Relationship between the degree of antioxidant protection and the level of malondialdehyde in high-performance Polish Holstein-Friesian cows in peak of lactation

Aleksandra Kapusta⦿, Beata Kuczyńska⦿, Kamila Puppel*⦿

Cattle Breeding Division, Animal Breeding & Production Department, Warsaw University of Life Sciences, Warsaw, Poland

⦿ These authors contributed equally to this work.
* kamila_puppel@sggw.pl

Abstract

Lipid peroxidation can be described as a process under which free radicals attack carbon double bonds of omega-3 and omega-6 fatty acids. Whereas the end products of this process are reactive aldehydes, such as malondialdehyde (MDA). Lipid peroxidation leads to adverse changes in the nutritional value of milk; therefore, higher degree of antioxidant protection (DAP) ensures higher stability of dairy products by effecting their high antioxidative potential. Therefore, the purpose of this study was to demonstrate the relationship between the DAP and the level of MDA in high-performance Polish Holstein-Friesian cows in peak of lactation. Sixty-three Polish Holstein-Friesian cows were selected to the experiment according to: parity (all in the 2nd lactation), phase of lactation (peak of lactation), cytological quality of milk (somatic cell count < 150 thousand/ml) and without diagnosed metabolic diseases. The data obtained were analyzed statistically by two–way ANOVA, and Tukey's post-hoc test. After analysis of performance the cows were divided into 3 groups (twenty one cows in each group) based on milk yield and MDA concentration. The study revealed a significant effect of the lactation performance of cows on MDA levels in milk ($P \leq 0.01$). The highest concentration of MDA (61.137 nM/mL) was shown in milk of cows yielding between 50.00 and 55.80 kg/day. The highest concentration of fat was found in milk in which the MDA level ranged from 48 to 86 nM/mL. Whereas, the inverse relationship was demonstrated in case of protein concentration. The highest level of protein was found in cows with MDA levels in the range of 18–28 nM/mL ($P \leq 0.01$). The lowest MDA level (in the range of 18–28 nM/mL) was associated with the highest concentration of vitamin E, β-carotene, total antioxidant status (TAS) and DAP, measured in both milk and plasma. The obtained results show that lipid peroxidation leads to adverse changes in the nutritional value of milk; the highest DAP (7.89 x 10^{-3}) was found in the cows with the lowest MDA concentration in milk.

Introduction

Lipid peroxidation is a biological lipid oxidation, chain and free radical process, that results in the formation of peroxides of omega-3 and omega-6 fatty acids. This reaction consists of three stages: initiation, propagation, and termination; typical steps of free radical reactions and reinitiation. Products of lipid peroxidation include reactive aldehydes such as malondialdehyde and 4-hydroxynonenal [1]. Malondialdehyde is produced during the peroxidation of polyunsaturated fatty acids by the action of reactive oxygen species, as a result of the depletion of antioxidant systems. There are two forms of MDA: endogenous—from lipid peroxidation; and exogenous–delivered with food [2,3]. MDA modifies the physical structures of cell membranes and is indirectly involved in the synthesis of protein, DNA, and RNA. In addition, it has mutagenic and carcinogenic properties. MDA has been frequently used for many years as a biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid [4,5]. Ayala et al. [5] reported, that the thiobarbituric acid (TBA) test is predicated upon the reactivity of TBA toward MDA to yield an red adduct; this test was first used to demonstrate autoxidative degradation of fats.

An antioxidant is a substance that, even in low concentrations compared to a substance that is susceptible to oxidation, significantly delays or prevents by minimizing oxidative damages in cells and biomolecules [1]. Antioxidants also reduce the risk of atherosclerosis, protect against ischemic heart disease, reduce the risk of cancer, slow down the course of Alzheimer's disease, and protect the body from environmental pollution [6]. Typical exogenous antioxidants supplied with food or in specially formulated supplements are: vitamins A, C, and E, and carotenoids (α- and β-carotene). Several forms of carotenoids may be found in cow's milk: all-trans β-carotene, α-carotene, δ-carotene, zeaxanthin, and β-cryptoxanthin; with all-trans β-carotene occurring in the highest concentration—750 g/kg [7,8]. β-Carotene (provitamin A) reduces the risk of breast, lung, stomach, and uterine cancer [9], protects against myocardial infarction and stroke [10]. Rice-Evans et al. [11] have shown that carotenoids exhibit antioxidant properties and suppress free radicals by transferring free electrons, or by forming complexes with them.

One of the biologically-active compounds commonly known as vitamin E, is α-tocopherol. Tocopherol family consists of four representatives: α, β, δ, and Δ; all of them occur in the "D" form. D-α-tocopherol (RRR-α-tocopherol) is the most common and most active form, but not only is it antioxidant, similar properties are attributed to the tocopherol gamma form, as the only tocopherol to protect against nitrogen oxides [1]. Vitamin E (tocopherol) prevents: oxidation of LDL cholesterol fraction, heart disease, and cancer [7,12,13,14,15], it strengthens the immune system, inhibits the development of Alzheimer's disease, helps control blood sugar, protects the eye lens from damage caused by free radicals, and retards the development of Parkinson's disease [9]. Vitamin E is one of the most important antioxidants soluble in milk fat. It prevents lipid peroxidation by trapping a singlet oxygen, hydroxyl and superoxide radicals [7]. By entering the free radical reaction, a tocopherol is converted into a tocopherol radical; and ascorbic acid participates in its regeneration (by reducing it). Vitamin E and carotenoids protect fatty compounds against oxidation in the blood and within the cell membranes, and play a significant role in the communicative and defense functions of cells against free radicals [16,17].

