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Production Traits, Blood Metabolic Profile, and Antioxidative Status of Dairy Goats Fed a Red Corn Supplemented Feed Mixture

Zvonko Antunović [1], Željka Klir Šalavardić [1], Josip Novoselec [1,*], Zvonimir Steiner [1], Mislav Đidara [1], Valentina Pavić [2], Lidija Jakobek Barron [3], Mario Ronta [1] and Boro Mioč [4]

[1] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Trg Sv. Trojstva 3, 31000 Osijek, Croatia; zantunovic@fazos.hr (Z.A.); zklir@fazos.hr (Ž.K.Š.); zsteiner@fazos.hr (Z.S.); mdidara@fazos.hr (M.Đ.); mronta@fazos.hr (M.R.)

[2] Department of Biology, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8, 31000 Osijek, Croatia; vpavic@biologija.unios.hr

[3] Faculty of Food and Technology Osijek, Josip Juraj Strossmayer University of Osijek, Trg Sv. Trojstva 3, 31000 Osijek, Croatia; lidija.jakobek@ptfos.hr

[4] Department of Animal Science and Technology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia; bmioc@agr.hr

* Correspondence: jnovoselec@fazos.hr; Tel.: +385-31-554906

Abstract: This study investigated the effect of red corn in the feed mixture of dairy goats on production traits, blood metabolic profile, and antioxidative status. The study was conducted on 30 French Alpine dairy goats. The feed mixture for the goats in the control group (CC) contained yellow corn (100%). In the first experimental group (RC50), yellow corn was partially (50%) replaced by red corn (RC), and in the second experimental group (RC100), yellow corn was completely (100%) replaced by red corn. No significance variations ($p > 0.05$) were determined in production traits of dairy goats between dietary treatments. A significant increase in hemoglobin (84.43, 100.00 and 106.55 g/L), mean corpuscular hemoglobin (7.98, 9.70 and 12.54 pg), and mean corpuscular hemoglobin concentration (293.57, 357.50 and 462.78 g/L) was found in the RC groups, and a decrease in erythrocytes in the RC100 compared with the RC50 group of goats (from 8.71 to 10.45 $\times$ 10^{12} L). A significant increase in blood superoxide dismutase (SOD) activity in the RC groups was found (0.29, 0.53, and 0.44 U/mL). The results indicate maintaining production traits and a moderate effect on blood metabolic profile (most hematologic parameters) as well as a positive antioxidative effect RC.

Keywords: lactating goats; corn with increased anthocyanins; milk performance; blood metabolic profile

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1. Introduction

Domestic animals are pressured to increase their production, i.e., financial profit of their farmers. This is why an increase in cellular respiration and production of free radicals and oxidative stress often occurs, afterward [1]. This is the primary cause of various metabolic disorders in animals, especially in the transition period, which leads to impairment of welfare, production properties, and quality of obtained products [2]. However, various antioxidants can be used for prevention [3,4]. The prohibition on the use of antibiotics and the interest for the use of various phyto-additives as food supplements such as flavonoids, including anthocyanins, is growing [5]. Flavonoids can promote the growth and development of animals and improve the health and quality of animal products [3]. Feeding anthocyanin- and antioxidant-rich forages to sheep and dairy cows can improve performance and product quality [6]. Anthocyanins are water-soluble glycosides of polyhydroxy and polymethoxy derivatives of 2-phenyl-benzopyrylium or flavylium salts responsible for the colors like red, purple, and blue presented in many fruits, vegetables, and cereal kernels as well as included in a class of flavonoids [7]. Further efforts

by scientists are being made toward the use of anthocyanins in natural sources, through the consumption of certain feeds rich in their content. Corn is the most commonly concentrated fodder used in animal feeding. However, the different varieties of corn contain significantly different concentrations of anthocyanidins, as blue, red, and purple corn have higher concentration of anthocyanidins (up to 325 mg/100 g dry matter of corn), while yellow corn is rich in carotenoids (up to 823 µg/100 g dry matter of corn) [8]. Anthocyanins, besides giving various striking colors, also have a positive health influence [9]. However, the regulatory mechanisms in the metabolic pathway for anthocyanins are not yet fully elucidated [10]. It is known that anthocyanins as one of the flavonoids are absorbed in the gut poorly and thus concentrations in tissues are too low to obtain an effective antioxidant defense [4]. Changxing et al. [11] reported that anthocyanins have excellent antioxidant, anti-inflammatory, antimicrobial, and anticancer effects as well are proven to be safe and potent feed additives. In the available literature, we found only one paper that investigated the application of red corn in weaned lamb feeding [12], while there is no research on goats. Therefore, for the discussion, we used research conducted with purple corn in ruminant nutrition while monitoring the content of anthocyanins and polyphenols in them. Tian et al. [13] propose the use of purple corn in ruminant nutrition due to improved antioxidant resistance, better transfer of anthocyanins to goat's milk, and the lack of impact on milk composition. The present study investigates the effect of different levels of red corn in the feed mixture of dairy goats on productive traits (especially milk yield and composition), blood metabolic profile, and antioxidant status.

