.223	0.621	0.066
ALB (g/L)	12.62	11.65	11.40	0.645	0.732	0.027	0.021
TP (g/L)	18.64	17.93	18.30	0.741	0.947	0.054	0.015
GUK (mmol/L)	0.28 [a]	0.19 [b]	0.16 [b]	0.014	0.001	0.460	0.377
Urea (mmol/L)	8.95	8.65	8.45	0.274	0.834	0.174	0.010
SCC (log)	5.95 [a]	5.85 [a]	5.15 [b]	0.077	<0.001	0.052	0.411

[a,b] Means in rows with different letters differ significantly ($p < 0.05$). CON: control group; GSC5: grape seed cake 5%, GSC10: grape seed cake 10%; D: diet, S: time of sampling, D × S: interaction (diet × sampling); SEM: standard error of mean; BCS: body condition score; TP: total proteins; ALB: albumins; GUK: glucose; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: γ-glutamyl transferase; SCC: somatic cells count.

Table 3. Hematological parameters of goats fed diets with different amounts of GSC.

Parameter	Diets			SEM	*p* Value			*Reference Values* *
	CON	GSC5	GSC10		D	S	D × S	
RBC ($\times 10^{12}$ L)	8.47	9.47	9.20	0.283	0.305	0.831	0.672	8.00–18.00
WBC ($\times 10^{9}$ L)	10.06	9.61	10.48	0.422	0.733	0.563	0.803	4.00–13.00
HGB (g/L)	67.09	78.76	71.95	2.078	0.073	0.892	0.723	80.00–120.00
HCT (L/L)	0.24	0.27	0.26	0.006	0.222	0.869	0.624	0.22–0.38
MCH (pg)	8.27	8.37	7.85	0.135	0.230	0.052	0.796	5.20–8.00
MCV (fL)	30.10	28.71	28.57	0.486	0.262	0.059	0.328	16.00–25.00
MCHC (g/L)	276.39	293.10	275.29	3.443	0.068	0.357	0.835	300.00–360.00

CON: control group; GSC5: grape seed cake 5%; GSC10: grape seed cake 10%; D: diet; S: time of sampling; D × S: interaction (diet × sampling); SEM: standard error of mean; RBC: erythrocytes; WBC: number of leukocytes; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: average hemoglobin content in erythrocytes; MCHC: mean hemoglobin concentration in erythrocytes; * [30].

The activities of blood enzymes did not vary depending on feeding treatment, except for SOD activity, which was significantly increased in GSC5 and GSC10 (Table 5). The milk sampling period had a significant influence on GPx activity in goats' blood.

The $TBARS_{450}$ content and DPPH scavenging activity did not differ significantly when influenced by the feeding treatment, although the $TBARS_{450}$ content was decreased in the milk of goats fed diets supplemented with GSC (Table 6). Milk from GSC10 goats had a significantly higher activity of SOD. Furthermore, when compared to goat milk from C and GSC5, milk from GSC10 had a significantly higher activity of GR. The milk sampling period had a significant influence on DPPH scavenging as well as the activities of SOD and GR.

Table 4. Blood biochemical parameters in goats fed diets with different amounts of GSC.

Parameter (mmol/L)	Diets			SEM	*p* Value			*Reference Values* *
	CON	GSC5	GSC10		D	S	D × S	
Urea	9.28	9.35	9.57	0.215	0.870	0.575	0.714	4.00–8.60
TP (g/L)	70.44	74.97	73.58	1.332	0.820	<0.001	0.232	62.00–79.00
ALB (g/L)	25.20	28.50	26.52	0.563	0.060	0.783	0.838	29.00–43.00
GLOB (g/L)	45.23	46.47	47.06	0.906	0.701	0.362	0.740	35.00–57.00 [1]
Ca	2.21	2.18	2.10	0.028	0.221	0.017	0.503	2.30–2.90
P-inorganic	3.29	3.26	3.39	0.098	0.286	0.056	0.550	1.00–2.40
Mg	1.44	1.50	1.41	0.023	0.286	0.056	0.550	0.80–1.30
Fe (µmol/L)	23.60	22.64	16.96	1.233	0.078	0.850	0.609	11.60–38.10 [1]
GUK	4.26	4.23	4.37	0.060	0.619	0.072	0.699	2.40–4.00
CHOL	2.64	3.02	2.84	0.078	0.159	0.938	0.991	1.00–3.00
HDL	1.44	1.70	1.49	0.039	0.616	0.314	0.754	1.05–1.76
LDL	1.11	1.23	1.33	0.070	0.420	0.916	0.906	0.77–1.25
TGC	0.17	0.20	0.21	0.012	0.440	0.022	0.537	0.20
NEFA	0.16	0.19	0.19	0.012	0.410	<0.001	0.392	>0.2 [2]
BHB	0.54 [a]	0.50 [a]	0.41 [b]	0.016	0.003	0.150	0.750	0–1.20

