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Original Article

Biomarkers of Oxidative Stress During the Transition Period in Romanian Dairy Cows Breeds

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Abstract

In modern days when production demands continue to escalate within the dairy industry oxidative stress continues to be a problem in transition cows. This study was designed to evaluate the plasma profile of oxidative stress biomarkers at Romanian dairy cows breeds during the transition period from gestation to lactation. In order to achieve this objective 100 dairy cows belonging to Romanian Spotted breed and Brună of Maramureş breed grouped in three physiological states, have been evaluated for oxidative stress by measuring the activity of enzymatic antioxidants glutathione-peroxidase (GPx) and superoxide dismutase (SOD) respectively. The results of our study conducted on Romanian dairy cows breeds conclude that during early lactation or just after parturition the cows experience more oxidative stress and low antioxidant defense than advanced gestation cows. Also a statistical analysis was performed using "t" test and ANOVA which revealed that dairy cows experiencing oxidative stress during periparturient period.

Keywords: oxidative stress, transition period, GPx, SOD, dairy cows.

1. Introduction

Oxidative stress was initially defined by a serious imbalance between oxidation and antioxidants, "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage" [16, 17].

Exposure to biologically generated free radicals and reactive oxygen species (ROS) are natural occurrences in living organisms. Normally reactive oxygen species (ROS) and antioxidants remain in balance, but when this balance is disrupted as a consequence of ROS overproduction or depletion of antioxidants, oxidative stress (OS) occurs [1, 6].

ROS are formed continuously as normal by-products of cellular metabolism and, in low concentrations, they are essential for several physiological processes, including protein phosphorylation, transcription factors activation, cell differentiation, apoptosis, oocyte maturation, steroidogenesis, cell immunity, and cellular defense against microorganisms [14, 8, 1].

Oxidative Stress (OS) plays a key role in several pathological conditions connected with animal production, reproduction and welfare [13]. Antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecules [11].

The role of antioxidants in health and disease was studied extensively in both human and animal medicine [14, 21, 7].

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Endogenous antioxidants can be divided into three major groups: the first group comprises enzymatic antioxidants including superoxide dismutase (SOD) and glutathione-peroxidase (GPx), and represents the main form of intracellular antioxidant defense, second group includes non-enzymatic protein antioxidants that are primarily found in plasma and are mainly represented by sulfhydryl (SH) groups of albumin, and the third group is represented by the non-enzymatic low-molecular weight antioxidants such as glutathione,α-tocopherol, β-carotene and uric acid [14, 12].

Dairy cows undergo substantial metabolic and physiological adaptations during the transition from pregnancy to lactation [18].

Several more recent studies support the concept that oxidative stress is a significant underlying factor to dysfunctional host immune and inflammatory responses that can increase the susceptibility of dairy cattle to a variety of health disorders, particularly during the transition period [2, 4, 5, 19, 22].

2. Material and Method

This study involved the collection of blood samples from dairy cows belonging to breeds raised in Romania (Romanian Spotted-Simmental type, Brună of Maramureş) at different stages, regarding the transition from gestation to lactation, in order to compare the differences between these stages in terms of oxidative stress.

Evaluation of oxidative stress it was carried out by measuring the activity of enzymatic antioxidants, glutathione-peroxidase (GPx) and superoxide dismutase (SOD) respectively.

Glutathione peroxidase (GPx) is part of a enzymes family that catalyze the degradation of organic hydroperoxide resulting from normal metabolic processes and ensure the protection of proteins, lipids and nucleic acids against the molecules oxidation using as an electron donor glutathione or in some cases thioredoxin and glutaredoxin. GPx is a selenium-dependent enzyme, which is found in the cytosol (70%), but also in mitochondria (30%).

Superoxide dismutase (SOD) is considered in terms of biochemical processes the most important oxidative enzyme, characteristic of aerobic life and is present in all living cells [3]. It catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen [15].

Animals, nutrition and husbandry

Experiments were conducted during the year 2014 in two dairy cows farms located in Transylvania region with different exploitation and growth conditions and with different dairy cows breeds. The first farm (Farm A) exploited a population of dairy cows belonging to the Romanian Spotted breed-Simmental type in an conventional system and the second farm (Farm B) exploited a population of Brună of Maramureş dairy cows in an organic farming system. The biological material from Farm A was represented by 50 dairy cows with an average 305 days normalized milk production of 5620.96 kg/cow distributed as follows: 17 dairy cows in the first lactation, 15 dairy cows in second lactation and 18 individuals in the third lactation. Regarding the biological material from Farm B, this was represented also from 50 animals with an average 305 days normalized milk production of 3180.38 kg/cow distributed as follows: 16 dairy cows in the first lactation, 19 dairy cows in second lactation and 18 dairy cows in the third lactation.

