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PROCESSES OF LIPID PEROXIDATION AND ANTIOXIDANT DEFENSE IN DAIRY COWS DEPENDING ON LACTATION PERIOD AND SEASON

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The aim of the study was to investigate the intensity of lipid peroxidation processes and the activity of enzymatic and non-enzymatic antioxidant defense system in dairy cows in different seasons during lactation cycle. The diene conjugates, lipid hydroperoxides and TBA-reactive substances concentration in blood of cows as indicators of lipid peroxidation state, and superoxide dismutase, glutathione peroxidase activities and concentration of vitamins A and E in blood as indexes of antioxidant defense state were determined in winter and in summer in clinically healthy dairy cows of the Ukrainian Black-and-White dairy breed (15 animals in each season). Blood samples were taken four times: three weeks before the expected date of calving, in early lactation (days 10–16), in mid lactation (days 80-90) and in late lactation (days 268–285). The highest level of lipid peroxidation intensity was noted at the beginning of lactation, and during the same period the activity of the antioxidant system was low. The opposite relationship was found at the peak of lactation, namely low intensity of free-radical oxidation and high activity of antioxidant defense. Antioxidant system in dairy cows undergoes higher strain during winter compared with summer season.

Key words: dairy cows, lipid peroxidation, antioxidant system, lactation period, season

Lipid peroxidation is a normal physiological process that takes place in all tissues of living organisms, but it remains at a low level with stable concentration of radicals, which are involved in sustaining homeostasis. Increase in activity of free radical oxidation in physiological conditions is considered to be an adaptive response of organism to stress factors (Reis and Spickett, 2012; Volinsky and Kinnunen, 2013). Coincidentally, residual radicals are the most active factor causing cell membrane injury, since reactive oxygen species (ROS) induce and enhance free radical peroxidation, involving in the process oxygen in red blood cells, deposited in tissues (Baumann et al., 2013; Power et al., 2013). It was determined that intensity of free-radical reactions in organism is influenced by both endogenous and exogenous factors (Bernabucci et

al., 2005; Lien et al., 2008; Celi, 2010; Ganaie et al., 2013; Overton and Yasui, 2014). In particular, character and intensity of metabolic processes related to milk synthesis change during lactation under the influence of different factors (pregnancy, season of the year, ration content, etc.). Intensification of free-radical oxidative reactions leads to activation of antioxidant cell enzymes and phosphogluconate pathway. Constant presence of a reserve pool of fatty acids is necessary for the system of lipid repair to function. Use of fatty acids in animal organism is associated with increased utilization of oxygen; deficit of the latter under the influence of stress factors leads to disorders of utilization of free fatty acids, which in turn may provoke their accumulation and initiate a range of pathological processes (Sharma et al., 2011; Dizdaroglu and Jaruga, 2012; Jóźwik et al., 2012; Rahal et al., 2014).

A significant number of scientific papers available worldwide, especially the recent ones which investigate oxidative stress intensity, gives evidence that this problem is relevant, many-sided and controversial. It should be mentioned that majority of scientific papers spotlight investigations on intensity of peroxidation processes exceptionally during critical period of calving or describe influence of diseases of different origin on course of free-radical reactions (Sharma et al., 2011; Tanha et al., 2011; Turk et al., 2013; Sordillo et al., 2014; Konvičná et al., 2015). Our study objective was to assess intensity of lipid peroxidation processes and activity of enzymatic and non-enzymatic antioxidant defense system within lactation cycle of dairy cows during various seasons.

Material and methods

The study was performed in compliance with General Ethical Principles of Animal Experiments (Ukraine, 2001) and international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1985).

Study material consisted of clinically healthy dairy cows. Animals were kept on a farm in Lviv region of Ukraine. Mixed housing and grazing system is common for this farm, i.e. from October to April cows are kept confined and from May to September animals are on pasture fields.

All animals in this study were of Ukrainian Black-and-White dairy breed, in their 2nd to 5th lactation with average yield of 5800 kg milk for a previous lactation. Two groups were compared, each consisting of 15 cows. The first group was observed during winter season, and the second one during summer period. Blood samples were taken four times in each group of cows: three weeks before the expected date of calving, in early lactation (days 10–16), in mid lactation (days 80–90) and in late lactation (days 268–285).

Blood was withdrawn from jugular vein of cows before morning feeding. Intensity of lipid peroxidation was evaluated taking into consideration concentration of lipid hydroperoxides (LHP) in plasma and serum levels of diene conjugates (DC) and TBA-reactive substances. State of enzymatic antioxidant defense was assessed taking into account activity of superoxide dismutase (SOD), glutathione peroxidase (GPX),

and non-enzymatic antioxidant system was evaluated on the basis of the levels of vitamins A and E in blood of cows.

