sciendo

J Vet Res 68, 409-417, 2024
DOI:10.2478/jvetres-2024-0052

Effect of chokeberry pomace as a feed additive for high-producing dairy goats on oxidative stress parameters and quality of milk

Monika Szymańska-Czerwińska[1], Maima Matin[1]✉, Krzysztof Niemczuk[1]✉, Nina Strzałkowska[1], Zbigniew Osiński[2], Karina Horbańczuk[1], Agnieszka Wierzbicka[1], Jarosław Olav Horbańczuk[1], Atanas Georgiev Atanasov[1,3], Artur Jóźwik[1]

[1]Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, 05-552 Jastrzębiec, Poland
[2]Department of Hygiene of Feeding Stuffs, National Veterinary Research Institute, 24-100 Puławy, Poland
[3]Ludwig Boltzmann Institute Digital Health and Patient Safety, Medical University of Vienna, 1090 Vienna, Austria
m.matin@igbzpan.pl, k.niemczuk@igbzpan.pl

Received: May 29, 2024 *Accepted: September 6, 2024*

Abstract

Introduction: Chokeberry pomace, rich in polyphenolic compounds, holds potential to be a valuable feed additive for enhancing the antioxidative capacity and overall quality of milk. This study explores the impact of dietary inclusion of chokeberry pomace on oxidative stress parameters and other milk quality parameters in high-producing dairy goats. **Material and Methods:** Twenty-seven goats were allocated into three groups: a control group provided standard feed and two experimental groups provided feed supplemented with 15 g or 30 g of chokeberry pomace per kilogram. Milk samples were analysed for physicochemical traits, a range of enzyme activities and antioxidant properties. **Results:** Supplementation with chokeberry pomace significantly reduced milk fat content, enhanced antioxidative properties and increased most of the quantified enzyme activities. Total polyphenol content and reduced glutathione levels were significantly higher in the supplemented groups, correlating with improved antioxidative potential of the milk. **Conclusion:** Chokeberry pomace in goat diets enhances milk's antioxidative properties and upregulates its enzymatic activity profile, suggesting a potential strategy to improve the nutritional quality and health benefits of goat milk. The study underscores the utility of chokeberry pomace as a feed additive that might not only benefit animal health but also contribute to enhanced milk quality.

Keywords: dairy goats, chokeberry pomace, feed additives, oxidative stress, milk quality.

Introduction

Phenolic compounds constitute the most important bioactive components of fruits with strong antioxidative properties (4). The black chokeberry (*Aronia melanocarpa*) contains an extraordinary number of polyphenolic compounds in high levels: dominant are proanthocyanidins and the second group by size are anthocyanins and phenolic acids, which have antioxidant properties. The strong antioxidative action of black chokeberry is known and was reported in studies in humans and some species of animals (13).

Poland is a leader in the production of chokeberry on a global scale. Annually, 1.5 million tonnes of fruit are processed in the domestic fruit industry, of which 30–40 thousand tonnes are chokeberries. The effective utilisation of chokeberry pomace remaining after the fruit is juiced by using it as animal feed fits the model of sustainable agriculture and has a positive impact on environmental conservation. In the available literature, data are lacking about the effect of chokeberry pomace on oxidative stress parameters in goat's milk and on its quality. Oxidative stress is central to many diseases, reproductive disorders and reduced milk yield in ruminants (23). One of the approaches to minimising it is including chokeberry as a livestock feed ingredient to enrich and improve the quality of their milk. Another reason for implementing fodder modification is to increase the efficiency of breeding animals and maintain antioxidative/oxidative homeostasis.

Goat meat and milk are valued in modern society for their nutritional content and potential health benefits. Goat's milk is regarded as a high-quality, valuable, healthy, tasty, and easily digestible nutritional product, diverse health benefits are ascribed to it, and it is rich in diverse macro- and micro-nutrients (16, 29). Moreover,

it is more abundant in amino acids, vitamins, minerals, and its proteins and fats are digested more easily and faster than cow's milk (29). One of the main characteristics of goat's milk that has contributed to its appeal as an alternative to cow's milk is its lower allergenicity (7). This explains why it is also becoming more and more popular among consumers. Milk from goats kept on large-herd farms, where the focus is mainly on volume production, and nutrition is from industrial feed products, cannot be guaranteed to have any health-promoting properties and nor will its consumption be certain to bring the expected results. Ameliorating industrial animal feed with plants including fruiting plants exploits an important source of diverse compounds with health-promoting bio-effects (4).

The aim of this study was to establish the impact of different amounts of chokeberry pomace addition to a diet for high-producing dairy goats on a range of parameters associated with oxidative stress, metabolic health and the quality of milk.

Material and Methods

Livestock and diet. The experiment was performed with 27 three- or four-year-old dairy goats of the Polish white improved breed which were in middle-stage lactation and in the second or third year of lactation. The goats were kept on a specialised farm in the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences and were maintained in a loose barn and fed with corn and wilted grass silage along with mineral-vitamin premix according to INRA (Institut National de la Recherche Agronomique, now Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Paris, France) guidelines. The animals also had free access to water. They were under veterinary care which included screening for infections or inflammatory changes in the mammary gland such as mastitis. The animals were divided into three dietary groups: a control group (C), fed with standard maintenance feed prepared in accordance with INRA requirements; and two experimental feeding groups differing in the amount of chokeberry pomace added to the feed – group A1 on standard maintenance feed with 15 g of chokeberry pomace added per kilogram and group A2 on standard maintenance feed with 30 g of chokeberry pomace added per kilogram. Commercial chokeberry pomace obtained by a drying process and free of artificial preservative substances was obtained from JAR-PASZ (Jaworzno, Poland) and was characterised by a minimum of 90% dry matter, maximally 4% raw ash content, 13% total protein and maximally 14% humidity. Each group included nine dairy goats. The three indicated diets were supplied to the animals for 60 d, whereby all animals were fed individually using a controlled system for feed supply. All operations were conducted in accordance with relevant international and national guidelines concerning animal welfare. Since the present study was on supplementation with an established feed additive and the animals were maintained and milk was collected on established commercial premises for goat's milk production, according to relevant national guidelines ethical committee approval was not needed for the procedures carried out.