Sampling took place between March and June, when the climate condition are ideal for dairy cows, with a maximum average temperature recorded in June (17.5^{o} C) and a minimum average temperature recorded in March (3.4^{o} C). This issue was particularly important due to heat stress because it is well known that heat increase the production of ROS [4].

During the experimental period all animals were kept under identical conditions. The animals were feed ad libitum with a diet consisted in a daily mix feed (Table 1).

Thus in each of the two herds involved in this study the dairy cows were grouped into three physiological statuses: (1) Prepartum from -1 month until delivery, (2) Early lactation from 0-7 days after delivery, and (3) Lactation from 25 to 35 days after parturition.

Blood sampling, analytical determinations and statistical procedure

Blood samples were taken by jugular venipuncture, 5 ml of blood from all the animals, into Li-heparinised tubes.

Erythrocytes glutathione peroxidase activity was determined photometrically, on heparinized whole blood, using a commercial kit (Ransel, Randox Laboratories) and semiautomatic biochemistry analyzer Master Plus Touch Screen.

Superoxide dismutase activity was determined kinetically at 505 nm lambda wavelength from heparinised plasma at 37^{o} C, with the spectrophotometer Uv-Vis Screen Master Touch. Heparinised plasma was purified for three times with 0.9% NaCl solution, after each wash the product was centrifuged for 10 minutes at 3000 rot/min with the elimination of supernatant. The

final results were reported in units per gram of hemoglobin (U.g-1 Hb).

All statistical analysis were performed with the GraphPad Prism v. 6.01 for Windows software package. To compare the mean values of GPx and SOD recorded in different physiological state, the Student-t test Welch corrected was used. Also to get an accurate analysis, ANOVA One Way was used in order to compare more than two means among the different stages of the transition period.

The confidence interval was established at 95%.

Table 1. Chemical composition of the diet supplied in the present study

Specification	M.U.	Farm A	Farm B
Dry matter	%	43,24	56,24
Crude protein	% D.M.	13,90	8,14
Neutral detergent fibre	% D.M.	27,15	36,80
Acid detergent fibre	% D.M.	14,54	19,87
Ether extract content	% D.M.	5,80	5,52
Ashes	% D.M.	7,2	6,74
PDIE	g/kg D.M.	104,25	61,05
PDIN	g/kg D.M.	102,21	59,86
Milk fodder units	UFL/kg D.M.	0,9	0,88

Note: DM-dry matter; PDIE-protein supplied when energy is limited in the rumen; PDIN-protein supplied when nitrogen is limited in the rumen; UFL-"Unite Fouragere Lait "

3. Results and Discussions

Table 2 shows the results of the oxidative stress markers in all the physiological stages of dairy cows, belonging to Romanian Spotted and Brună of Maramureş breeds.

Mean values of GPx and SOD reveals variations depending on the physiological state in both Romanian dairy cows breeds taken in this study. Thus the mean values of GPx in late gestation period has presented the highest value of 83.33 ± 1.89 U/g Hb at Romanian Spotted breed and 76.13 ± 0.60 U/g Hb at Brună de Maramureş breed. In early lactation period, the mean values of GPx decreases at the lowest level with a value of 75.12 ± 1.45 U/g Hb at Romanian Spotted breed and 71.30 ± 1.30 U/g Hb at Brună of Maramureş breed, level that in the next period of lactation increases.

Also, data from table 2 regarding the mean values of SOD reveals almost a similar pattern that we found for GPx. Thus, the lowest level of SOD was recorded in early lactation, when the mean values was 1731.18 ± 42.65 U/g Hb at Romanian Spotted breed and 1646.96 ± 29.25 U/g Hb at Brună of Maramureş breed. The highest levels of SOD were found also in late gestation period, with a mean values of 1925.57 ± 49.46 U/g Hb at Romanian Spotted breed and 1756.33 ± 18.48 U/g Hb at Brună of Maramureş breed.