CON: control group; GSC5: grape seed cake 5%; GSC10: grape seed cake 10%; D: diet; S: time of sampling; D × S: interaction (diet × sampling); SEM: standard error of mean; TP: total proteins, ALB: albumins; GLOB: globulins; GUK: glucose; CHOL: cholesterol; HDL: HDL cholesterol; LDL: LDL cholesterol; TGC: triglycerides; NEFA: non-esterified fatty acids; BHB: β-hydroxybutyrate; [a,b] means in rows with different letters differ significantly ($p < 0.05$).* [31]; [1] [32]; [2] [33].

Table 5. Blood enzyme activities of goats fed diets with different amounts of GSC.

Parameter (U/L)	Diets			SEM	*p* Value		
	CON	GSC5	GSC10		D	S	D × S
AST	125.51	121.35	119.76	3.296	0.772	0.725	0.570
ALT	27.34	26.10	26.82	0.895	0.857	0.425	0.974
GGT	46.52	50.17	48.35	1.165	0.466	0.965	0.972
CK	172.87	154.05	166.90	4.719	0.274	0.621	0.953
GPx	768.25	829.09	849.90	23.662	0.330	0.006	0.961
SOD (U/mL)	0.26 [b]	0.48 [a]	0.51 [a]	0.021	0.001	0.126	0.851
GR	90.48	83.90	82.16	2.512	0.336	0.500	0.411

CON: control group; GSC5: grape seed cake 5%; GSC10: grape seed cake 10%; D: diet; S: time of sampling; D × S: interaction (diet×sampling); SEM: standard error of mean; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: γ-glutamyl transferase; CK: creatine kinase; GPx: glutathione peroxidase; SOD: superoxide dismutase; GR: glutathione reductase; [a,b] means in rows with different letters differ significantly ($p < 0.05$).

Table 6. Antioxidant status of milk of goats given diets with various amounts of GSC.

Parameter	Diets			SEM	*p* Value		
	CON	GSC5	GSC10		D	S	D × S
$TBARS_{450}$	0.039	0.036	0.035	0.0017	0.724	0.604	0.233
DPPH scavenging (%)	92.87	92.19	92.14	0.247	0.240	<0.001	0.425
SOD (U/mL)	3.41 [a]	4.31 [ab]	5.30 [b]	0.250	0.002	0.002	0.765
GPx (U/L)	426.64	441.17	472.75	36.043	0.866	0.302	0.200
GR (U/L)	3.37 [a]	3.25 [a]	6.57 [b]	0.426	<0.001	0.047	0.072

CON: control group; GSC5: grape seed cake 5%, GSC10: grape seed cake 10%; D: diet, S: time of sampling, D × S: interaction (diet × sampling); SEM: standard error of the mean; $TBARS_{450}$—Thiobarbituric acid reactive substances; DPPH—radical scavenging activity at final milk concentration 10 uL/mL; SOD—superoxide dismutase; GPx—glutathione peroxidase; GR—glutathione reductase; [a,b] means in rows with different letters differ significantly ($p < 0.05$).