Determination of lipid hydroperoxides concentration was performed using spectrophotometric technique according to V.V. Myronchyk (Влізло et al., 2012). Concentration of diene conjugates was assessed using the method of I.D. Stalna based on determination of absorbance of heptane-isopropanol extract of lipids (Влізло et al., 2012). The level of TBA-reactive substances was measured using spectrophotometric technique proposed by S.N. Korobejnikov (Влізло et al., 2012). Activity of superoxide dismutase was determined according to the method described by E. Ye. Dubinina et al., and glutathione peroxidase was measured using the technique of V.M. Moin (Влізло et al., 2012). Determination of the levels of vitamins A and E in blood of cows was performed using liquid chromatography technique (Влізло et al., 2012).

Obtained results were processed statistically using Microsoft Excel (2007). Arithmetic mean value and standard error of the mean value, significance of the difference between two sets of variation and correlation coefficient were calculated.

Results

The conducted investigation of blood samples taken from dairy cows in winter period revealed the highest intensity of lipid peroxidation processes at the beginning of lactation (Table 1). In particular, the level of lipid peroxidation products after calving was significantly increased (2-fold increase of the level of diene conjugates and lipid hydroperoxides ($P<0.001$), and 1.4-fold increase of TBA-reactive substances ($P<0.05$)). Subsequently the level of primary and secondary lipid peroxidation products gradually decreased up to the peak of lactation with further lowering till the end of lactation. At the end of lactation the level of diene conjugates decreased by 44% ($P<0.001$), lipid hydroperoxides by 42% ($P<0.01$) and TBA-reactive substances by 32% ($P<0.001$) in comparison with the beginning of lactation.

Similar dynamics of the content of lipid peroxidation products in blood of cows was revealed during the summer period (Table 1). In particular, after calving the level of diene conjugates increased by almost 33% ($P<0.05$), lipid hydroperoxides by 8%, and TBA-reactive substances by 50% ($P<0.01$). At the end of lactation the level of these substances gradually decreased by 50% ($P<0.001$), 170% ($P<0.001$) and 11%, respectively. Regardless of the similar dynamics to this in the winter season, during the summer period content of primary and secondary products of lipid peroxidation was significantly lower. In particular, the level of diene conjugates was lower by 17–27% ($P<0.05$–0.01), lipid hydroperoxides by 25–52% ($P<0.05$–0.001) and TBA-reactive substances by 4–36% ($P<0.05$–0.01).

Activity of antioxidant enzymes is in definite correlation with intensity of redox processes in tissues of animals. It was revealed that the dynamics of changes in activity of antioxidant enzymes in cow's blood was similar during winter and summer periods of keeping. The lowest activity of superoxide dismutase and glutathione pero-

xidase in blood was three weeks before calving (Table 2). Increase in enzyme activity was observed after calving and it lasted up to the peak of lactation. In particular, the activity of superoxide dismutase increased by 71–140% (P<0.001), and activity of glutathione peroxidase by 91–97% (P<0.001) depending on period of keeping, when compared to antepartum period.

Table 1. Plasma concentration of diene conjugates (μmol/l), lipid hydroperoxides (U/ml) and TBA--reactive substances (μmol/l) in cows, in relation to lactation cycle phases and seasons; n=15

Lactation cycle phases	Parameter	Season					
		Winter			Summer		
		Diene conjugates (mmol/l)	Lipid hydroperoxides (U/ml)	TBA-reactive substances (mmol/l)	Diene conjugates (mmol/l)	Lipid hydroperoxides (U/ml)	TBA--reactive substances (mmol/l)
3 weeks before calving	M	3.7	1.8	2.4	4.9	2.5	1.8
	m	0.27	0.24	0.47	0.64	0.41	0.32
Early lactation	M	7.8	3.6	3.4	6.5**	2.7*	2.7**
	m	0.50	0.40	0.19	0.14	0.06	0.16
	1. P<	0.001	0.001	0.05	0.05	0.5	0.01
Mid lactation	M	5.3	2.4	3.6	4.3**	1.5***	2.3*
	m	0.27	0.16	0.32	0.25	0.27	0.58
	1. P<	0.001	0.01	0.01	0.1	0.01	0.1
	2. P<	0.001	0.01	0.1	0.001	0.001	0.5
Late lactation	M	4.4	2.1	2.3	3.2*	1.0**	2.4
	m	0.15	0.26	0.08	0.32	0.16	0.19
	1. P<	0.1	0.5	1	0.05	0.01	0.1
	2. P<	0.001	0.01	0.001	0.001	0.001	0.1
	3. P<	0.05	0.5	0.001	0.01	0.1	1.0