Table 2. Mean values and variability of GPx and SOD according to physiological state at Romanian dairy cows breeds

Physiological status	Breed	MU	n	GPx		SOD	
				X ± sx	V%	X ± sx	V%
Late gestation	RSB	U /g Hb	50	83.33 ± 1.89	16.04	1925.57 ± 49.46	18.16
	BM		50	76.13 ± 0.60	5.57	1756.33 ± 18.48	7.44
Early lactation	RSB	U / g Hb	50	75.12 ± 1.45	13.65	1731.18 ± 42.65	16.47
	BM		50	71.30 ± 1.30	12.99	1646.96 ± 29.25	12.56
Lactation	RSB	U / g Hb	50	81.19 ± 2.19	19.07	1874.23 ± 60.31	22.75
	BM		50	75.66 ± 1.03	9.63	1748.66 ± 23.01	9.30

Note: RSB – Romanian spotted breed (Simmental type); BM – Bruna of Maramureş

A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in both humans and dairy cows [10, 4, 5, 20, 19]. The lower GPx blood level found in postpartum period is considered an indicator of oxidative stress because when GPx levels drops plasma lipid peroxidation occurs [15]. Thus quickly after birth the GPx levels from blood decrease while the level of reactive oxygen species increased.

This represent a strong evidence that dairy cattle show an increase level of oxidative stress after parturition. Also the low levels of SOD found in early lactation we put on the increased levels of reactive oxygen species. Thus because GPx and SOD activity decreased after partirition as a consequence, antioxidant defense mechanisms also declined.Our research findings about variation of GPx and SOD levels in the transition period at Romanian dairy cows breeds are confirmed by previous studies conducted on other cattle breeds [15, 6, 4, 19, 9]. In order to establish the existing statistical differences, GPx and SOD average values obtained were compared with Student- t test and the results are presented below in Table 3.

Table 3. Differences and statistical significance between mean values regarding GPx and SOD, according to physiological status at romanian dairy cows breed (Student test – Welch correction and ANOVA One Way analysis)

Physiological status	Breed	n	GPx		SOD	
			d	P	d	P
Late gestation vs. Early lactation	RSB	50	3.446	0.0009 ***	2.976	0.0037 **
	BM	50	3.352	0.0013 **	3.161	0.0022 **
Late gestation vs. Lactation	RSB	50	0.7398	0.4612 ns	0.6582	0.5120 ns
	BM	50	0.3943	0.6944 ns	0.2599	0.7955 ns
Early lactation vs. Lactation	RSB	50	2.311	0.0233 *	1.937	0.0560 ns
	BM	50	2.616	0.0104 *	2.733	0.0075 **
ANOVA (F test)	RSB	50	5.197	0.0066 **	3.852	0.0234 *
	BM	50	6.783	0.0015 **	6.476	0.0020 **

Note: RSB – Romanian spotted breed (Simmental type); BM – Bruna of Maramureş

Regarding Romanian spotted breed, in the case of GPx mean values, the differences between late gestation period (83.33 ± 1.89 U/g Hb) and early lactation period (75.12 ± 1.45 U/g Hb) reveals that they are highly statistical significant in favor of the late gestation period, and the differences between early lactation period (75.12 ± 1.45 U/g Hb) and the lactation period (81.19 ± 2.19 U/g Hb) is statistically significant (P=0.0233). The differences between mean values of GPx in late gestation period (83.33 ± 1.89 U/g Hb) and lactation period (81.19 ± 2.19 U/g Hb) is not statistically significant (P=0.4612). Concerning SOD mean values, the differences between late gestation period (1925.57 ± 49.46 U/g Hb) and early lactation period (1731.18 ± 42.65 U/g Hb) reveals that they are distinct statistically significant in favor of the late gestation period, and the differences between early lactation period (1731.18 ± 42.65 U/g Hb) and the lactation period (1874.23 ± 60.31 U/g Hb) is not statistically significant. The differences between mean values of SOD in late gestation period (1925.57 ± 49.46 U/g Hb) and lactation period (1874.23 ± 60.31 U/g Hb) is also not statistically significant (P=0.5120).