4. Discussion

There is a growing interest of scientists exploring the potential of using grape waste products in the feeding of small ruminants. Flavonoids and proanthocyanidins, as bioactive compounds obtained from wine industry by-products, are used to enhance the immune response. They modulate the immune system of ruminants via binding to proteins, as well as to boost interference at the active site, thus exerting antioxidant effects [34]. Feeding goats with polyphenol-rich diets leads to the presence of proline-rich proteins (PRPs) in their saliva [35], as well as in a better capacity of saliva to bind tannins [36]. In the present research, supplementation of GSC to goats' diets resulted in a slight, nonsignificant increase in the quantity of milk, fat, and protein milk content but a significant decrease in SCC in the milk of the GSC10 group (Table 2). In a study on sheep fed a diet supplemented with 5% grape seeds during mid-pregnancy and lactation, Pascual-Alonso et al. [37] found similar content of milk fat, protein, and somatic cell count. Smaller changes in the lactose content in sheep milk were determined in all feeding treatments. A study by Gessner et al. [38] with dairy cows administered pomace meal extract and grape seeds describes the effect of grape phenols on enhancing the flow of protein into the small intestine, which results in an increase in milk production. Similarly, high doses of winery by-products (grape seeds and pomace) could reduce the breakdown of dietary protein [39]. When compared to CON, significantly higher activity of SOD and reduced SCC were found in the milk from GSC10 goats in the present study. Likewise, when compared to CON and GSC5, GSC10 had a significantly lower concentration of GUK in the milk. Alba et al. [15] supplemented grape residue flour (GRF) in the amount of 1% and 2% (10 and 20 g/kg) to sheep diets and determined a non-significant increase in milk quantity without changes in its composition (except for reduced BSC and increased fat content). The SCC was significantly lowered in the milk from sheep fed diet with 2% grape residue flour (by 18.01%) and nonsignificant lowered in groups fed with 1% (by 10.20%), which was linked to the anti-inflammatory capability caused by the ingredients present in the dietary supplement. The aforementioned changes align with the results obtained in the present study, where a reduction in SCC in milk by 13.5% was determined in the GSC10 group. The reduction in milk GUK in the GSC groups in the present study may be related to slightly higher milk production and higher lactose content in the milk. The reason for the high turnover of glucose in lactating ruminants is that the demand for the udder is high, and glucose is almost the only precursor of lactose [40]. Mokni et al. [41] investigated grape seed and skin supplemented to lactating sheep feed in the amount of 20% and proved increased milk production, but without significant differences in the composition of milk, except for the increased content of urea, Fe and Ca. Resconi et al. [42] did not confirm the influence of grape seeds (5%) in feeding lactating sheep on the quality of milk and meat of sheep and their lambs. Similarly, Manso et al. [43] and Nudda et al. [44] also did not confirm changes in the quantity of milk produced by sheep fed diet with grape pomace. The absence of a significant increase in the amount of milk in the goats of the experimental groups that consumed feed mixtures with added GSC can be connected with similar ME content in feed mixtures (Table 1). Mokni et al. [41] obtained similar results with sheep but with significantly higher milk production influenced by feeding sheep diets supplemented with grape seed and skin (20%). In the present research, a non-significant increase in GPx activity was observed in the milk and blood of goats fed diets supplemented with GSC. Alba et al. [15] also reported increased activity of GPx in sheep blood, which was more pronounced in sheep fed a diet supplemented with 2% GRF, and the authors associated this with the presence of polyphenols in grape residue.

In this research, the determined blood metabolic profile (concentrations of hematological and biochemical indicators) was within reference values [31–33,45,46], with a slight influence on the milk sampling period, which was expected (Tables 3 and 4). We determined a lower content of HGB, MCHC, ALB, and Ca as well as higher value of MCV, urea, and P. The content of hematological indicators was not influenced by GSC supplemented in goats' diets. When feeding sheep diet supplemented with grape pomace, Nudda et al. [47]

obtained similar results for hematochemical indicators in blood. The present research resulted in a non-significant increase in TGC and CHOL concentrations and a significant decrease in BHB in goats' blood, which points out the fact that feeding mixtures of the experimental groups were enriched with GSC ether extract (Table 1). Specifically, it is known that grape seeds have higher polysaccharide content, which can lower serum BHB concentrations and also inhibit the mobilization of adipose tissue and liver glycogen in the GSC10 group [48]. The amount of NEFA in the serum indicates how fat is metabolized, and the goats from the GSC groups' consistent concentration might be connected to the absence of alterations in CHOL. Similar results for blood TGC in investigation with dried pomace feeding of wethers were confirmed by Juráček et al. [49]. In a study on sheep fed a diet supplemented with 5% grape seeds during mid-pregnancy and lactation, Pascual-Alonso et al. [37] found higher concentrations of NEFA and CK activity in the blood. Alba et al. [15] published similar conclusions referring to concentrations of TGC in the blood of sheep fed diets supplemented with grape residue flour. It is very well known that both flavonoids and proanthocyanidins are free radical scavengers; they promote vasodilation by inhibiting phospholipase, cyclooxygenase, and lipoxygenase. They also reduce the peroxidation process of lipids [50]. This research did not confirm the variation in enzyme activities in goats' blood that depended on the feeding treatment, except for SOD activity, which was significantly increased in GSC5 and GSC10 (Table 5). SOD is an enzyme that is essential to the body's defence against oxidative stress because it removes superoxide radicals from inside cells [51]. When feeding sheep diets supplemented with grape residue flour (10 and 20 g/kg), Alba et al. [15] also found that there was an increase in GPx, a decrease in lipid peroxidation, an increase in total antioxidant status (TAS), and enhanced SOD activity in the serum. In this study, the absence of notable alterations in the activity of other antioxidant enzymes in the blood of goats could be linked to the restricted absorption of anthocyanins in small ruminants relative to monogastric animals [52]. Previous studies have shown a high correlation between the oxidation of dairy products and the yellow colour of TBARS at 450 nm, which have been associated with the oxidation of monounsaturated fatty acids [27,53,54]. The objectives of the TBARS analysis were to assess potential lipid oxidation in milk. Milk lipids are susceptible to chemical and physical changes, including autooxidation, oxidation, and the development of trans fatty acids, aldehydes, ketones, and lactones. These changes can have adverse effects on the properties of dairy products, such as undesirable odour, flavour, and colour. By conducting TBARS analysis, it becomes possible to quantify the extent of lipid oxidation, thereby evaluating the quality and stability of dairy products. The TBARS are indeed primary by-products of lipid peroxidation. Previous studies demonstrated that antioxidants, such as polyphenols found in grape extracts or grape pomaces, seeds, skins, and stems, have the capability to regulate and reduce the levels of TBARS [55]. Polyphenols gain their antioxidant properties via scavenging free radicals and preventing lipid peroxidation reactions, thus mitigating the formation of TBARS. Consequently, the incorporation of grape-derived antioxidants into food products may help preserve their quality by minimizing lipid oxidation and subsequent TBARS formation. While polyphenols have low plasma and tissue levels and limited bioavailability in healthy animals, they still exhibit a favourable effect on antioxidative capacity, particularly in stressed animals [56]. The content of $TBARS_{450}$ and DPPH scavenging activity determined in this research did not differ significantly depending on the feeding treatment, although the content of $TBARS_{450}$ was decreased in milk of goats fed with GSC supplementation (from 0.039 to 0.035; Table 6).