2. Materials and Methods

2.1. Experimental Design

The study was conducted in accordance with the Declaration of Helsinki, by obeying legal provisions determined by the Animal Protection Act (Republic of Croatia Official Gazette No. 133 (2006), No. 37 (2013), and No. 125 (2013)), and approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek (644-01/23-01/23 from 14 March 2023).

The study was conducted with 30 French Alpine dairy goats during lactation on a family farm in Osijek-Baranja County in Croatia. The goats were approximately 4 years old and in their third lactation. All the goats included in the study were healthy and in good physical condition. The research goats were selected from a flock of 60 animals. The goats were divided into three groups of 10 animals each, depending on their nutrition. In each experimental group, there was the same number of goats with singles and twins. The goats were housed in the barn throughout the study, and milking took place in the milking parlor in a separate room. All selected goats had kidded within seven days. The experiment started 60 days after kidding. Eight days before the experiment started goats had an adaptation period and were fed with experimental diet. In total, the experiment lasted 30 days. The index of body condition score (BCS) was determined using a scale of 1 to 5 points according to Santucci and Maestrini [14]. This scale uses points from 1 (thin) to 5 (obese), with intervals of 0.25. All personnel dealing with live goats have been trained and educated.

2.2. Feedstuff Analysis and Nutrition

The goats were given individual feed mixtures (1.2 kg per day) according to their requirements according to NRC [15]. Feeding with prepared feed mixtures was conducted in separate feeding troughs in the milking parlor during twice-daily milking by a machine. Furthermore, the goats had hay (*ad libitum*). During the research, 24 h before each goat's milk sampling (1st, 15th, and 30th days), the kids were separated from their mothers. Milk samples for the research were taken only during morning hand milking. A measuring cylinder was used to determine the morning milk yield of each goat. During the rest of the time, that is, except for the days of sampling, kids stayed with their mothers and suckled milk. Experimental diets differed in the type of corn. Yellow corn (100%) was

included in the control group diet. In the first experimental group (RC50), yellow corn was partially replaced by 50% red corn in the feed mixture. In the feed mixture of the second experimental group (RC100), the yellow corn was completely replaced by 100% red corn. Red corn is an old native Croatian variety. The feed samples (feed mixture, hay, and corn) were dried and ground to a powder using an ultra-centrifugal mill (heavy metal-free, Retsch ZM 200, Haan, Germany). The standard methods of AOAC [16] were used to determine the feed composition of goats. The feed mixture (g/kg feed mixture) contained: corn 600, barley 120, wheat flour 23, soybean meal (46% crude proteins) 100, extruded soybean 120, salt 4, calcium carbonate 3, mineral vitamin premix 30. Goats mineral vitamin premix contained: 21% Ca; 5% P; 6% Na; 5% Mg; 1,200,000 IU/kg vitamin A; 140,000 IU/kg vitamin D_3; 3500 mg/kg vitamin E; 600 mg/kg Fe (iron sulphate monohydrate); 490 mg/kg Cu (copper sulphate pentahydrate); 500 mg/kg Cu (in the form of chelates); 5 mg/kg Mn (manganese sulphate pentahydrate); 6500 mg/kg Zn (zinc oxide); 1500 mg/kg Zn (in the form of chelates); 60 mg/kg I (anhydrous calcium iodate); 40 mg/kg Co (cobalt carbonate monohydrate); 50 mg/kg Se (disodium selenite). Chemical compositions of the food are presented in Table 1. The methods used for the analysis of the nutritional values of feed are described in [12]. Extraction and determination of total polyphenols and anthocyanins from feed were performed according to the procedures and equipment described by [17]. Concentrations of total polyphenols and anthocyanins in the RC50 group were 513.06 and 217.32 mg/kg, while in the RC100 group they were 1276.70 and 485.40 mg/kg, respectively.