Cholesterol (5-Cholesten-3β-ol) is an alcohol with the general formula $C_{27}H_{45}OH$ and a sterol in the 17-carbon cyclopentane phenanthrene ring, linked to the C17 hydrocarbon chain with C10 and C13 hydroxyl groups, C3 hydroxyl group and one double bond between C5 and C6 atoms. Milk and dairy products are often regarded as examples of products with high levels of cholesterol having adverse effect on human health [18,19]. Tomaszewski [20] has shown

that cholesterol levels in milk clearly increase with increased yield. In contact with air, cholesterol is autoxidized, and the rate of the formation of cholesterol oxidation products depends mainly on storage conditions. Cholesterol oxidation products (PUCHs) are highly toxic and highly reactive substances which initiate the free radical processes that may result in atherogenic and cancerous lesions. The most bioactive of these are: b-sitosterol (5-cholesten-24bethyl-3b-ol), campesterol (5-cholesten-24b-methyl-3b-ol), and stigmasterol (5,22-cholestadien-24-ethyl-3b-ol) [19,21]. Whole milk contains 20–30 mg PUCh/kg [22]. They are produced by non-enzymatic reactions such as ROS action, lipid peroxidation, and enzyme activity. Oxidative processes have a detrimental effect on milk and dairy products, by shortening their shelf-life and deteriorating their nutritional quality [23,24]. Havemose et al. [25] found that higher concentrations of antioxidants contributed to delaying or eliminating protein oxidation processes. Proper nutrition and supplementation provide antioxidant protection. In dairy products, the degree of antioxidant protection is calculated as the molar ratio between antioxidants (α-tocopherol and β-carotene) and the level of oxidants (cholesterol). Lipid peroxidation leads to adverse changes in the nutritional value of milk and its products; therefore, higher DAP ensures higher stability of dairy products by effecting their high antioxidative potential.

The main objective of the experiment described in this manuscript was to demonstrate the relationship between the degree of antioxidant protection and the level of malondialdehyde in high-performance PHF cows at the peak of lactation.

Material and methods

The experiment was conducted at the experimental dairy farm of the Warsaw University of Life Sciences (WULS). All cows were handled in accordance with the regulations of the Polish Council on Animal Care, and the Warsaw University of Life Sciences Care Committee reviewed and approved the experiment and all procedures carried out in the study. At the time of sample collection the cows were under veterinary control and did not manifest any disease symptoms.

Animals, treatment and sampling

The experiment was carried out at the experimental dairy farm of the WULS. The cows were kept in a free-stall dairy shed and fed a total mixed ration (TMR) diet (Table 1).

Sixty-three Polish Holstein-Friesian cows were selected to the experiment according to: parity (all in the 2nd lactation), phase of lactation (peak of lactation; at 70±18 days in milk), cytological quality of milk (somatic cell count < 150 thousand/ml) and without diagnosed metabolic diseases. The cows were milked daily at 05.30 and 17.30 h and milk yield was recorded at each milking. Combined milk from morning and evening milking were placed in sterile bottles, immediately chilled (up to 5˚C), preserved with Mlekostat CC (Zekar Sp. z o.o., Brwinów, Poland) and submitted to the WULS Milk Testing Laboratory for chemical analysis.

Blood samples (10 mL) were taken from each cow (the day following the sampling of milk) by jugular venipuncture (by a veterinary doctor) into a heparinized tube, separated by centrifugation at room temperature (1,800×g, 15 min) andimmediately chilled (up to 5˚C) Next, they were transported to the to the Cattle Breeding Division (Milk Testing Laboratory of WULS) for analysis. It should be noted that the analyzes were carried out on the same day, and samples were collected from all animals on the same day.

Chemical analyses

Milk gross composition: fat, total protein and casein were determined by infrared spectrophotometry using Milko-Scan FT-120 analyzer (Foss Electric, Hillerod, Denmark).

Table 1. Ingredient and chemical composition of the total mixed ration (TMR).

	TMR diet
Ingredient [kg d^{-1}]	
Maize silage	24.0
Alfalfa silage	10.30
Corn silage	5.0
Soybean meal	1.80
Pasture ground chalk	0.10
VIT-RA BML- vitamin mix [1]	0.16
Salt	0.05
Rapeseed meal	2.50
Magnesium oxide	0.05
Chemical composition [g kg^{-1} DM]	
Ash	63.0
Crude protein	95.0
Fat	45.5
Acid detergent fiber	230.0
Neutral detergent fiber	360.0
Ca	9
P	5
Total, kg of DM (offered)	21.2
Daily intake (kg)	20.8
UFL(unit of milk production.) balance (%)	+5.25%
PDIN (protein digested in the small intestine when rumen-fermentable nitrogen is limiting)	+ 3.43%
PDIE (protein digested in the small intestine when rumen-fermentable energy is limiting)	-2.78%

[1]VIT-RA BML (values per kg): 150 g Ca, 100 g P, 50 g Na, 40 g Mg, 9000mg Zn, 7000 mg Mn, 1000 mg Cu, 100mg J, 50mg Se, 1 200 000 IU vitamin A, 120 000 IU vitamin D_3, 5 000 mg vitamin E, 93 mg vitamin K, 80 mg vitamin B_1, 160 mg vitamin B_6, 110 mg vitamin B_2, 1 000 µg vitamin B_{12} (PPH VITRA, Kusowo, Poland).

https://doi.org/10.1371/journal.pone.0193512.t001

Extraction of fat was performed according to Röse-Gottlieb procedure [26] at a room temperature. The determination of α-tocopherol (vitamin E) and β-carotene (BK) were established using an Agilent 1100 Series reverse phase high–performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel et al. [27]

The determination of MDA level were established using Tecan's NanoQuant Infinite M200 PRO (Tecan Austria GmbH, Grödig, Austria)analyzer at wavelength532nm. To 250 µL milk (in 2.0 ml Eppendorf cup), 25 µL 0.2% *2,6-bis(1,1-dimetyoetylo)-4-metylofenol* (BHT; in absolute ethanol) and 1 ml 5% trichloroacetic acid (aqueous; TCA; Sigma-Aldrich, Warsaw, Poland) was added and vortexed. After centrifugation at 14 000g for 10 minutes, 750 µL of clear supernatant was transferred to glass tube. A 500 µL 0.6% Thiobarbituric acid (aqueous; Sigma-Aldrich, Warsaw, Poland) was then added, mixed and incubated for 45 minutes in water bath in 90˚C. MDA was reacted with TBA in the presence of BHT to minimize formation of artifacts. After cooled on ice and centrifugation at 4 000g for 5 minutes, 200µL of clear supernatant was transferred to the microplate. This method is based on the reaction of free MDA (present in the sample) with TBA to generate a MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically (OD = 532 nm). The commonly used TBA test for measuring MDA production has been criticized for its lack of specificity and low reproducibility [28]. Notwithstanding, measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage.

The determination of Cholesterol level were established using Agilent 7890A GC and BP-5 column (Agilent Technologies, Waldbronn, Germany). Fatty acid methylation was performed according to the trans estrification method EN ISO 5509 [29]. After centrifugation at 3000 g for 3 minutes, 50 μL *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA; Sigma-Aldrich, Warsaw, Poland), and 50 μL pirydyne (Sigma-Aldrich, Warsaw, Poland) were added, then mixed and incubated for 20 minutes in water bath in 75˚C. After cooling, 500 μL n-heksane (Sigma-Aldrich, Warsaw, Poland) was added and transferred to the vials. The separation was performed at pre programmed temperature: 250˚C for 4 min and 300˚C for 15 min. Helium at a flow rate of 25 cm/sand constant pressure was used as the carrier gas.