When compared to CON, significantly higher activity of SOD was determined in GSC10 goat milk. In contrast to CON and GSC5, GSC10 had significantly higher activity of GR in milk. A significantly greater GR activity in the milk of goats fed with 10% GSC in food indicates the stimulation of oxidation-reduction potential. Similar results were found in the research with dried apple pomace by Bartel et al. [57]. GR is the primary enzyme involved in the metabolism of glutathione and plays a crucial role in the glutathione redox cycle, which sustains appropriate amounts of reduced cellular GSH [58]. For example,

adding grape extracts from different cultivars to goat feed confirmed increased GR activity in the liver tissue of goats [59].

According to Santos et al. (2014) [60], dairy cows fed silage with the greatest addition of grape residue had milk with a positive antioxidant influence. Significant rises in SOD activity in the serum and GPx in milk were confirmed by Alba et al. [15]. In an experiment with pigs fed a diet with an additional 5% GSC throughout the 24-day finishing period, Taranu et al. [14] found no influence on blood biochemistry or pig characteristics, but they did find a modulatory effect on the antioxidative status (substantially reduced TBARS). Paraskevakis [61] identified the beneficial effects of polyphenols on the oxidative status of goats. This is attributed to the direct antioxidative effect of polyphenols, stemming from their absorption in the gastrointestinal tract and subsequent tissue deposition [62].

5. Conclusions

Considering the preservation of goat milk production and quality, as well as the metabolic profile of goats' blood, a significant increase in antioxidant enzymes in milk (SOD and GR) and in blood (SOD) confirms that using grape seed cake rich in polyphenols as a supplement in goats' feed is justified because it reduces oxidative stress caused by lactation, which is very stressful for animals. Supplementation of 10% GSC rich in polyphenols to the diet fed to lactating goats can be recommended because GSC can prevent oxidative stress and reinforce the antioxidative response of animals. By bolstering the antioxidative response and mitigating oxidative stress during this demanding production period, GSC supplementation may contribute to the overall well-being of lactating goats. Further research should explore the effects of various groups of polyphenols in the diet of lactating goats.

Author Contributions: Investigation, writing—original draft preparation conceptualization, visualization, Z.A.; writing—review and editing, V.P.; investigation, formal analysis, methodology, J.N.; investigation, statistical analyses, visualization, Ž.K.Š.; formal analysis and methodology, visualization, M.Đ., V.P., M.D., Z.S. and L.J.B.; field investigation, M.R.; investigation, visualization, B.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek (644-01/22-01/03 from 30 June 2022). It was carried out in accordance with the Declaration of Helsinki and followed the legal provisions determined by the Animal Protection Act (Republic of Croatia Official Gazette No. 133 (2006), No. 37 (2013) and No. 125 (2013)).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The research was carried out with the Innovative Breeding and Technological Processes in Animal Production (no. 1126) research team at the Faculty of Agrobiotechnical Sciences Osijek.

Conflicts of Interest: The authors declare no conflicts of interest.

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