Table 1. Chemical composition of feed mixture, hay, yellow corn, and red corn used in investigation with dairy goats.

Chemical Content (g/kg DM)	Feed Mixture	Yellow Corn	Red Corn	Hay
DM	912	905	907	932
Crude protein	157	100	105	91
Crude fiber (g/kg DM)	36	25	23	328
Crude ash (g/kg DM)	30	13	14	67
EE (g/kg DM)	51	38	37	8
ME (MJ/kg DM)	12	-	-	7
Polyphenols * (total), mg/kg	144.77	179.87	298.69	-
Anthocyanins ** (total), mg/kg	0	125.37	253.04	-

DM—dry matter; * The concentration of polyphenols in RC50 and RC100 was 513.06 and 1276.70 mg/kg, respectively; ** The concentration of anthocyanins in RC50 and RC100 was 217.32 and 485.40 mg/kg, respectively.

2.3. Milk Sampling and Analysis

At the 1st, 15th, and 30th day of the research, two milk samples (one for chemical analyses and other for biochemical parameters) were taken from each goat at morning (7:00 a.m.) during routine hand milking. Total morning milk yield of each goat was determined using a measuring cylinder. Immediately after the collection of these two milk samples, a milk sample (30 mL) from each goat was placed in plastic bottles containing 0.3 mL of azidiol, cooled to 4 °C, and prepared for analysis. The chemical composition of the goat's milk was determined using the MilkoScan FT 6000 analyzer (Foss Electric, Hillerød, Denmark) according to the principle of infrared spectroscopy in accordance with HRN ISO 9622:2017 [18]. The somatic cell count (SCC) was determined according to the Fluor-electronic method HRN ISO 13366-2/Ispr.1:2007 [19] using a Fossomatic 5000 analyzer (Foss Electric, Hillerød, Denmark). To determine the biochemical parameters, the milk was centrifuged at 5000× *g* (30 min) after fat separation and the milk plasma was frozen at −80 °C until analysis. In blood serum, biochemical parameters (aspartate aminotransferase—AST; alanine aminotransferase—ALT; gamma-glutamyl transferase—GGT; iron—Fe; calcium—Ca; and phosphorus—P) were determined using the Olympus AU 400 biochemical analyzer (Olympus, Tokyo, Japan), while the activity of SOD was determined using RANSOD (Randox Laboratories, Crumlin, UK) on the same Olympus AU 400 biochemical analyzer (Olympus, Tokyo, Japan).

2.3.1. Antioxidant Indicators

Fresh goat's milk samples were stored at −80 °C. The extraction of the antioxidant compounds followed the method described in the paper of Alyaqoubi et al. [20] with some modifications. Fresh milk (1 g) was added to 10 mL extraction solution (1 N HC1/95% ethanol (*v*/*v*, 15/85)) in brown 50 mL bottles and shaken for 1 h at 30 °C in a rotary shaker (Brunswick™ Innova® 43/43R—Console Incubator Shaker, Eppendorf AG, Hamburg, Germany) at 300 rpm. The mixture of solvent and samples was then centrifuged at 4400× *g* (Hermle Z 326 K, Hermle Labortechnik GmbH, Wehingen, Germany) at 5 °C for 40 min. The supernatant liquids were stored at −20 °C in the dark until further analysis for DPPH radical scavenging activity and $TBARS_{450}$. The extraction was performed three times.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

In a modification of the methods of [21], the DPPH radical scavenging test was carried out to determine the total antioxidant activity of milk extracts. Briefly, an aliquot of 200 µL of milk extracts was added to 1.8 mL of a 0.2 mM methanol DPPH radical solution, so the final concentration was 0.18 mM DPPH. The mixture was shaken vigorously using a vortex mixer and then left to stand for 30 min in the dark at room temperature. Negative control was carried under the same conditions, which contained 200 µL of milk extraction solution and 1800 µL of 0.2 mM DPPH in methanol. All measurements were performed three times. Absorbance was measured at 517 nm using a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA). The percentage of DPPH scavenging activity was determined using Equation (1):

$$\text{DPPH scavenging activity (\%)} = ((Ab + As) - Am)/Ab \times 100 \quad (1)$$

where Ab is the absorbance of the negative control, 0.18 mM DPPH radical methanolic solution with the milk extraction solution, As is the absorbance of the milk sample control, and Am is the absorbance of samples of tested milk extracts (final concentration 10 µL/mL) and DPPH radical.