Total antioxidant status were established by RANDOX application using an NanoQuant Infinietie M200Pro analyzer (Tecan Austria GmbH, Grödig, Austria). ABTS® incubation with peroxidase (metmyoglobin) leads to the formation of the ABTS⁺ radical cation. This substance is blue-green and can be detected at a wavelength of 600 nm. The antioxidants present in the sample decrease the formation of the blue-green color, in proportion to their concentration.

HX-FeIII–metmyoglobin, X-[Fe^{IV} = 0]–ferrylmyoglobin, ABTS®- 2,2-Azino-di[3ethyl-benzthiazoline sulphonate] (RANDOX materials). U/L shoves concentration of TAS.

$$HX - Fe^{III} + H_2O_2 \rightarrow X - [Fe^{IV} = 0] + H_2O$$

$$ABTS^{®} + X - [Fe^{IV} = 0] \rightarrow ABTS^{®+} + HX - Fe^{III}$$
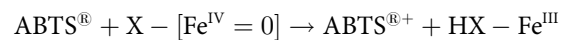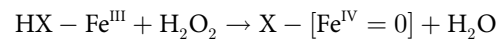
DAP was calculated from the molar ratio between antioxidants and oxidants according to Puppel et al. [21] from Pizzoferrato et al. [30]

$$DAP = \frac{\sum_{i=1}^{n} AC_i \ (n°moles)}{OT \ (n°moles)}$$

Where: AC_i is the antioxidant compound, OT is the oxidation target (cholesterol) and *i* is the number of components.

Statistical analysis

After preliminary analyses (analysis of cows performance and MDA concentration), the cows were divided into 3 groups (twenty one cows in each group) based on milk yield:

1. 36.80–39.90 kg/d
2. 40.00–49.20 kg/d
3. 50.00–55.80 kg/d

and MDA concentration in milk (twenty one cows in each group):

1. 18–28 nM/mL
2. 29–47 nM/mL
3. 48–86 nM/mL

The analyses were performed using SPSS 23.0 [31]. The data obtained were analyzed statistically by two–way ANOVA, and Tukey's post-hoc test. Significant differences were present among the means at a 95.0% confidence level. Data were presented as least squares means with standard error of the mean.

The statistical model was:

$$Yijkl = \mu + Ai + Bj + (Ai \times Bj) + eijk$$

Where: Y_{ijkl} is the dependent variable; μ is the overall mean; A_i is the milk yield effect (i = 1, 2, 3,); B_j is the MDA level effect (j = 1, 2, 3,); $(A_i \times B_j)$ is the interaction between milk yield and MDA level; e_{ijk} is the residual error.

Results and discussion

Continuous genetic selection has improved the milk yield per cow in the past few decades. The increase in production should be viewed with concern because: the increase in milk yield has been accompanied by declining fertility, increasing locomotive and metabolic problems and declining longevity [32]. Ingvartsen et al [33]showed an unfavourable genetic correlation between milk yield and incidence of ketosis (0.26–0.65), ovariancyst (0.23–0.42), mastitis (0.15–0.68) and lameness (0.24–0.48).

Malondialdehyde, the lipid peroxidation end product is one of the most reliable and often used indexes of oxidative stress. The study demonstrated the high impact of the milking performance of cows on MDA levels in milk. The highest concentration of MDA—61.137 nM/mL–was shown in milk of cows from the 3rd group; yielding between 50.00 and 55.80 kg/d (Fig 1). This proves the negative effect of high milk yield of the cows on their milk quality. Cows producing large amounts of milk are more likely to be exposed to free radicals and therefore to free radical peroxidation processes which result in MDA production [34]. Castillo et al. [35] showed that after the peak of lactation, the metabolic status stabilizes (with mean values of 28.87 ± 5.33 μm/L for MDA) and MDA level decreases. Additionally Sharma et al [34], stated
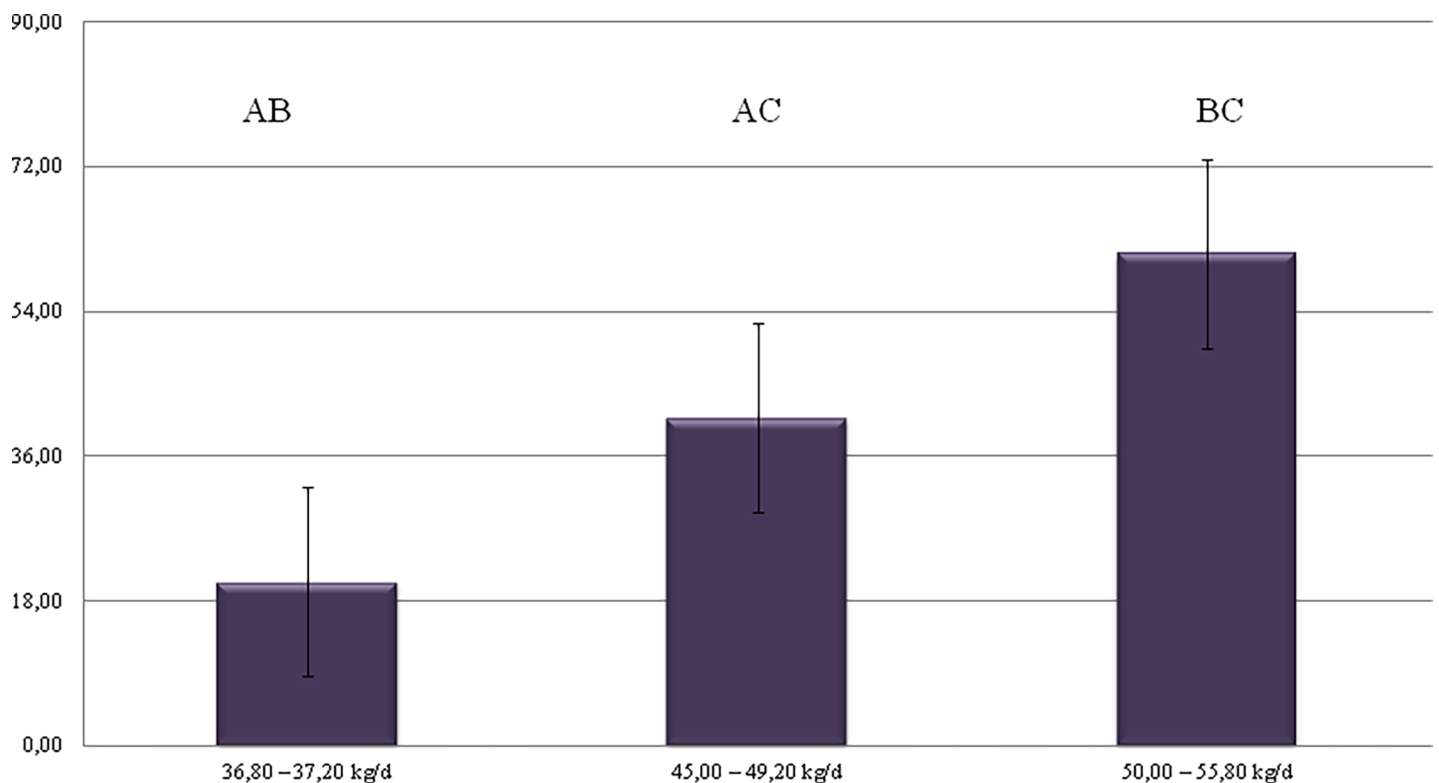