Notes: In this and in following tables * – P<0.05; ** – P<0.01; *** – P<0.001, differences are statistically significant in comparison with winter period;

1. P< – degree of confidence, in comparison with antepartum period;
2. P< – degree of confidence, in comparison with the beginning of lactation;
3. P< – degree of confidence, in comparison with the peak of lactation.

It is important to underline the fact that the activity of antioxidant enzymes in blood of dairy cows is significantly higher during summer season in comparison to the winter period (Table 2). For instance, after calving superoxide dismutase activity was higher by 30–44% (P<0.01–0.001), and the activity of glutathione peroxidase was increased by 27–67% (P<0.05–0.01), depending on the physiological state.

The conducted statistical analysis revealed medium and strong negative correlation between the activity of antioxidant enzymes and the level of lipid peroxidation products in cow's blood during winter (from r= –0.6 to r= –0.9) and summer (from r= –0.6 to r= –0.9) periods of keeping. Moreover, the strongest negative correlation was registered at the beginning (r= –0.7 – –0.8) and at the peak (r= –0.8 – –0.9) of lactation in the course of both periods.

Table 2. Activity of superoxide dismutase (% reaction block/1 g Hb) and glutathione peroxidase (mmol/min/1 g Hb) in cow's blood in relation to lactation cycle phases and seasons; n=15

Lactation cycle phases	Parameter	Season			
		Winter		Summer	
		SOD	GPX	SOD	GPX
3 weeks before calving	M	19.6	191.5	18.8	236.5
	m	1.34	11.92	1.24	25.67
Early lactation	M	26.7	241.3	34.7**	401.9**
	m	1.40	33.58	2.29	30.06
	1. P<	0.001	0.01	0.001	0.001
Mid lactation	M	33.5	366.4	45.1***	464.9*
	m	2.29	42.64	1.63	14.93
	1. P<	0.001	0.001	0.001	0.001
	2. P<	0.01	0.01	0.01	0.1
Late lactation	M	27.0	361.5	38.8***	486.5*
	m	1.12	63.86	1.18	19.51
	1. P<	0.01	0.01	0.001	0.001
	2. P<	1.0	0.1	0.1	0.05
	3. P<	0.01	1.0	0.01	0.5

Table 3. Concentration of vitamins A and E (μmol/l) in cow plasma in relation to lactation cycle phases and seasons; n=15

Lactation cycle phases	Parameter	Season			
		Winter		Summer	
		A	E	A	E
3 weeks before calving	M	1.28	5.8	2.95***	10.6**
	m	0.076	0.78	0.198	0.63
Early lactation	M	1.43	7.8	3.39***	13.1**
	m	0.059	1.41	0.318	1.56
	1. P<	0.1	0.1	0.1	0.1
Mid lactation	M	1.53	10.9	2.54***	21.2**
	m	0.186	1.63	0.157	3.03
	1. P<	0.1	0.01	0.05	0.01
	2. P<	0.5	0.1	0.01	0.05
Late lactation	M	1.19	8.6	2.52**	14.1**
	m	0.127	0.82	0.392	0.62
	1. P<	0.5	0.05	0.1	0.1
	2. P<	0.1	0.5	0.1	0.5
	3. P<	0.1	0.1	1.0	0.1

The conducted laboratory examination of the serum levels of retinol and tocopherol in dairy cows showed that their highest concentration was registered during periods of maximum milk production and the lowest concentrations of retinol and tocopherol were observed at the end of lactation and during dry period, respectively (Table 3). At the peak of lactation concentration of retinol was higher by 20% in winter period and by 14% ($P<0.05$) during summer in comparison with antepartum

period. The level of tocopherol was higher by 88% ($P<0.01$) and 100% ($P<0.01$), respectively. Despite the similar character of changes, absolute levels of retinol and tocopherol were higher during grazing period – retinol 1.7–2.4-fold ($P<0.01$–0.001) and tocopherol 1.6–1.9-fold ($P<0.01$)) in comparison with winter period.

The obtained results revealed medium and strong negative correlation between the concentration of investigated vitamins and lipid peroxidation products in plasma of cows ($r= -0.5 - -1.0$).