Milk sampling. The goats were machine-milked twice a day according to standard procedures. Prior to milking, the goat's teats were washed with clean tap water and dried with a single-use paper towel. Before attaching the milking machine to the teats, the first three to four streams of milk from both teats of each goat were discarded onto a strip cup and examined for any sign of mastitis. Morning and evening milk samples were mixed and sent for analyses. Milk was analysed by the Milk Laboratory of the Institute of Genetics and Animal Biotechnology, Jastrzębiec, Poland. The somatic cell count (SCC) was determined using the Bactocount IBCM (Bentley Instruments, Chaska, MN, USA). Each milk sample was also analysed for fat, protein (Prot), casein (Cas), lactose (Lac), total solid, (TS), solids-not-fat (SNF), urea, freezing point (FP), free fatty acid (FFA), density (D), natural acidity (NA) and citric acid (Cit acid) content. The composition and parameters of milk were estimated using the MilkoScan FT2 device (Foss Electric, Hillerod, Denmark).

Enzyme activities of carbohydrate-metabolizing enzymes, aminopeptidases, and acid phosphatase. Substrates for determining the lysosomal enzyme activities were supplied from Sigma-Aldrich (St. Louis, MO, USA). The activities of β-glucuronidase (β-GRD; Enzyme Commission (EC) number 3.2.1.31), α-galactosidase (α-GAL; EC 3.2.1.22), β-glucosidase (β-GLU; EC 3.2.1.21), β-galactosidase (β-GAL; EC 3.2.1.23), β-N-acetylhexosaminidase (HEX; EC 3.2.1.52) and mannosidase (MAN; EC 3.2.1.24) were assayed according to the procedures described by Barrett and Heath, using p-nitrophenyl-β-D-glucuronide, p-nitrophenyl -α-D-glucopyranoside, p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl-β-D-galactopyranoside, N-acetyl-β-D-glucosaminide and p-nitrophenyl-β-D-mannopyranoside as the respective substrates upon which incubation was undertaken at 37°C (3). The activities of acid phosphatase (AcP; EC 3.1.3.2) were also assessed based on 4-nitrophenyl derivative conversion at 420 nm with a Cary 50 Bio UV-VIS spectrophotometer (Varian, part of Agilent Technologies, Mulgrave, VA, Australia) also applying Barrett and Heath's method (3). The activities of the three quantified aminopeptidases, alanyl aminopeptidase (AlaAP; EC 3.4.11.2), leucyl aminopeptidase (LeuAP; EC 3.4.11.1) and arginyl aminopeptidase (ArgAP – EC 3.4.11.6), were assessed in the obtained supernatants as described by McDonald and Barrett (15), as Fast Blue BB salt (4-benzoyloamino-2, 5-diethoxybenzene-diazinium chloride) derivatives at 540 nm using the Cary 50 Bio spectrometer (Varian). The enzyme activities were expressed in nmol/mg of total protein, per h. To adjust for protein amounts, the milk's protein content was determined as described by Krawczyński and Osiński (12), using bovine serum albumin as a standard.

Vitamin C assay. The vitamin C (Vit C) content of milk was determined according to the procedure described by Omaye (19) and modified by Jóźwik *et al.* (10) with a Lambda Bio-20 spectrophotometer (PerkinElmer, Waltham, MA, USA).

Reduced glutathione determination. Reduced glutathione (GSH) in the milk samples was assayed with an OxisResearch Bioxytech® GSH/GSSG-412 test (Portland, OR, USA). Before analysis, the milk samples were frozen with 1-methyl-2-vinylpyridium trifluoromethane sulphonate at −80°C, and thawed afterward in order to initiate erythrolysis. Then, the released GSH was measured according to the kit manufacturer's instructions. Absorbance values at λ412 and reaction kinetics data were obtained with a Bio-Tek Synergy4 microplate reader (Winooski, VT, USA). The evaluation of the obtained results was performed with the Gen5 program (BioTek), and finally the glutathione levels were expressed as μM values.

Potential to scavenge the free 1,1-diphenyl-2-picrylhydrazyl radical. The potential of milk samples to scavenge the free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assessed according to the method described by Brand-Williams *et al.* (5), which uses a synthetic DPPH radical. First, 1 mL of milk sample was mixed with 10 mL of cold (4°C) ultra-pure ethanol and the mixture was homogenised. The obtained homogenates were then sparged with nitrogen and sealed, before being extracted for 2 h in an ultrasonic bath at 40°C. After extraction, the tubes were cooled, and the samples were further centrifuged at 4°C and 4,000 × *g* for 15 min. Next, 0.5 mL of supernatant was mixed with an equal part ethanolic solution of DPPH (0.5 mM), previously diluted to achieve an absorbance of around 0.9 at a wavelength of λ = 517 nm. The obtained mixture was then thoroughly blended and incubated in a dark and cool environment for 30 min in order to stabilise the colour. Extinction measurements were finally done with a Cary 50 Bio UV-VIS spectrophotometer at a wavelength of λ = 517 nm with Cary WinUV software.

Determination of total phenolic content. To determine total polyphenol (Pol) content, 1 mL of the milk samples was homogenised in 10 mL of ultra-pure methanol containing 1% acetic acid chilled to 4° C. After that, the tubes with samples were sparged with nitrogen and sealed, which was followed by extraction for 2 h in an ultrasonic bath at 40°C. The samples were next cooled and allowed to settle in the dark. The total phenol content was then determined using the modified procedure described by Škerget *et al.* (26), employing spectrophotometric assessment of the colorimetric reaction of oxidation and reduction. The Folin-Ciocalteu reagent was used as an oxidising reagent (1). The reconstituted extracts (0.5 mL) were then transferred to 6-mL test tubes and mixed with 2.5 mL of Folin-Ciocalteu reagent which had been diluted 10-fold with demineralised water (Sigma-Aldrich, Buchs, Switzerland). The samples were then thoroughly mixed for 8 min and received the addition of 2 mL of saturated sodium carbonate solution. After that, incubation at 40°C for 30 min was carried out (until the development of a stable blue colour). The absorbance was next measured at 765 and 735 nm against a blank sample of 0.5 mL of double-distilled H_2O. The results were evaluated using a calibration curve prepared based on the absorbance of a gallic acid standard in the range 0–0.5 mg/mL. The results were finally expressed as mg of gallic acid equivalents (GAE)/g milk.