Fig 1. Changes in levels of MDA in milk, depending on cows performance.

https://doi.org/10.1371/journal.pone.0193512.g001

that dairy cows have more oxidative stress and low antioxidant defense during early lactation or just after parturition and it could by reason for their increased susceptibility to production diseases (e.g. mastitis, metritis, retention of fetal membranes etc.).

The study showed a significant connection between MDA concentrations and composition of cows' milk (Fig 2). The highest concentration of fat (4.63%) was found in milk in which the MDA level ranged from 48 to 86 nM/mL. Whereas, the inverse relationship was demonstrated in case of protein concentration. The highest level of this component (3.26%) was found in cows with MDA levels in the range of 18–28 nM/mL. On the other hand, Suriyasathaporn et al [36] reported, that results from Pearson's correlation coefficients showed that MDA was significantly associated only with BMSCC (bulk tank SCC).

In healthy animals, the antioxidant system protects tissues from the attack of free radicals. There are three types of antioxidants: these of the first line of defense (superoxide dismutase, glutathione peroxidase, and celuroplasmin), these of the second line of defense (vitamin E, β-carotene) which remove newly created free radicals before they can trigger chain reactions, and these of the third line of defense (DNA repair enzymes, and methionine sulfoxide reductase) [1]. A kit for determination of the total level of antioxidants—TAS, enables the assessment of an integrated antioxidant system that covers all biological components which exhibit antioxidant activity. The obtained results show the high impact of MDA concentrations on the antioxidant potential of cow's milk. The lowest MDA level was associated with the highest concentration of vitamin E (1.174 mg/L), β-carotene (0.387 mg/L) and TAS measured in both milk and plasma (1.384 and 1.423 mmol/L, respectively; Fig 3). Similar relationships has been shown by Pintea et al. [37] with regard to β-carotene. TAS and MDA levels are usually negatively correlated; i.e. once lipid peroxidation (MDA) increases, the antioxidant potential decreases. This correlation is especially visible in sick cows. Endometritis was associated with higher degree of oxidative stress (decreasing TAS and increasing MDA content) [2].
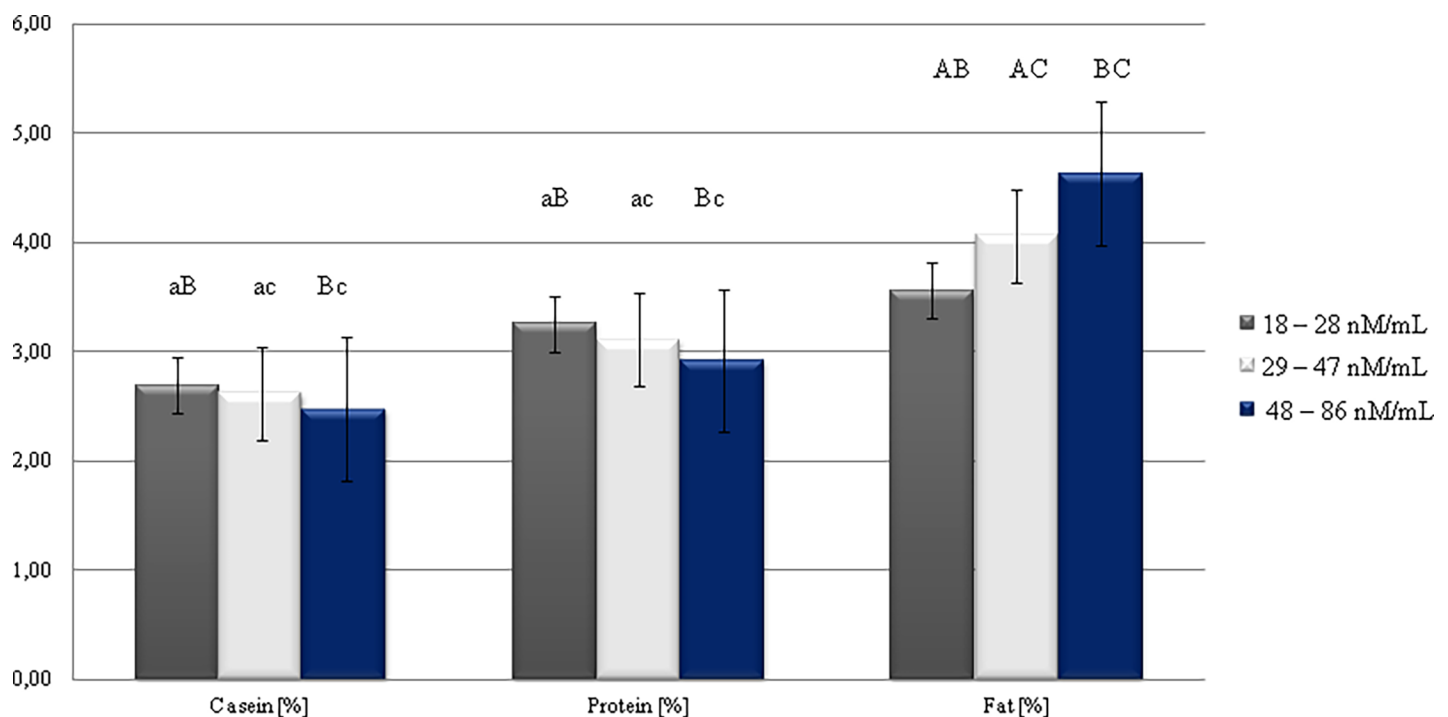