Thiobarbituric Acid Reactive Substances ($TBARS_{450}$)

Slightly modified methods from Oancea et al. [22] based on the absorbance values (at 450 nm, 532 nm and 600 nm) from Sun et al. [23] with a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA) were used to measure $TBARS_{450}$. Determinations were performed after defrosting of milk samples overnight in the refrigerator. Each sample used for analytical determination consisted of 1.5 mL of defrosted milk, and 0.5 mL of 0.1% TCA was used for the protein-removal step. After centrifugation at 3000× *g* for 5 min at 4 °C (Hermle Z 326 K, Hermle Labortechnik GmbH, Wehingen, Germany), the supernatants were incubated with 1 mL of TBA/TCA reagent (0.5% TBA in a 20% TCA) for 90 min at 80 °C.

After incubation and cooling of samples on ice, the absorbance values were read at different wavelengths, specific for milk degradation products (450 nm—saturated aldehydes, 532 nm—MDA and 600 nm—nonspecific absorption). The results were expressed as the absorbance values at 450 nm after subtraction of non-specific absorption at 600 nm.

2.4. Blood Sampling and Analysis of Blood Metabolic Profile

Blood samples were taken from the jugular vein of each goat (10 mL) into a sterile vacuum tube Venoject® (Sterile Terumo Europe, Leuven, Belgium) containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for hematologic analysis. After blood collection, samples were transferred on ice at 0–6 °C to the laboratory of the Department of Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences, Josip Juraj Strossmayer University of Osijek, Osijek Croatia. The EDTA tubes containing the anticoagulant were inverted several times to ensure adequate mixing of the blood. Hematological parameters in goat whole blood, such as leukocyte (WBC, 10^9 L) and erythrocytes count (RBC, 10^{12} L) as well as the content of hemoglobin (HGB, g/L), hematocrit (HCT,

L/L), mean corpuscular volume (MCV, fL), the mean hemoglobin content in erythrocytes (MCH, pg), and the mean hemoglobin concentration in the erythrocytes (MCHC, g/L) were determined using the Sysmex PocH-100iV automated three-differential hematology analyzer (Sysmex Europe GmbH, Hamburg, Germany). Blood samples collected in sterile vacuum tubes (Venoject®, Leuven, Belgium) were centrifuged within 10 min at 1609.92× *g* by centrifuge ROTOFIX 32A (Hettich GmbH & Co. KG, Tuttlingen, Germany) and the serum samples obtained were analyzed in the Olympus AU400. In the blood serum, the concentration of biochemical parameters was determined and showed in the reagent code, such as calcium (Ca, mmol/L; OSR 60117), phosphorus-inorganic (P-inorg, mmol/L; OSR6122), magnesium (Mg, mmol/L; OSR6189), iron (Fe, μmol/L; OSR6186), urea (mmol/L; OSR6134), glucose (GUK, mmol/L; OSR6140) total proteins (TPROT, g/L; OSR6132), albumin (ALB, g/L; OSR6102), cholesterol (CHOL, mmol/L; OSR6116), LDL—low-density lipoprotein (LDL, mmol/L; OSR6183), HDL—high-*density* lipoprotein (HDL, mmol/L; OSR6187), triglycerides (TGC, mmol/L; OSR60118); β-hydroxybutyrate (BHB, mmol/L; RB1007), and non-esterified fatty acids (NEFA, mmol/L; FA115). Also, in the blood serum, activities of enzymes were determined and showed in the reagent code, such as alanine aminotransferase (ALT, U/L; OSR 6007), aspartate aminotransferase (AST, U/L; OSR6009), γ-glutamyl transferase (GGT; U/L; OSR6020), and glutathione reductase (GR; U/L; GR2368) and were measured by using Olympus System reagents (Lismeehan, Ireland). The globulin content (GLOB) was calculated as the difference between the total protein and albumin content. In blood serum, the activity of glutathione peroxidase (GPx, U/L; RA505) was determined using a Ransel® kit (RANSEL, RANDOX, London, UK), and the activity of total superoxide dismutase (SOD, U/mL; SD125) was determined using a Ransod® kit (RANDOX, San Diego, CA, USA) on an automatic Olympus AU 400 (Olympus, Tokyo, Japan) analyzer.