Statistical evaluation. The data were presented as mean ± standard deviation (SD). Prior to the statistical analysis, the data for SCC were transformed to a logarithmic value (log). The normality of all data was checked by a Shapiro–Wilk test in STATISTICA 13 software (Dell, now TIBCO, Palo Alto, CA, USA). If the data for multiple-group comparisons had normal distribution, the statistical significance was calculated by the software using the two-factor fixed model analysis of variance procedure, followed by a least significant differences post-hoc test for significance analysis, and a P-value ≤ 0.05 was determined to be statistically significant. The relationship between the technological parameters of milk and enzymes was analysed by Pearson's correlation analysis using the data of all analysed groups.

Results

The results of the quantification of the physicochemical parameters of milk are presented in Table 1. The log SCC remained at a constant level in groups supplemented with chokeberry and changes were not observed, while in the control group a higher level was seen.

A lower fat level was recorded in the milk samples of both groups supplemented with chokeberry. However, it should be noted that the group given more chokeberry (A2 group) produced milk lower in fat content (P-value < 0.001) and density (P-value = 0.036). A significantly (P-value = 0.026) lower FP was found in the A1 group's milk at the end of the experiment compared to the control group's at the beginning of the experiment. A significant FP difference (P-value = 0.012) was noted over the course of the experiment between A1 group milk and A2 group milk.

Significant decrease in the NA of milk was observed; these were in the A1 group's samples, between the control group's and the A1 group's samples and between the control group's and the A2 group's samples over the course of the experiment, as well as between the final control group milk samples and the final A2 group milk samples (P-values < 0.001–0.023). Moreover, a decrease was also found within the control group (P-value < 0.001), which might possibly be due to exogenous (environmental) factors that bore effect during the experimental period.

No statistically significant differences were observed in the levels of Cas, Lac, TS, SNF, urea, FFA or Cit acid content.

Table 1. Physicochemical parameters of milk in the control and experimental groups at the start and finish of chokeberry supplementation to dairy goats

Physico-chemical parameters of milk	Amount of additive	Mean ± standard deviation		Statistical significance (P-value)				
		Start	Finish	Within group A1, A2 and C	Between C and A1 and C and A2	Between C and A1	Between C and A2	Between A1 and A2
				Start to finish	Finish	Start to finish		Finish
Log somatic cell count	15g (A1) 30g (A2) control (C)	2.65 ± 0.57 2.63 ± 0.24 2.68 ± 0.39	2.68 ± 0.17 2.61 ± 0.20 2.90 ± 0.20	0.831 0.912 0.187	0.184 0.070	0.992	0.619	0.626
Fat (%)	15g (A1) 30g (A2) control (C)	2.94 ± 0.42 3.30 ± 0.41 2.88 ± 0.32	2.73 ± 0.20 2.56 ± 0.36 2.68 ± 0.50	0.251 <0.001* 0.264	0.757 0.511	0.403	0.066	0.316
Protein (%)	15g (A1) 30g (A2) control (C)	2.85 ± 0.24 2.76 ± 0.20 2.66 ± 0.27	2.82 ± 0.14 2.94 ± 0.27 2.74 ± 0.29	0.793 0.106 0.093	0.883 0.359	0.162	0.013*	0.271
Casein (%)	15g (A1) 30g (A2) control (C)	2.01 ± 0.22 1.98 ± 0.18 1.93 ± 0.27	1.87 ± 0.19 2.06 ± 0.26 1.96 ± 0.25	0.189 0.482 0.784	0.438 0.371	0.604	0.227	0.085
Lactose (%)	15g (A1) 30g (A2) control (C)	4.80 ± 0.25 4.83 ± 0.14 4.89 ± 0.15	4.76 ± 0.20 4.82 ± 0.22 4.82 ± 0.19	0.708 0.828 0.484	0.550 0.946	0.184	0.418	0.573
Total solids (%)	15g (A1) 30g (A2) control (C)	10.97 ± 0.64 11.28 ± 0.60 10.79 ± 0.54	10.86 ± 0.32 10.84 ± 0.62 10.88 ± 0.73	0.707 0.112 0.732	0.946 0.881	0.777	0.835	0.934
Solids not fat (%)	15g (A1) 30g (A2) control (C)	8.13 ± 0.41 8.08 ± 0.29 8.01 ± 0.31	7.99 ± 0.25 8.15 ± 0.45 8.06 ± 0.34	0.404 0.671 0.797	0.684 0.566	0.877	0.389	0.309
Urea (mg/L)	15g (A1) 30g (A2) control (C)	241.56 ± 76.05 234.89 ± 67.15 257.67 ± 92.03	257.67 ± 102.27 199.50 ± 46.60 210.25 ± 52.78	0.652 0.312 0.202	0.202 0.765	1.000	0.100	0.100
Freezing point (°C)	15g (A1) 30g (A2) control (C)	−0.592.89 ± 11.59 −0.589.8 ± 99.09 −0.601.67 ± 4.12	−0.584.11 ± 20.34 −0.600.80 ± 17.55 −0.599.63 ± 13.78	0.187 0.094 0.764	0.026* 0.859	0.010*	0.893	0.012*
Free fatty acids (mmol/100 g fat)	15g (A1) 30g (A2) control (C)	0.61 ± 0.11 0.60 ± 0.10 0.58 ± 0.38	0.79 ± 0.43 0.63± 0.17 0.59 ± 0.17	0.140 0.780 0.923	0.113 0.713	0.084	0.630	0.191
Density (g/mL)	15g (A1) 30g (A2) control (C)	1023.96 ± 1.65 1023.24 ± 0.85 1022.90 ± 1.60	1023.37 ± 1.48 1024.75 ± 1.95 1023.66 ± 1.25	0.416 0.036* 0.308	0.691 0.138	0.518	0.011*	0.054
Natural acidity (^{0}T)	15g (A1) 30g (A2) control (C)	16.89 ± 1.62 16.78 ± 0.67 17.44 ± 1.42	14.44 ± 2.01 15.80 ± 1.32 13.75 ± 1.83	0.001* 0.170 <0.001*	0.354 0.007*	<0.001*	0.023*	0.059
Citric acid (%)	15g (A1) 30g (A2) control (C)	0.10 ± 0.03 0.09 ± 0.02 0.09 ± 0.03	0.09 ± 0.01 0.09 ± 0.03 0.09 ± 0.03	0.849 0.953 0.863	0.863 0.815	1.0	0.953	0.953