Fig 2. Changes in milk gross composition, depending on MDA concentration.
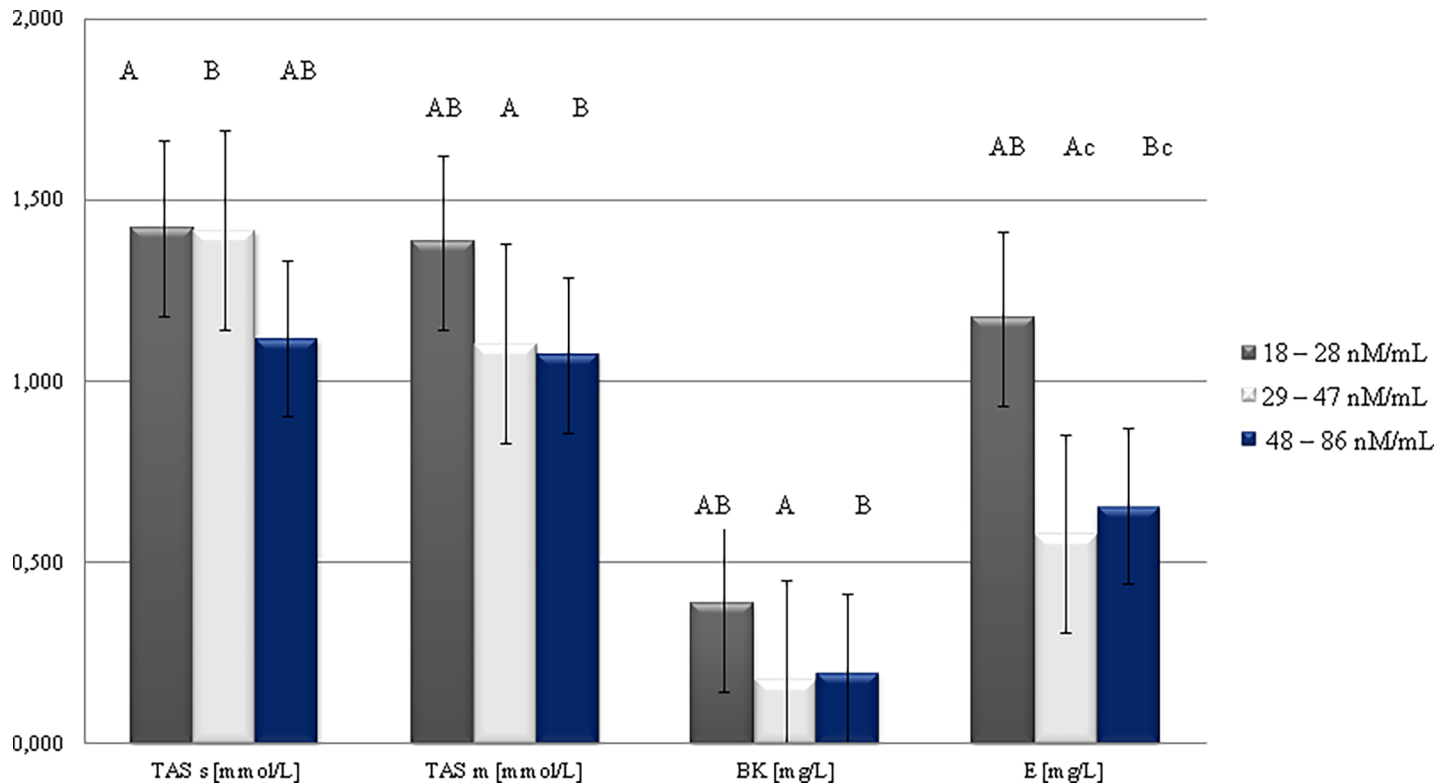
Fig 3. Changes in antioxidant capacity of milk depending on MDA concentration.

In contact with air, cholesterol undergoes autooxidation, and the rate of the formation of cholesterol oxidation products depends mainly on storage conditions [38]. Studies have shown the significant effect of MDA concentrations on cholesterol synthesis in milk fat (Fig 4). The highest level of cholesterol—298.43 mg/100 g of fat, was found in the cows with the highest concentration of MDA in milk; what is also confirmed by results of Pearson correlation (Table 2). Sparse information may be found in the available literature about the correlation between MDA and cholesterol levels. However other researches confirmed a correlation between cholesterol level and stage of lactation [39]. The highest content of cholesterol was found at the peak of lactation, which is in agreement with the highest concentration of MDA in milk determined in our study. Strzałkowska et al. [40] confirmed that cholesterol content of milk increased with the progress of lactation. Similar results were obtained by Son et al. [41].