2.5. Statistical Analyses

Data for milk yield, milk composition, blood metabolic profile parameters, and antioxidant status for each goat and sampling period were analyzed by repeated measure using the PROC MIXED procedure of SAS 9.4 [24] according to the following model:

$$Yijk = \mu + di + hij + wk + dwik + eijk,$$

where:

μ = overall mean,
di = fixed effect of diet (i = CC, RC50, RC100),
hij = animal within diet as subject (j = CC, RC540, RC100),
wk = fixed effect of period during lactation (k = 1–3),
dwik = interaction between diet and period (diet × period), and
eijk = random error variation (residual error).

Means were compared using Tukey's honestly significant difference test [24], and $p \leq 0.05$ was indicated as significant. The values for SCC were logarithmically transformed to obtain a linear value that approximates the normal distribution.

3. Results

Table 2 shows the body weight, body condition score, and milk yield and composition of milk of dairy goats during lactation when fed feed mixtures containing different levels of red corn.

No significant ($p > 0.05$) variations were determined in milk yield and composition between dietary treatments. The timing of sampling and the interaction between feed and timing of sampling did not affect the parameters studied, except for urea concentration depending on the lactation stage (Table 2). It is evident that the activity of enzymes (AST, ALT and GGT) in milk showed an insignificant decrease ($p > 0.05$) when feeding with a higher content of red corn in the feed mixtures (RC50 and RC100) compared to the CC group

Table 2. Production traits and milk composition of dairy goat fed feed mixtures with different levels of red corn.

Traits	Diets			SEM	*p*-Value		
	CC	RC50	RC100		D	T	D*T
Morning milk yield (g)	1461.47	1323.54	1367.18	54.176	0.555	0.443	0.779
Body weight (kg)	46.10	48.22	47.83	0.712	0.465	0.991	0.993
BCS (point)	2.76	2.90	2.80	0.051	0.577	0.687	0.856
Milk composition							
Fat (g/100 g)	3.14	3.22	3.09	0.083	0.840	0.939	0.980
Protein (g/100 g)	2.84	2.89	3.00	0.029	0.086	0.569	0.995
Lactose (g/100 g)	4.45	4.48	4.53	0.022	0.800	0.285	0.335
NFDM (g/100 g)	8.34	8.43	8.58	0.042	0.101	0.650	0.688
AST (U/L)	171.29	130.43	145.66	11.812	0.415	0.734	0.614
ALT (U/L)	386.68	346.95	352.00	46.341	0.904	0.460	0.784
GGT (U/L)	364.92	360.19	327.88	15.362	0.584	0.766	0.446
Fe (µmol/L)	17.21	15.79	13.76	1.261	0.603	0.774	0.769
Ca (mmol/L)	41.03	45.30	45.84	1.673	0.417	0.081	0.484
P-inorg (mmol/L)	28.47	32.63	33.96	1.200	0.154	0.172	0.435
Urea (mg/dL)	19.88	22.51	23.06	0.257	0.572	0.032	0.911
SSC (log)	5.43	5.51	5.37	0.049	0.645	0.940	0.562

CC—control corn; RC50—red corn 50%, RC100—red corn 100%; D—diet, T—time of sampling, D*T—interaction (diet × time of sampling), SEM—standard error of mean; BCS—body condition score; NFDM—non-fat dry matter; AST—aspartate aminotransferase, ALT—alanine aminotransferase, GGT—γ-glutamyl transferase; SSC—somatic cell count.

From the results of the analysis (Table 3), the increase in HGB, MCH, and MCHC was found in feeding goats with an increased content of RC in both groups (RC50 and RC100) compared with CC, and a decrease in RBC in RC100 compared with RC50. These parameters were affected by dietary treatment and time of milk sampling and their interaction.

Table 3. Hematologic parameters in dairy goat fed feed mixtures containing red corn.