* – statistically significant differences at P-value ≤ 0.05; A1 and A2 – dairy goat groups given feed supplemented with chokeberry pomace; C – control dairy goat group

The results of aminopeptidase activity assessment are presented in Table 2. Significant differences (P-value < 0.001) were found in all tested aminopeptidase activity levels between the beginning and the end of chokeberry supplementation both within the supplemented groups and within the control group. A significant increase (P-value = 0.014) in LeuAP activity between the control group at the beginning of the experiment and A1 group at the end of supplementation was observed. There was also significant difference (P-value = 0.011) between the A1 and the A2 group at the end of the experiment for LeuAP activity, while for AlaAP and ArgAP there were no significant differences between the experimental groups after supplementation.

The results of lysosomal enzyme activity assessment are presented in Table 3. Chokeberry supplementation resulted in a significant increase in AcP activity in the A1 and A2 groups. The activity of this enzyme at the end

of the experiment in the A2 group was significantly higher (P-value < 0.001) than in the control group. Acid phosphatase activity in the A1 group at the end of supplementation was significantly lower (P-value = 0.001) than its activity in the A2 group.

There was a significant (P-value < 0.001) increase in the activity of all tested glycoside enzymes in both supplemented groups between the beginning and the end of the experiment. Beta glucuronidase activity in the A1 and the A2 group increased significantly (P-value < 0.001) between the beginning and the end of supplementation. It should be noted that the increase in activity was much higher in the A2 group at the beginning of the experiment, and that it then increased further. Beta glucuronidase activity was also significantly higher in the A1 and A2 groups (P-value < 0.001) at the end of the experiment than it was in the control group before the start of supplementation. The differences in β-GRD activity in the final phase of the experiment between the A1 and the A2 group were also significant (P-value = 0.050).

A significant potentiation of β-GAL activity (P-value < 0.001) was evident in both groups supplemented with chokeberry. This was seen between the beginning and the end of supplementation.

Table 2. The activity of aminopeptidases in milk in the control and experimental groups at the start and finish of chokeberry supplementation to dairy goats

Enzymes	Amount of additive	Mean ± standard deviation		Statistical significance (P-value)				
		Start	Finish	Within group A1, A2 and C	Between C and A1 and C and A2	Between C and A1	Between C and A2	Between A1 and A2
				Start to finish	Finish	Start to finish		Finish
Alanyl-aminopeptidase (nmol/mg protein/h)	15g (A1)	2.96 ±0 .37	3.65 ± 0.40	<0.001*	0.248	<0.001*	<0.001*	0.302
	30g (A2)	2.91 ± 0.13	3.80 ± 0.40	<0.001*	0.853			
	control (C)	2.85 ± 0.24	3.83 ± 0.23	<0.001*				
Leucyl-aminopeptidase (nmol/mg protein/h)	15g (A1)	2.98 ± 0.35	3.53 ± 0.25	<0.001*	0.014*	<0.001*	<0.001*	0.011*
	30g (A2)	2.90 ± 0.12	3.87 ± 0.36	<0.001*	0.965			
	control (C)	2.87 ± 0.23	3.88 ± 0.31	<0.001*				
Arginyl-aminopeptidase (nmol/mg protein/h)	15g (A1)	3.01 ± 0.37	3.59 ± 0.42	0.001*	0.676	<0.001*	<0.001*	0.642
	30g (A2)	2.94 ± 0.15	3.66 ± 0.41	<0.001*	0.982			
	control (C)	2.88 ± 0.24	3.66 ± 0.29	<0.001*				

* – statistically significant differences at P-value ≤ 0.05; A1 and A2 – dairy goat groups given feed supplemented with chokeberry pomace; C – control dairy goat group

Table 3. The activity of lysosomal enzymes in milk in the control and experimental groups at the start and finish of chokeberry supplementation to dairy goats

Enzymes	Amount of additive	Mean ± standard deviation		Statistical significance (P-value)				
		Start	Finish	Within group A1, A2 and C	Between C and A1 and C and A2	Between C and A1	Between C and A2	Between A1 and A2
				Start to finish	Finish	Start to finish		Finish
Acid phosphatase (nmol/mg protein/h)	15g (A1)	17.78 ± 2 .87	20.29 ± 1.83	0.005*	0.069	0.001*	<0.001*	0.001*
	30g (A2)	17.28 ± 0.82	23.28 ± 2.01	<0.001*	<0.001*			
	control (C)	17.33 ± 1.36	18.64 ± 1.23	0.146				
β-glucuronidase (nmol/mg protein/h)	15g (A1)	0.99 ± 0.14	1.26 ± 0.10	<0.001*	<0.001*	<0.001*	<0.001*	0.051
	30g (A2)	0.97 ± 0.05	1.35 ± 0.12	<0.001*	<0.001*			
	control (C)	0.96 ± 0.08	1.00 ± 0.06	0.413				
β-galactosidase (nmol/mg protein/h)	15g (A1)	4.10 ± 0.29	5.08 ± 0.32	<0.001*	<0.001*	<0.001*	<0.001*	0.014*
	30g (A2)	4.01 ± 0.24	5.50 ± 0.56	<0.001*	<0.001*			
	control (C)	3.95 ± 0.41	3.66 ± 0.10	0.092				
β-glucosidase (nmol/mg protein/h)	15g (A1)	3.99 ± 1.15	5.10 ± 0.36	<0.001*	0.038*	0.003*	<0.001*	0.050*
	30g (A2)	4.18 ± 0.17	5.67 ± 0.51	<0.001*	<0.001*			
	control (C)	4.17 ± 0.55	4.45 ± 0.50	0.349				
α-glucosidase (nmol/mg protein/h)	15g (A1)	4.27 ± 0.26	5.14 ± 0.33	<0.001*	<0.001*	<0.001*	<0.001*	0.230
	30g (A2)	4.41 ± 0.22	5.37 ± 0.52	<0.001*	<0.001*			
	control (C)	4.28 ± 0.46	4.39 ± 0.50	0.566				
N-acetylhexosaminidase (nmol/mg protein/h)	15g (A1)	9.00 ± 1.12	10.18 ± 0.81	0.004*	0.021*	0.001*	<0.001*	<0.001*
	30g (A2)	9.07± 0.63	11.64 ± 0.95	<0.001*	<0.001*			
	control (C)	8.77 ± 0.68	9.22 ± 0.60	0.266				
Mannosidase (nmol/mg protein/h)	15g (A1)	2.23 ± 0.31	2.53 ± 0.23	0.008*	0.421	0.020*	<0.001*	0.186
	30g (A2)	2.19 ± 0.07	2.67 ± 0.21	<0.001*	0.038*			
	control (C)	2.27 ± 0.24	2.44 ± 0.22	0.133				