Oxidative processes have a negative impact on milk and dairy products. In dairy products, the degree of antioxidant protection is calculated from the molar ratio between levels of antioxidants and oxidants. Lipid peroxidation caused by the oxidative stress, leads to adverse changes in the nutritional value of milk and milk products. Changes in the DAP are shown in Fig 5. The obtained result demonstrate the negative effect of MDA concentrations on DAP. The highest DAP (7.89×10^{-3}) was found in the cows with the lowest MDA concentration in milk (Fig 5). Sharma et al [34], reported that in cattle oxidative stress and induction of the immune response increases nutrient requirements. Pizzoferrato et al. [30] proved that the highest content of DAP was based on grazing than when cut herbage was utilized indoors by animals. During grazing on pasture, more natural antioxidants from the forage are introduced into the ruminant's body. They prevent lipid peroxidation and thus the formation of MDA, and cause DAP to increase. This confirms the negative relationship between MDA level and DAP found in our study.
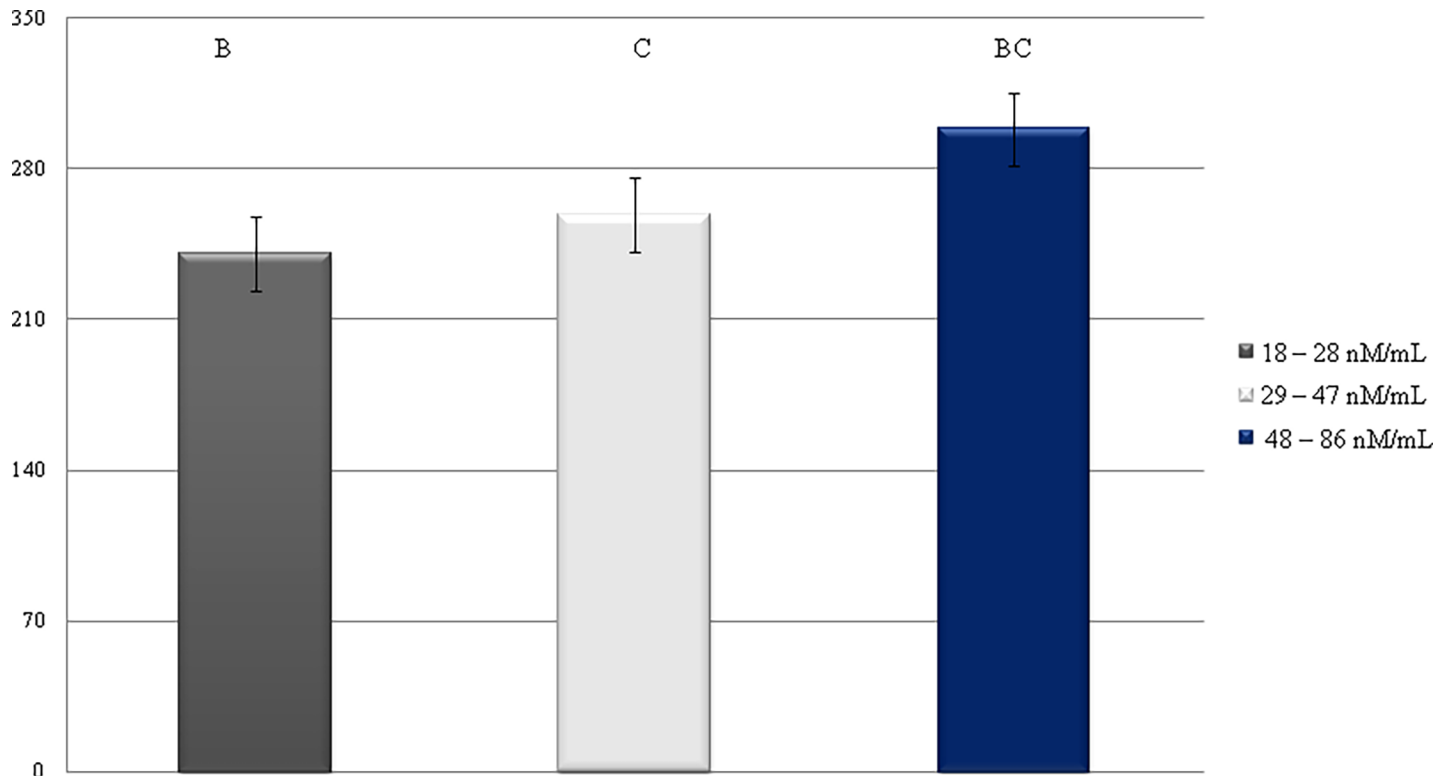
Fig 4. Changes in level of cholesterol milk depending on MDA concentration.

The study showed a significant correlation between MDA concentrations and individual components (Table 2). As shown by study results, the high concentrations of MDA was associated with lowered level: TASs, TASm, BK, E and DAP.

Table 2. Pearson correlations between individual components.

	Milk yield	Casein	Protein	Fat	FY	MDA	TASs	TASm	BK	E	Cholesterol	DAP
Milk yield	1	NS	-0.351**	NS	0.254*	0.902**	-0.412**	-0.390**	-0.565**	-0.593**	0.556**	-0.689**
Casein	NS	1	0.942**	NS	NS	-0.252*	NS	NS	0.265*	NS	NS	NS
Protein	-0.351**	0.942**	1	NS	NS	-0.361**	NS	NS	0.322**	NS	NS	0.281*
Fat	NS	NS	NS	1	-0.845**	NS	NS	NS	NS	NS	NS	NS
FY	0.254*	NS	NS	-0.845**	1	NS	NS	NS	NS	NS	NS	NS
MDA	0.902**	-0.252*	-0.361**	NS	NS	1	-0.494**	-0.370**	-0.529**	-0.509**	0.695**	-0.645**
TASs	-0.412**	NS	NS	NS	NS	-0.494**	1	NS	0.391**	0.317*	-0.252*	0.386**
TASm	-0.390**	NS	NS	NS	NS	-0.370**	NS	1	NS	0.303*	-0.339**	0.370**
BK	-0.565**	0.265*	0.322**	NS	NS	-0.529**	0.391**	NS	1	0.521**	-0.403**	0.772**
E	-0.593**	NS	NS	NS	NS	-0.509**	0.317*	0.303*	0.521**	1	-0.343**	0.901**
Cholesterol	0.556**	NS	NS	NS	NS	0.695**	-0.252*	-0.339**	-0.403**	-0.343**	1	-0.589**
DAP	-0.689**	NS	0.281*	NS	NS	-0.645**	0.386**	0.370**	0.772**	0.901**	-0.589**	1

MDA- malondialdehyde; TASm- Total antioxidant status determined in milk; TASs- Total antioxidant status determined in serum; BK- β-carotene; E- α-tocopherol;