Parameter	Diets			SEM	*p*-Value		
	CC	RC50	RC100		D	T	D*T
RBC ($\times 10^{12}$ L)	9.74 ^{ab}	10.45 ^{a}	8.71 ^{b}	0.228	0.015	0.180	0.843
WBC ($\times 10^{9}$ L)	9.74	8.99	9.74	0.350	0.313	0.382	0.901
HGB (g/L)	84.43 ^{b}	100.00 ^{a}	106.55 ^{a}	5.212	<0.001	<0.001	<0.001
HCT (L/L)	0.27	0.28	0.26	0.006	0.169	0.015	0.728
MCH (pg)	7.98 ^{c}	9.70 ^{b}	12.54 ^{a}	0.661	<0.001	<0.001	<0.001
MCV (fL)	27.24	27.25	30.54	0.992	0.300	0.207	0.474
MCHC (g/L)	293.57 ^{c}	357.50 ^{b}	462.78 ^{a}	18.755	<0.001	<0.001	<0.001

CC—control corn; RC50—red corn 50%; RC100—red corn 100%; D—diet; T—time of sampling; D*T—interaction (diet × time of sampling); SEM—standard error of mean; RBC—erythrocytes, WBC—number of leukocytes; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean hemoglobin content in erythrocytes; MCHC—mean hemoglobin concentration in erythrocytes; $^{a–c}$ Values within a row with different superscripts differ significantly at $p < 0.05$.

Analysis of blood metabolic profile (biochemical parameters and enzyme activities) in dairy goats concerning the consumption of feed mixtures with red corn did not reveal statistically significant ($p > 0.05$) differences, except for the concentrations of urea, glucose, and Mg influence of milk sampling time (Table 4).

In the RC100 group, a significant decrease in $TBARS_{450}$ values in milk compared with CC was found, as well as a smaller increase in the DPPH scavenging, but without significant differences in RC50 and RC100 compared with CC. A sampling time affected $TBARS_{450}$ concentration significantly as well (Table 5). The TBARS450 results were expressed as the

absorbance values at 450 nm after subtraction of non-specific absorption at 600 nm, while the absorbance values at 532 nm were insignificant.

Table 4. Blood biochemical parameters and enzyme activities of dairy goat fed with feed mixtures containing red corn.

Parameter	Diets			SEM	*p*-Value		
	CC	RC50	RC100		D	T	D*T
Urea (mmol/L)	7.58	7.92	7.44	0.220	0.626	<0.001	0.488
TPROT (g/L)	74.74	77.13	73.71	0.867	0.295	0.788	0.505
ALB (g/L)	27.56	27.83	26.46	0.439	0.442	0.760	0.541
GLOB (g/L)	47.18	49.30	47.25	0.616	0.314	0.454	0.741
Ca (mmol/L)	2.31	2.29	2.24	0.024	0.463	0.097	0.720
P-inorganic (mmol/L)	2.25	2.11	2.20	0.065	0.696	0.975	0.375
Mg (mmol/L)	1.30	1.30	1.29	0.011	0.960	0.009	0.273
Fe (µmol/L)	25.57	24.90	24.33	0.950	0.869	0.489	0.314
GUK (mmol/L)	3.55	3.49	3.60	0.045	0.636	0.031	0.803
CHOL (mmol/L)	2.74	3.09	2.67	0.080	0.099	0.656	0.801
HDL (mmol/L)	1.64	1.83	1.63	0.044	0.143	0.907	0.891
LDL (mmol/L)	1.03	1.18	0.95	0.041	0.098	0.316	0.736
TGC (mmol/L)	0.17	0.18	0.19	0.011	0.734	0.235	0.921
NEFA (mmol/L)	0.11	0.14	0.10	0.009	0.311	0.157	0.863
BHB (mmol/L)	0.71	0.67	0.57	0.024	0.071	0.562	0.766
AST (U/L)	130.53	121.43	137.93	3.423	0.187	0.512	0.914
ALT (U/L)	27.71	24.59	27.54	0.679	0.136	0.326	0.878
GGT (U/L)	45.29	43.92	43.44	1.027	0.753	0.681	0.506

CC—control corn; RC50—red corn 50%; RC100—red corn 100%; D—diet, T—time of sampling; D*T—interaction (diet × time of sampling); SEM—standard error of mean; TPROT—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; AST—aspartate aminotransferase; ALT—alanine aminotransferase, GGT—γ-glutamyl transferase.

Table 5. Antioxidative status of milk and blood of dairy goats fed with feed mixtures containing red corn.