* – statistically significant differences at P-value ≤ 0.05; A1 and A2 – dairy goat groups given feed supplemented with chokeberry pomace; C – control dairy goat group

Table 4. The activity of antioxidant non-enzymatic compounds in milk in control and experimental groups at the start and finish of chokeberry supplementation to dairy goats

Non-enzymatic compounds	Amount of additive	Mean ± standard deviation		Statistical significance (P-value)				
		Start	Finish	Within group A1, A2 and C	Between C and A1 and C and A2	Between C and A1	Between C and A2	Between A1 and A2
				Start to finish	Finish	Start to finish		Finish
(2,2-diphenyl-1-picrylhydrazyl) (% of remaining DPPH)	15g (A1)	80.03 ± 3.60	85.77 ± 2.68	<0.001*	<0.001*	<0.001*	<0.001*	0.097
	30g (A2)	80.95 ± 1.54	87.47 ± 1.32	<0.001*	<0.001*			
	control (C)	80.80 ± 1.49	80.37 ± 1.52	0.684				
Vitamin C (mg/100mL)	15g (A1)	1.88 ± 0.22	2.10 ± 0.34	0.059*	0.978	0.414	0.337	0.901
	30g (A2)	1.91 ± 0.24	2.11 ± 0.27	0.107	0.882			
	control (C)	1.99 ± 0.25	2.09 ± 0.28	0.444				
Reduced glutation (μM)	15g (A1)	24.46 ± 4.09	55.56 ± 10.60	<0.001*	<0.001*	<0.001*	<0.001*	0.029*
	30g (A2)	22.84 ± 2.98	66.50 ± 20.59	<0.001*	<0.001*			
	control (C)	21.76 ± 2.33	29.64 ± 7.77	0.132				
Polyphenols (mg/GAE/mL)	15g (A1)	3.78 ± 0.32	6.97 ± 1.14	<0.001*	<0.001*	<0.001*	<0.001*	0.001*
	30g (A2)	3.51 ± 0.38	8.11 ± 1.01	<0.001*	<0.001*			
	control (C)	3.68 ± 0.44	3.81 ± 0.51	0.712				

* – statistically significant differences at P-value ≤ 0.05; A1 and A2 – dairy goat groups given feed supplemented with chokeberry pomace; C – dairy goat group; DPPH – 1,1-diphenyl-2-picrylhydrazyl; GAE – gallic acid equivalent

There was a significant augmentation of β-GLU activity (P-value < 0.001) in both groups supplemented with chokeberry at the end of the experiment. The greatest significant difference between the initial activity level and the final activity level was in the group given 30 g chokeberry. Significantly higher β-GLU activity was demonstrated after supplementation and compared to the control group. A significant difference (P-value = 0.050) between the A1 and the A2 groups was noted at the end of supplementation.

After supplementation with chokeberry, both the A1 and A2 groups showed significant differences in HEX (P-value < 0.001), but the highest enzyme activity was observed in the A2 group. Significant difference between the three levels of chokeberry supplementation (P-value < 0.001) were also found.

As it did for the other glycoside enzymes, chokeberry supplementation resulted in a significant increase in α-GLU activity (P-value < 0.001). The highest increase in enzyme activity occurred in the A2 group after the end of supplementation.

Mannosidase activity increased significantly in both experimental groups after the addition of chokeberry to their feed. Interestingly, there were no significant differences in MAN activity in milk between the 15-g- and 30-g-supplemented groups.

Table 4 shows the results for non-enzymatic parameters. An increase in DPPH depending on the amount of chokeberry added in the feed, the highest level of DPPH (P-value < 0.001) was observed in the A2 group. However, no significant differences were found between the supplemented groups.

The addition of chokeberry did not significantly affect the level of Vit C in milk. Meanwhile, it had a positive effect on the oxidation-reduction potential of milk, which was manifested by an increase of GSH in the experimental groups. It was also shown that the level of GSH depends on the lactation period, which was manifested by an increased trend in GSH activity also in the control group.

It was shown that a higher level of chokeberry addition in the feed resulted in a significant increase in the total level of polyphenols in milk compared to the control group (P < 0.001). A significant relationship with the chokeberry dose was also found (P < 0.001).

Figure 1 correlates the milk physicochemical parameters with the activity of lysosomal enzymes and other tested non-enzymatic antioxidant parameters. There was a significant negative correlation between the fat level and the activity of other parameters except DDPH (r = −0.223). The acidity parameter correlated negatively with the activity of some lysosomal enzymes: β-GRD, MAN and GSH (Fig. 1). Natural acidity was also strong negatively correlated with AlaAP, LeuAP and ArgAP. A low positive correlation was also found between the level of FFA and DPPH activity, and between milk density and β-GLU and HEX activity.

The total level of Pol in all experimental groups and also in the control group was slightly positively correlated with D (r = 0.283) and slightly negatively correlated with the level of fat in milk (r = −0.394). The remaining physicochemical parameters of milk did not show any correlation with the level of Pol.