DAP- degree of antioxidant protection; FY- fat yield

** The correlation significant at the 0.01 level (two-sided)

* The correlation significant at the 0.05 level (two-sided)
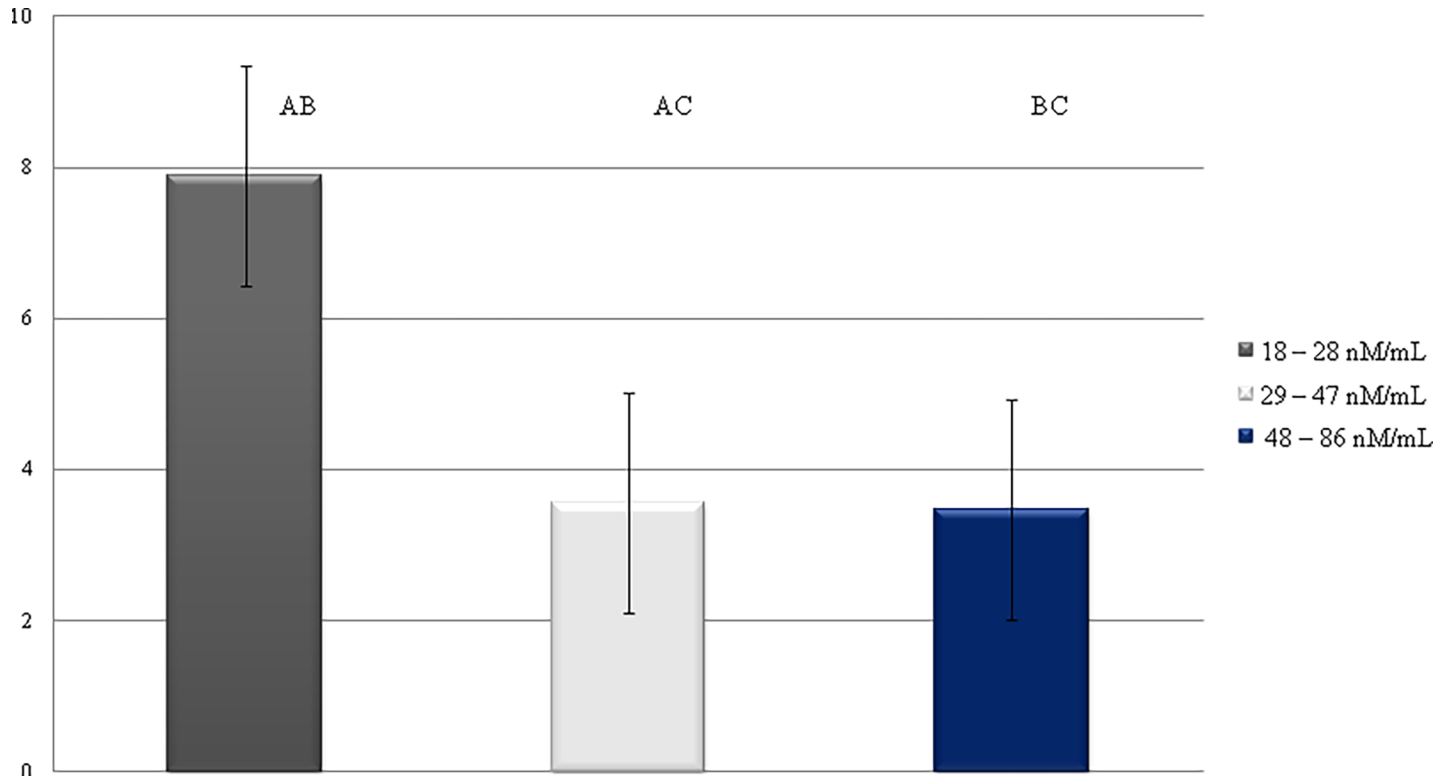
NS- not significant

Fig 5. Changes in level of DAP, depending on MDA concentration.

https://doi.org/10.1371/journal.pone.0193512.g005

Conclusion

The results of this study enable formulating the following conclusions:

1. Milk yield of cows has a high impact on MDA levels in milk;

2. The level of cholesterol in milk is connected with the milk yield of cows;

3. As the concentration of MDA increases, the antioxidant capacity of milk decreases;

Supporting information

S1 File. The results obtained for individual cows.
(PDF)

Acknowledgments

Author Contributions

Conceptualization: Aleksandra Kapusta, Kamila Puppel.

Data curation: Beata Kuczyńska, Kamila Puppel.

Formal analysis: Kamila Puppel.

Investigation: Aleksandra Kapusta, Beata Kuczyńska, Kamila Puppel.

Methodology: Aleksandra Kapusta, Kamila Puppel.

Project administration: Beata Kuczyńska, Kamila Puppel.

Supervision: Beata Kuczyńska.

Validation: Beata Kuczyńska.

Writing – original draft: Aleksandra Kapusta, Kamila Puppel.

Writing – review & editing: Aleksandra Kapusta, Kamila Puppel.

References

1. Puppel K, Kapusta A, Kuczyńska B. The etiology of oxidative stress in the various species of Animals, a review. J. Sci. Food. Agric. 2015; 95: 2179–2184. https://doi.org/10.1002/jsfa.7015 PMID: 25418967

2. Heidarpour M, Mohri M, Fallah-rad AH, Dehghan Shahreza F, Mohammadi M. Oxidative stress and trace elements before and after treatment in dairy cows with clinical and subclinical endometritis. Revue. Méd. Vét. 2012; 163(12): 628–633.

3. Puppel K, Nałęcz-Tarwacka T, Kuczyńska B, Gołębiewski M, Kordyasz M. Effect of different fat supplements on the antioxidant capacity of cows' milk. Archiv Tierzucht. 2013; 56(17): 178–190.

4. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Method Enzymolog. 1990; 186: 407–421.

5. Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: production, metabolism, and signaling mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev, 2014; https://doi.org/10.1155/2014/360438

6. Ziemlański Ś, Wartanowicz M. Rola antyoksydantów żywieniowych w stanie zdrowia i choroby. Pedr. Współ. Gastroentr. Hep. Żyw. Dziecka. 1999; 1(2/3): 97–105.

7. Celi P. Biomarkers of oxidative stress in ruminant medicine. Immunopharmacol. Immunotoxicol. 2011; 33:233–240.

8. Nozière P, Graulet B, Lucas A, Martin P, Grolier P, Doreau M. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Technol. 2006; 131: 418–450.

9. Pressman AH, Buff S. Witaminy i minerały. Zakład Poligraficzno-Wydawniczy POZKAL, 2006. Warszawa.

10. O'Grady MN, Monahan FJ, Fallon RJ, Allen P. Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. J. Anim. Sci. 2001; 79: 2827–2834. PMID: 11768111

11. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants in vivo? Free Radic. Res. 1997; 26: 381–398. PMID: 9167943

12. Diplock AT. The role of vitamin E and selenium in the prevention of oxygen-induced tissue damage. In: Spallholz JE, Martin JL, Ganther HE editors. Selenium in biology and medicine. Hartford, CT: AVI Publishing. 1981; 303.

13. Bendich A. Antioxidant micronutrients and immune responses. Ann. New York Acad. Sci. 1990; 587: 168–169.

14. Chew BP, Wong TS, Michal JJ. Uptake of orally administered β-carotene by blood plasma, leukocytes, and lipoproteins in calves. J. Anim. Sci. 1993; 710–730.

15. Kaneai N, Fukui K, Koike T, Urano S. Changes in the levels of CAM kinase II and synapsin I caused by oxidative stress in the rat brain, and its prevention by vitamin E. Adv. Biosci. Biotechnol. 2012; 3: 1199–1205.