Parameter	Diets			SEM	*p*-Value		
	CC	RC50	RC100		D	T	D*T
Milk							
$TBARS_{450}$	0.025 ^{a}	0.022 ^{ab}	0.019 ^{b}	0.017	0.022	<0.001	0.186
DPPH scavenging (%)	87.78	91.17	92.54	1.107	0.187	0.099	0.324
SODm (U/mL)	10.37	11.45	11.63	0.668	0.735	0.532	0.616
Blood serum							
GPx (U/L)	1633.48	1669.75	1531.92	99.589	0.726	<0.001	0.043
SODb (U/mL)	0.29 ^{b}	0.53 ^{a}	0.44 ^{a}	0.025	0.001	0.356	0.916
GR (U/L)	96.16	95.36	95.06	2.561	0.990	0.003	0.512

CC—control corn; RC50—red corn 50%; RC100—red corn 100%; D—diet; T—time of milk sampling; D*T—interaction (diet × time of sampling); SEM—standard error of mean; $TBARS_{450}$—Thiobarbituric acid reactive substances; DPPH—radical scavenging activity at final milk concentration 10 uL/mL; SODm—milk superoxide dismutase; GPx—blood glutathione peroxidase; SODb—blood superoxide dismutase; GR—blood glutathione reductase; ^{a,b} Values within a row with different superscripts differ significantly at $p < 0.05$.

It can be seen that SODm activity in milk increased when fed with a higher content of red corn in the feed mixtures (RC50 and RC100) compared to the CC group but without significant differences. A significant ($p \leq 0.05$) effect was found on the activity of antioxidant enzymes in the blood of dairy goats fed with different proportions of RC, namely an increase in SODb activity in the RC50 and RC100 groups and a significant effect of sampling time on GPx and GR activity in the blood (Table 5).

4. Discussion

Lactation is a very challenging physiological process for an animal. Due to their high metabolic demands, goats and other small ruminants are prone to suffering from oxidative stress [13]. Both the productivity and health of ruminants are negatively influenced by the oxidative stress. Therefore, research on the addition of anthocyanins to the feed of small ruminants is very timely. However, the high content of polyphenols in food, especially astringent polyphenols, can bind nutrients and reduce their absorption in the digestive system causing reduced food consumption and impaired animal production properties [25]. Plants rich in anthocyanins influence the immune response associated with the inhibition of inflammatory processes by promoting the normalization of microflora in the digestive tract and reducing the permeability of the gastrointestinal barrier [26]. In this research, no significant changes were observed in the production traits of goats. The lactose content of milk was slightly increased in RC50 and RC100 goats compared to the CC group (from 4.45 to 4.53% DM). In a study by Tian et al. [27], no effects of feeding goats with anthocyanins from purple corn on dry matter intake, average daily gain of goats, glucose blood concentrations, urea nitrogen, total proteins, albumins, and DPPH activity were observed. In a study [13] conducted with lactating dairy goats, no change in milk quantity and composition was observed when feeding purple corn silage, except for a significant increase in lactose content in the goat milk compared to the group fed ordinary corn (from 4.50 to 4.55%). This may be related to fermentation in the rumen, in particular by inhibiting acetic acid and increasing the proportion of propionic acid in the rumen. The authors indicated that the anthocyanin sugars can be broken down in the digestive system and are therefore involved in lactose synthesis. The absence of changes in the activity of other enzymes such as GPx and GR could be explained by the poor absorption of anthocyanins by small ruminants compared with monogastric animals. SOD is the first line of defense among the intracellular enzymes that scavenge reactive oxygen species [28]. Consequently, anthocyanins are powerful antioxidants that regulate peroxidation reactions and control the production of free radicals in the goat's body [29]. Quadros et al. [6] suggested that anthocyanins can suppress oxidation resistance and enhance plasma SOD enzyme, resulting in reduced saturated fatty acids (SFA) and increased polyunsaturated fatty acid (PUFA) levels. Antunović et al. [12] found similar deviations in blood GPx and GR activities, and an insignificant increase in blood SOD activity in lambs fed mixtures with the addition of red corn. Hosoda et al. [30] pointed out that the cause might be a sufficient number of non-enzymatic antioxidants, as no serious oxidative stress was found in sheep. Tian et al. [31] found an increase in plasma SOD activity when goats were fed silage from anthocyanin-rich purple corn, while the activity of other antioxidant enzymes (GPx and catalase) in the goats' plasma did not change. These authors pointed out that the reason could be that the increased activity of SOD in the plasma of goats reduces the load on the antioxidant defense system so that other enzymes in the plasma do not change [32]. Matsuba et al. [33] found that when feeding dairy cows with purple corn silage, the activity of SOD in the blood also increased. Corn rich in anthocyanins is suitable to provide an antioxidant effect in dairy cattle due to the stability of anthocyanins in rumen fluid [34]. Hosoda et al. [28] found an increase in plasma activity SOD in lactating sheep fed purple corn and a significant increase in plasma activity SOD in cows [35] fed anthocyanin-rich corn silage. The antioxidant enzymes, such as SOD, have the ability to eliminate reactive oxygen and prevent cell damage, which can be associated with the use of purple corn extract that can penetrate cell membranes and stimulate the production of antioxidant enzyme [36]. Tian et al. [29], in a study on the effects of anthocyanin-rich purple corn pot silage in goat feed, found a potential to improve antioxidant status by improving SOD activity in blood plasma through modulation of antioxidant genes in the mammary gland. In the present study, a significant ($p \leq 0.05$) increase in blood SOD activity was found in the RC50 and RC100 groups compared to the control group, which corresponds with previously mentioned studies. Hematologic and biochemical parameters and enzyme activities in goat blood in the present investigation were within the reference values established for goats