The activity of all lysosomal enzymes was significantly and positively correlated with the total level of Pol in milk (Fig. 1.). The strongest correlation occurred for β-GRD activity (r = 0.838) and β-GAL (r = 0.831). A high correlation (r = 0.817) was also noted for GSH activity. For the remaining enzymes, the correlation ranged from 0.524 to 0.779.

Physicochemical parameters	Enzymes									Non-enzymatic antioxidant parameters				
	AlaAP	LeuAP	ArgAP	ACP	β-GRD	β-GAL	β-GLU	HEX	α-GLU	MAN	DPPH	Vit C	GSH	Pol
Log SCC	0.013	0.038	-0.048	-0.087	-0.139	-0.190	-0.134	-0.166	-0.114	0.069	-0.040	-0.237	-0.055	-0.109
Fat	-0.480	-0.477	-0.454	-0.480	-0.393	-0.301	-0.446	-0.427	-0.449	-0.467	-0.223	-0.337	-0.321	-0.394
Prot	0.231	0.252	0.202	0.104	0.204	0.111	0.151	0.209	0.027	0.082	0.132	0.245	0.125	0.228
Cas	0.032	0.048	-0.027	0.006	0.001	-0.040	-0.021	0.080	-0.122	-0.065	-0.059	0.166	-0.085	0.040
Lac	0.025	-0.039	0.002	-0.063	0.007	-0.102	0.046	0.012	-0.144	0.092	-0.067	-0.036	-0.134	-0.070
TS	-0.146	-0.150	-0.164	-0.262	-0.128	-0.161	-0.206	-0.168	-0.290	-0.226	-0.059	-0.122	-0.138	-0.129
SNF	0.063	0.041	0.023	-0.062	0.057	-0.034	0.000	0.058	-0.146	0.000	-0.007	0.093	-0.073	0.032
Urea	-0.111	-0.153	-0.106	-0.175	-0.050	0.004	-0.053	-0.096	-0.007	-0.073	-0.062	-0.199	-0.034	-0.155
Cit aci	-0.011	-0.049	-0.111	-0.049	0.016	0.061	-0.248	-0.059	0.048	-0.041	0.008	-0.114	0.008	-0.032
FP	-0.075	-0.046	-0.159	0.082	-0.112	-0.136	-0.083	0.008	-0.111	-0.109	0.038	-0.098	-0.098	-0.079
FFA	0.095	0.058	0.113	0.037	0.208	0.212	0.133	0.003	0.150	0.178	0.303	-0.122	0.257	0.135
D	0.235	0.240	0.233	0.232	0.259	0.151	0.272	0.313	0.054	0.225	0.162	0.154	0.132	0.283
NA	-0.614	-0.565	-0.538	-0.105	-0.350	-0.053	-0.207	-0.151	-0.210	-0.328	-0.153	-0.256	-0.297	-0.218
Pol	0.549	0.544	0.524	0.770	0.838	0.831	0.661	0.721	0.727	0.650	0.779	0.233	0.817	

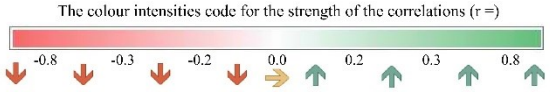

Fig. 1. Milk parameter correlation coefficients with the activity of lysosomal enzymes and other measured non-enzymatic antioxidant parameters AlaAP – aminopeptidase; LeuAP – leucyl aminopeptidase; ArgAP – arginyl aminopeptidase; AcP – acid phosphatase; β-GRD – β-glucuronidase; β-GAL – β-galactosidase; β-GLU – β-glucosidase; HEX – β-N-acetylhexosaminidase; α-GLU – α- galactosidase; MAN – mannosidase; DPPH – 1,1-diphenyl-2-picrylhydrazyl; Vit C – vitamin C; GSH – glutathione; Pol –polyphenol; Log SCC – logarithmic somatic cell count; Prot – protein; Cas – casein; Lac – lactose; TS – total solids; SNF – solids-non-fat; Cit acid – citric acid; FP – freezing-point; FFA – free fatty acid; D – density (of milk); NA – natural acidity

Discussion

The healthy food market is developing very dynamically. Consumers increasingly frequently look for food with high health benefits. Milk plays an important role in the human diet, and goat's milk is an alternative for people allergic to cow's milk protein. Using fruit pomace in animal feed conforms to the modern trend in sustainable agriculture and simultaneously may produce milk with beneficial properties desired by consumers.

The supplementation of the diet of high-production dairy goats with chokeberry pomace exhibits promising effects on oxidative stress parameters and the overall quality of milk, as demonstrated by our findings. This study contributes to the growing body of research evidence that underscores the potential health benefits of dietary polyphenol supplementation in livestock nutrition, particularly in the context of improving milk quality and animal welfare.

While chokeberry and chokeberry-derived by-products (such as pomace) are broadly studied as health-promoting nutraceuticals (11), the putative effects of chokeberry supplementation on the milk quality of livestock animals remained unexplored up to now. Chokeberry is known to be rich in polyphenolic compounds (11), and the supplementation with chokeberry pomace significantly enhanced the antioxidative capacity parameters (DPPH, GSH and total Pol level) of goat's milk (Table 4). The better antioxidative capacity of milk resulted in the stabilisation of log SCC, which remained materially unchanged through the chokeberry supplementation, while rising in the control group as is typical in the course of lactation (28). Therefore chokeberry pomace also improved the cytological quality of goat milk. These results align with the growing body of findings from previous studies which have highlighted the role of dietary polyphenols in boosting antioxidant defence mechanisms in dairy animals (9, 24).

Relatedly, our study noted significant improvements in the levels of non-enzymatic antioxidants such as GSH and Pol. The elevation in GSH level is particularly noteworthy, since GSH is a key antioxidant that plays a major role in neutralising free radicals and protecting mammalian cells from oxidative stress (2, 20). This finding is consistent with previous works that reported that dietary antioxidant supplementation could modulate reduced GSH levels in ruminants, leading to better stress resilience and health- and productivity-related outcomes (6, 18, 22). Moreover, our work also aligns particularly well with previous research involving dietary chokeberry pomace supplementation, which demonstrated an increase in reduced GSH blood levels in Polish Merino and Wrzosówka lambs (13).