16. Hai V Vu, Acosta T J, Yoshioka S, Abe H, Okuda K. Roles of prostaglandin F2alpha and hydrogen peroxide in the regulation of Copper/Zinc superoxide dismutase in bovine corpus luteum and luteal endothelial cells. Reprod. Biol. Endocrinol. 2012; 10: 87. https://doi.org/10.1186/1477-7827-10-87 PMID: 23101731

17. Gabryszuk M, Sakowski T, Metera E, Kuczyńska B, Rembiałkowska E. Wpływ żywienia na zawartość składników bioaktywnych w mleku krów z gospodarstw ekologicznych. Food Sci. Technol. Qual. 2013; 3(88): 16–26.

18. Manuelian CL, Currò S, Penasa M, Cassandro M, De Marchi M, Characterization of major and trace minerals, fatty acid composition, and cholesterol content of Protected Designation of Origin cheeses. J. Dairy Sci. 2017; 100(5): 3384–3395. https://doi.org/10.3168/jds.2016-12059 PMID: 28237598

19. Mayasari N, Chen J, Ferrari A, Bruckmaier RM, Kemp B, Parmentier HK et al. Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. J. Dairy Sci. 2017; 100(6): 4961–4975. https://doi.org/10.3168/jds.2016-11857 PMID: 28365121

20. Tomaszewski A. Kształtowanie się zawartości cholesterolu w mleku krów rasy czarno-białej. Zesz. Nauk. AR we Wrocławiu, seria: Rozprawy, 2005; 235.

21. Puppel K, Sakowski T, Kuczyńska B, Grodkowski G, Gołębiewski M, Barszczewski J et al. Degrees of Antioxidant protection: A 2-Year Study of the Bioactive Properties of Organic Milk in Poland. J. Food Sci. 2017; 82(2): 523–528. https://doi.org/10.1111/1750-3841.13594 PMID: 28125770

22. Sander B, Smith D, Addis P. Effects of processing stage and storage condition on cholesterol oxidation products in butter and Cheddar cheese. J. Dairy Sci. 1998; l(71): 3173–3178.

23. Krzyżewski J, Strzałkowska N, Bagnicka E, Jozwik A, Horbańczuk J. Wpływ antyoksydantów zawartych w tłuszczu pasz objętościowych na jakość mleka krów. Żywność Nauka Technologia Jakość 2012; 3 (82): 35–45.

24. Havemose MS, Weisbjerg MR, Bredie WLP, Poulsen HD, Nielsen JH. Oxidative stability of milk influenced by fatty acids, antioxidants, and copper derived from feed. J. Dairy Sci. 2006; 89: 1970–1980. https://doi.org/10.3168/jds.S0022-0302(06)72264-0 PMID: 16702260

25. Havemose MS, Weisbjerg MR, Bredie WLP, Nielsen JH. Influence of feeding different types of roughage on the oxidative stability of milk. Int. Dairy J. 2004; 14: 63–570.

26. Association of Official Analytical Chemists, Official Methods of Analysis, 15th edition, vol 1 AOAC, Arlington, VA (1990).

27. Puppel K, Kuczyńska B, Nałęcz-Tarwacka T, Gołębiewski M, Sakowski T, Kapusta A et al. Effect of supplementation of cows diet with linseed and fish oil and different variants of ß–lactoglobulin on fatty acid composition and antioxidant capacity of milk. J. Sci. Food Agric. 2016; 96: 2240–2248. https://doi.org/10.1002/jsfa.7341 PMID: 26174000

28. Wong S.H.Y., Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin. Chem. 1987; 33(2): 214–220.

29. EN ISO 5509. Animal and vegetable fats and oils- preparation of methyl esters of fatty acids (ISO 5509:2000).

30. Pizzoferrato L, Manzi P, Marconi S, Fedele V, Claps S, Rubino R. Degree of antioxidant protection: a parameter to trace the origin and quality of goat's milk and cheese. J. Dairy Sci. 2007; 90:4569–4574. https://doi.org/10.3168/jds.2007-0093 PMID: 17881677

31. IBM Corp. Released 2017: IBM SPSS Statistics for Windows, Version 23.0. Armonk, N.

32. Oltenacu PA, Broom DM. The impact of genetic selection for increased milk yield on the welfare of dairy cows. Anim. Welfare 2010; 19: 39–49.

33. Ingvartsen KL, Dewhurst RJ, Friggens NC. On the relationship between lactational performance and health: is it yield or metabolic imbalance that causes diseases in dairy cattle? A position paper. Livestock Prod. Sci. 2003; 83:277–308.

34. Sharma N, Singh NK, Singh OP, Pandey V, Verma PK. Oxidative Stress and Antioxidant Status during Transition Period in Dairy Cows. Asian-Aust. J. Anim. Sci. 2011; 24(4): 479–484.

35. Castillo C, Hernández J, Valverde I, Pereira V, Sotillo J, López Alonso M et al. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet.Sci. 2006; 80(2): 133–139. https://doi.org/10.1016/j.rvsc.2005.06.003 PMID: 16084546

36. Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T. Relationships among malondialdehyde, milk compositions, and somatic cell count in milk from bulk tank. Songklanakarin J. Sci. Technol. 2010; 32 (1): 23–26.

37. Pintea A, Zinveliu D, Pop R Al, Andrei S, Kiss E. Antioxidant status in dairy cows during lactation. Buletin USAMV-CN2006; 63: 130–135.

38. Min JS, Lee SO, Khan MI, Yim DG, Seol KH, Lee M et al. Monitoring the formation of cholesterol oxidation products in model system using response surface methodology. Lipids Health Dis. 2015; 14: 77, https://doi.org/10.1186/s12944-015-0074-6 PMID: 26201850

39. Turk R, Juretic D, Geres D, Turk N, Rekic B, Svetina A. Serum paroxonase activity in pregnant dairy cows. Proceedings of the IV Central European Buiatric Congress, Zagreb, Croatia. April 23–27, 2003; 400–405.

40. Strzałkowska N, Jóźwik A, Bagnicka E, Krzyżewski J, Horbańczuk J. Studies upon genetic and environmental factors affecting the cholesterol content of cow milk. I. Relationship between the polymorphic form of beta-lactoglobulin, somatic cell count, cow age and stage of lactation and cholesterol content of milk. Anim. Sci. Papers Rep. 2009; 27(2): 95–103.

41. Son J, Grant RJ, Larson LL. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 1996; 79(5): 822–830. https://doi.org/10.3168/jds.S0022-0302(96)76430-5 PMID: 8792281