under similar rearing conditions, except for the MCH content in RC50 and RC100, MCV in all groups, and MCHC (only in RC100), which were above the reference values [37–39] with a slight effect of period and interaction between diet and period, which was expected. A significant increase in HGB, MCH, and MCHC content with increasing RC content in both groups (RC50 and RC 100) compared with CC might be related to anthocyanins stabilizing erythrocyte membranes and inhibiting hemoglobin polymerization [40]. According to Youdim et al. [41], polyphenols protect against reactive oxygen species-induced hemolysis by increasing the integrity of red blood cells in conjunction with the inhibition of lipid peroxidation. In the research by Antunović et al. [12], which was carried out on the feeding of lambs after weaning, an increase in the HGB content was also found with an increase in the proportion of red corn in the feed. The effect of anthocyanins from the feed on the antioxidant response of the animal organism is well known. The antioxidant properties of phenols are attributed to their redox property, which allows phenols to act as reducing agents, hydrogen donors, and oxygen scavengers. In the present study, a significant decrease in $TBARS_{450}$ in milk was observed in the RC100 group compared with CC, as well as a smaller increase in the DPPH scavenging, but without significant differences in RC50 and RC100 compared with CC. These changes also indicate the antioxidative effect of nutritional treatments. Indeed, the anthocyanins in plasma can supply hydrogen atoms to DPPH [42] and thereby increase DPPH scavenging activity. In addition, antioxidants in the diet can reduce the content of superoxide anions, hydroxyl radicals, and peroxynitrite [43]. The aforementioned authors point out that it can inhibit the activities of enzymes that generate reactive oxygen species and increase the expression of antioxidant enzymes such as SOD. Tsuda et al. [44] performed a study with rats fed C3G anthocyanins (2 g/kg) for 14 days. A significant decrease in $TBARS_{450}$ formation during serum formation was observed, but no significant difference in serum lipid concentrations (CHOL, NEFA, TGC). Tian et al. [13] also found no changes in DPPH scavenging activity and GPx activity when feeding goats with purple corn silage due to the possible high toxicity of O_2 that was converted into low toxicity of H_2O_2 by SOD, thus facilitating oxidative stress. In the present research, we also did not determine significant ($p > 0.05$) differences in DPPH and GPx activity when feeding the goats with red corn.

5. Conclusions

From the present results, it can be concluded that the replacement of yellow corn with red corn had no effect on the production traits and blood metabolic profile of the goats, except for most of the blood hematologic parameters. Higher levels of HGB, MCH, and MCHC were observed with an increase in the content of CR in both groups (RC50 and RC100) compared to CC, as well as a decrease in RBC in RC100 compared to RC50. A significant increase in SOD activity in the blood of RC50 and RC100 groups was found. A significant decrease in $TBARS_{450}$ in milk was found compared to the CC group, as well as an increase in DPPH scavenging, but without significant differences compared to the CC group. These changes indicate an adequate antioxidative effect of red corn used in goat diet, since the SOD activity in blood, as well as antioxidant indicators ($TBARS_{450}$ and DPPH scavenging), were increased in goat milk, while the blood metabolic profile and production traits were maintained. Further studies should be undertaken to develop indicators for antioxidant status in the blood ($TBARS_{450}$, DPPH scavenging) and anthocyanin profiles in red corn and milk from lactating dairy goats.

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