In this investigation the synthesis of basic ingredients such as protein and lactose was at a constant level, while fat anabolism was less efficient. The fat content in milk decreased in chokeberry-supplemented goats and the larger chokeberry mass supplement had a significant impact on reducing the fat content. Interestingly, this research showed a negative correlation between the activity of the tested enzymes and the percentage of fat content in goat's milk. It can be assumed that the reduction of fat content brought about by chokeberry supplementation may be caused by the increased activity of lysosomal enzymes originating from mammary gland cells and bacteria present in the milk. In respect to milk fat content, the observed reduction associated with chokeberry supplementation (Table 1) raises important considerations regarding the nutritional and sensory qualities of milk. A reduced fat content can influence these qualities and the caloric value of milk, which are critical factors for consumer acceptance. However, from a health perspective, lower-fat milk may be preferable in diets aiming at an overall health improvement, weight loss and reduced fat intake (17, 30).

Interestingly, despite the increased activity of aminopeptidases, the experimental animals maintained

their metabolic balance, and the protein level in their milk was not raised by the chokeberry pomace supplementation. Higher aminopeptidase activity is typical during lactation, and the similar increased in activity of aminopeptidases in the control group may have been due to the increase in log SCC. On the other hand, the significant correlation between the content of polyphenols and the activity of aminopeptidases may indicate that chokeberry accelerates protein degradation processes, but may also be the cause of increased protein synthesis, as no changes in milk protein content were seen. A further interesting observation in the present work is the increased activities of aminopeptidases, AcP and carbohydrate-metabolising enzymes (Tables 2 and 3) in the milk samples of chokeberry-supplemented goats. Importantly, some of the boosted enzymes, particularly β-GRD, are crucial not just for metabolic processes but also for cellular detoxification (27). Of particular importance could also be the chokeberry pomace-induced increase in the enzymatic activity of β-GAL and α-GAL, which are involved in the degradation of the lactose that is frequently not tolerated by milk and dairy-product consumers (14, 25). On the other hand, reduction of lactose content did not occur. Therefore, potentiated activity of glycoside enzymes not only improves the physicochemical parameters of milk, but also its quality and nutritional value.

In respect of the conducted correlation study (Fig. 1), it was notable that overall, increased enzyme activities in the milk correlated positively with polyphenol content and negatively with fat content, which established potential causal links between these parameters, but also suggested a coordinated anabolism–catabolism programme shift in the studied population.

From a clinical perspective, the enhanced antioxidative properties of milk could be indicative of the improved health status of the dairy goats, with potential implications for the reduction of the incidence of diseases linked to oxidative stress, such as mastitis (8). A dietary supplementation approach using chokeberry pomace could possibly lower a farm's veterinary costs and reduce its use of antibiotics, aligning well with the goals of sustainable and ethical animal farming practices.

With respect to farming sustainability and environmental protection, the use of chokeberry pomace, a by-product of chokeberry juice production, as a feed additive is also contributory because it utilises agricultural waste. Such a dietary supplementation practice thus might not only provide health benefits to livestock animals but also build out the circular economy in agriculture, reducing waste and the environmental footprint of goat farming.

While the current study provides valuable insights into the benefits of chokeberry pomace in dairy goat nutrition, future research should explore the longer-term effects of such dietary modifications on animal health and milk production. It would also be beneficial to assess consumer acceptance of milk with altered fat content and antioxidative properties such as chokeberry-supplemented goats produce, as these factors play key roles in the commercial success of dairy products.

Conclusion

The supplementation of high-producing dairy goat diets with chokeberry pomace enhances the antioxidative properties of milk and improves various quality parameters (including reduction of fat content and enhancement of metabolic enzyme activities). These changes reflect the potential of chokeberry pomace as a natural antioxidant in improving animal health and milk quality, offering a promising strategy for sustainable dairy goat farming. The findings also suggest that chokeberry pomace could be a valuable feed additive in the context of precision livestock farming, where diet formulations induce specific metabolic patterns that could be optimised for health and productivity outcomes.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was funded by the Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Jastrzębiec, Poland within the "Leading National Research Centre" award made to project No. KNOW/IGHZ/RPB/WEW/2016/05.

Animal Rights Statement: None required.

References

1. American Oil Chemists' Society: Official methods and recommended practices of the American oil chemists' society, Fourth Edition, American Oil Chemists' Society, Urbana, IL, USA 1990.
2. Aquilano K., Baldelli S., Ciriolo M.R.: Glutathione: new roles in redox signaling for an old antioxidant. Front Pharmacol 2014, 5, 196, doi: 10.3389/fphar.2014.00196.
3. Barrett A.J., Heath M.F.: Lysosomal enzymes. In: *Lysosomes, A Laboratory Handbook*, edited by J.T. Dingle,North-Holland Publishing Company, New York, NY, USA, 1977, pp. 19–146.
4. Benvenuti S., Pellati F., Melegari M., Bertelli D.: Polyphenols, Anthocyanins, Ascorbic Acid, and Radical Scavenging Activity of Rubus, Ribes, and Aronia. J Food Sci 2004, 69, FCT164–FCT169, doi: 10.1111/j.1365-2621.2004.tb13352.x.
5. Brand-Williams W., Cuvelier M.E., Berset C.: Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol 1995, 28, 25–30, doi: 10.1016/S0023-6438(95)80008-5.
6. Brzezinska-Slebodzinska E., Miller J.K., Quigley J.D., Moore J.R., Madsen F.C.: Antioxidant Status of Dairy Cows Supplemented Prepartum with Vitamin E and Selenium. J Dairy Sci 1994, 77, 3087–3095, doi: 10.3168/jds.S0022-0302(94)77251-9.
7. El-Agamy E.I.: The challenge of cow milk protein allergy. Small Rumin Res 2007, 68, 64–72, doi: 10.1016/j.smallrumres.2006.09.016.
8. Ghasemian K.O., Safi S., Rahimi F.A., Bolourchi M.: Study of the relationship between oxidative stress and subclinical mastitis in dairy cattle. Iran J Vet Res 2011, 12, 350–353, doi: 10.22099/ijvr.2011.89.
9. Gobert M., Martin B., Ferlay A., Chilliard Y., Graulet B., Pradel P., Bauchart D., Durand D.: Plant polyphenols associated