Discussion

The performed study showed that the intensity of free radical oxidation depends on physiological state and season. At the beginning of lactation both in winter and in summer seasons the level of TBA-reactive substances, diene conjugates and lipid hydroperoxides in blood of cows reached the highest level. Our results are consistent with the data of other investigators, available in literature (Mudron and Konvičná, 2006; Castillo et al., 2006; Konvičná et al., 2015). Content of lipid peroxidation products was significantly lower in blood during summer period in comparison with winter season. This implies that maintenance of cows on pasture field in summer period is associated with an increase in activity of antioxidant enzymes, has a beneficial effect on their resistance and manifests itself in a decrease of intensity of free-radical oxidation in cow's organism. Intensification of peroxidation processes leads to activation of antioxidant enzyme synthesis, which manifests itself through the increase of their activity in blood of lactating cows. The highest activity of SOD and GPX was observed at the peak of lactation, while at the beginning of lactation it was low. There are some data in literature (Adela et al., 2006) in which the highest activity of SOD was registered in the third week of lactation, and the highest activity of GPX was noticed during the second week. The results of other authors are consistent with our suggestions (Stelletta et al., 2004; Castillo et al., 2006). According to data published by Festila et al. (2012), the lowest activity of antioxidant enzymes was detected at the beginning of lactation, however, fluctuations within the first 35 days of lactation were insignificant. It should be noted that during the summer season the enzyme activity was higher in comparison with the winter period. Significant differences in activity of enzymatic antioxidant system were described also by other investigators (Stelletta et al., 2004).

Statistical analysis of the obtained results showed medium and strong negative correlation dependence between the content of lipid peroxidation products and the activity of antioxidant enzymes.

Apart from enzymatic antioxidant system, its non-enzymatic part (water- and fat-soluble chemical substances reacting with oxygen radicals and their derivatives) also combats active oxygen species (Sies, 1993; Heyland et al., 2005; Ginter et al., 2014; Pincemail et al., 2014). In particular, vitamins A and E, as main fat-soluble antioxidants participate in inhibition of lipid peroxidation. The lowest plasma concentration of retinol and tocopherol in dairy cows was registered before calving and at the end of lactation; and the highest content of these vitamins

was detected at the peak of lactation. These results are also consistent with results of other investigators, in particular Goff and Stabel (1990), Herdt and Smith (1996) and Sivertsen et al. (2005). The conducted research showed that activity of enzymatic and non-enzymatic parts of the antioxidant system was significantly higher during summer period in comparison with winter season. Thus, plasma concentration of retinol and tocopherol throughout all physiological periods was significantly higher during summer period ($P<0.01 – 0.001$).

Considering the obtained results, we may conclude that antioxidant system in dairy cows undergoes maximum strain during the period between calving and the peak of milk production, especially in winter.

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Procesy peroksydacji i obrony antyoksydacyjnej u krów mlecznych w zależności od stadium laktacji i sezonu

STRESZCZENIE

Celem pracy była analiza intensywności peroksydacji lipidów i aktywności mechanizmów antyoksydacyjnych krów mlecznych w zależności od stadium laktacji i sezonu.

Koncentracje koniugatów dienowych, hydronadtlenków lipidów i związków reagujących z kwasem tiobarbiturowym jako wskaźniki peroksydacji lipidów oraz aktywność dysmutazy ponadtlenkowej, peroksydazy glutationowej i koncentrację witamin A i E jako wskaźniki obrony antyoksydacyjnej oznaczono we krwi klinicznie zdrowych krów w sezonie letnim i zimowym. W każdym sezonie koncentrację wskaźników oznaczano u 15 krów rasy ukraińskiej biało-czarnej w czterech terminach: trzy tygodnie przed spodziewanym terminem porodu, w początkowym stadium laktacji (10–16 dzień), w szczycie laktacji (80–90 dzień) oraz w końcowym okresie laktacji (268–285 dzień).

Najwyższy poziom peroksydacji lipidów stwierdzono na początku laktacji, w tym okresie aktywność układu antyoksydacyjnego była niska. Przeciwną zależność stwierdzono w stadium szczytu laktacji, charakteryzowała się niską intensywnością utleniania wolnych rodników i wysoką aktywnością obrony antyoksydacyjnej. Aktywność systemu antyoksydacyjnego była wyższa u krów mlecznych w sezonie zimowym w porównaniu do aktywności systemu w sezonie letnim.

Słowa kluczowe: krowy, peroksydacja lipidów, system antyoksydacyjny, sezon, stadium cyklu laktacyjnego