with vitamin E can reduce plasma lipoperoxidation in dairy cows given n-3 polyunsaturated fatty acids. J Dairy Sci 2009, 92, 6095–6104, doi: 10.3168/jds.2009-2087.
10. Jóźwik A., Strzałkowska N., Bagnicka E., Grzybek W., Krzyżewski J., Poławska E., Kołataj A., Horbańczuk J.O.: Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows. Czech J Anim Sci 2012, 57, 353–360.
11. Jurendic T., Scetar M.: *Aronia melanocarpa* Products and By-Products for Health and Nutrition: A Review. Antioxidants 2021, 10, 1052, doi: 10.3390/antiox10071052.
12. Krawczyński J., Osiński T.: *Laboratoryjne metody diagnostyczne* (in Polish). Państwowy Zakład Wydawnictwa Lekarskich, Warsaw, 1967.
13. Lipinska P., Atanasov A.G., Palka M., Jozwik A.: Chokeberry Pomace as a Determinant of Antioxidant Parameters Assayed in Blood and Liver Tissue of Polish Merino and Wrzosówka Lambs. Molecules 2017, 22, 1461, doi: 10.3390/molecules22111461.
14. Mahoney R.R.: Modification of Lactose and Lactose-Containing Dairy Products with β-Galactosidase. In: *Developments in Dairy Chemistry*, Volume 3, Lactose and Minor Constituents, Springer, Dordrecht, the Netherlands, 1985. pp. 69–109, doi: 10.1007/978-94-009-4950-8_3.
15. McDonald J.K., Barrett A.J.: *Mammalian Proteases: A Glossary and Bibliography,* Volume 2, Exopeptidases, Academic Press, Orlando, FL, USA, 1986, doi: 10.1007/978-94-009-4950-8_3.
16. Nayik G.A., Jagdale Y.D., Gaikwad S.A., Devkatte A.N., Dar A.H., Dezmirean D.S., Bobis O., Ranjha M., Ansari M.J., Hemeg H.A., Alotaibi S.S.: Recent Insights Into Processing Approaches and Potential Health Benefits of Goat Milk and Its Products: A Review. Front Nutr 2021, 8, 789117, doi: 10.3389/fnut.2021.789117.
17. Nolan-Clark D., Mathers E., Probst Y., Charlton K., Batterham M., Tapsell L.C.: Dietary consequences of recommending reduced-fat dairy products in the weight-loss context: a secondary analysis with practical implications for registered dietitians. J Acad Nutr Diet 2013, 113, 452–458, doi: 10.1016/j.jand.2012.10.017.
18. Oconitrillo M., Wickramasinghe J., Omale S., Beitz D., Appuhamy R.: Effects of Elevating Zinc Supplementation on the Health and Production Parameters of High-Producing Dairy Cows. Animals 2024, 14, 395, doi: 10.3390/ani14030395.
19. Omaye S.T., Turnbull J.D., Sauberlich H.E.: Chapter 1, Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. In: *Methods in Enzymology*, Volume 62, edited by D.B. McCormick, L.D. Wright, Elsevier, Amsterdam, the Netherlands, 1979.
20. Pietta P.G.: Flavonoids as Antioxidants. J Nat Prod 2000, 63, 1035–1042, doi: 10.1021/np9904509.
21. Podgorska E., Sniegocka M., Mycinska M., Trybus W., Trybus E., Kopacz-Bednarska A., Wiechec O., Krzykawska-Serda M., Elas M., Krol T., Urbanska K., Slominski A.: Acute hepatologic and nephrologic effects of calcitriol in Syrian golden hamster (*Mesocricetus auratus*). Acta Biochim Pol 2018, 65, 351–358, doi: 10.18388/abp.2018_2626.
22. Pogge D.J., Hansen S.L.: Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. J Anim Sci 2013, 91, 4303–4314, doi: 10.2527/jas.2012-5638.
23. Safa S., Kargar S., Moghaddam G.A., Ciliberti M.G., Caroprese M.: Heat stress abatement during the postpartum period: effects on whole lactation milk yield, indicators of metabolic status, inflammatory cytokines, and biomarkers of the oxidative stress. J Anim Sci 2019, 97, 122–132, doi: 10.1093/jas/sky408.
24. Serra V., Salvatori G., Pastorelli G.: Dietary Polyphenol Supplementation in Food Producing Animals: Effects on the Quality of Derived Products. Animals 2021, 11, 401, doi: 10.3390/ani11020401.
25. Singh P., Arora S., Rao P.S., Kathuria D., Sharma V., Singh A.K.: Effect of process parameters on the β-galactosidase hydrolysis of lactose and galactooligosaccharide formation in concentrated skim milk. Food Chem 2022, 393, 133355, doi: 10.1016/j.foodchem.2022.133355.
26. Škerget M., Kotnik P., Hadolin M., Hraš A.R., Simonič M., Knez Ž.: Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 2005, 89, 191–198, doi: 10.1016/j.foodchem.2004.02.025.
27. Sperker B., Backman J.T., Kroemer H.K.: The role of beta-glucuronidase in drug disposition and drug targeting in humans. Clin Pharmacokinet 1997, 33, 18–31, doi: 10.2165/00003088-199733010-00003.
28. Strzałkowska N., Jóźwik A., Bagnicka E., Krzyżewski J., Horbańczuk K., Pyzel B., Horbańczuk J.O.: Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation. Anim Sci Pap Rep 2009, 4, 311–320.
29. Tiwari G., Chauhan A., Sharma P., Tiwari R.: Nutritional Values and Therapeutic Uses of *Capra hircus* Milk. Int J Pharm Investig 2022, 12, 408–417, doi: 10.5530/ijpi.2022.4.71.
30. Wang S., Liu Y., Cai H., Li Y., Zhang X., Liu J., Sun R., Fang S., Yu B.: Decreased risk of all-cause and heart-specific mortality is associated with low-fat or skimmed milk consumption compared with whole milk intake: A cohort study. Clin Nutr 2021, 40, 5568–5575, doi: 10.1016/j.clnu.2021.09.012.