Regarding Brună of Maramureş breed, according to physiological state in the case of GPx mean values, the differences between late gestation period (76.13 ± 0.60 U/g Hb) and early lactation period (71.30 ± 1.30 U/g Hb) reveals that they are distinct statistically significant in favor of the late gestation period, and the differences between early lactation period (71.30 ± 1.30 U/g Hb) and the lactation period (75.66 ± 1.03 U/g Hb) is statistically significant (P=0.0104). The differences between mean values of GPx in late gestation period (76.13 ± 0.60 U/g Hb) and lactation period (75.66 ± 1.03 U/g Hb) is not statistically significant (P=0.6944). In the case of SOD mean values, the differences between late gestation period (1756.33 ± 18.48 U/g Hb) and early lactation period (1646.96 ± 29.25 U/g Hb) reveals that they are distinct statistically significant in favor of the late gestation period , and the differences between early lactation period (1646.96 ± 29.25 U/g Hb) and the lactation period (1748.66 ± 23.01 U/g Hb) is also distinct statistically significant. The differences between mean values of SOD in late gestation period (1756.33 ± 18.48 U/g Hb) and lactation period

(1748.66 ± 23.01 U/g Hb) is not statistically significant (P=0.7955).

However, for simultaneously compare existing differences between calculated mean of oxidative stress biomarkers found in all the three physiological statuses, we conducted and ANOVA analysis for comparing sample at variances level, considering this issue relevant for the scientific approach.

When testing variances differences between the three physiological state regarding GPx and SOD at Romanian spotted breed by ANOVA test, they were distinct statistically significant ($P < 0.01$) in the case of GPx and statistically significant for SOD ($P < 0.05$), the value of F obtained in the comparison was 5.197 for GPx and 3.852 for SOD. At Brună of Maramureş breed, the value of F obtained in the comparison was 6.783 for GPx and 6.476 for SOD and in both cases they were distinct statistically significant ($P < 0.01$).

4. Conclusions

The results of our study conducted on Romanian dairy cows breeds conclude that during early lactation or just after parturition the cows experience more oxidative stress and low antioxidant defense than advanced pregnant cows, and this seemed to be the origin of different production diseases and other health problems. Low levels of SOD and GPx found in our experiment at early lactation confirm that oxidative stress increases during the transition period from gestation to lactation, this aspect being revealed also statistically.

The antioxidant requirements of cows will likely increase as production demands continue to escalate within the dairy industry. Hence it is imperative to develop preventive actions in order to counteract the effect of oxidative stress encountered after parturition, and one alternative is to use the antioxidant supplementation from one month prior parturition until second month after parturition.

References

[1] Agarwal A., S. Gupta, R. Sharma, 2005, Role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology 3: 28, doi:10.1186/1477-7827-3-28.

[2] Allison R.D., R.A. Laven, 2000, Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Vet. Rec., 147: 703–708.

[3] Andrei S., S. Matei, N. Fiţ, C. Cernea, S. Ciupe, S. Bogdan, I.S. Groza, 2011, Glutathione peroxidase activity and its relationship with somatic cell count number of colony forming units and protein content in subclinical mastitis cows milk. Rom. Biotechn. Letters, 16(3): 6209 – 6217.

[4] Bernabucci U., B. Ronchi, N. Lacetera, A. Nardone, 2005, Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017–2026.

[5] Castillo C., J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira, J.L. Benedito, 2005, Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 169: 286–292.

[6] Celi P., 2011, Oxidative stress in ruminants. In 'Studies on veterinary medicine, 5: 191-231. L. Mandelker, P Vajdovich, Humana Press, Totowa, NJ.

[7] Celi P., 2011, Biomarkers of oxidative stress in ruminant medicine. Immunopharmacology and Immunotoxicology, 33(2): 233-240.

[8] Dröge W., 2002, Free radicals in the physiological control of cell function. Physiol. Rev., 82: 47–95.

[9] Feştilă Iulia Eugenia 2012. Cercetări privind însuşirile fiziologice şi influenţa unor factori asupra profilul metabolic la vacile de lapte. Teză de doctorat USAMV Cluj- Napoca.

[10] Gitto E., R.J. Reiter, M. Karbownik, D.X. Tan, P. Gitto, S. Barberi, I. Barberi, 2002, Causes of oxidative stress in the pre- and perinatal period. Biol. Neonate 81:146-157.

[11] Halliwell B., J.M.C Gutteridege, 2007, Free Radicals in Biology and Medicine, 4th ed. Oxford University Press.

[12] Halliwell B., J.M.C. Gutteridge, 1989, Free radicals in biology and medicine. 2nd ed. Oxford, UK: Oxford University Press.

[13] Lykkesfeldt J., O. Svendsen, 2007, Oxidants and antioxidants in disease: oxidative stress in farm animals. The Veterinary Journal 173: 505-511.

[14] Miller J.K., E. Brzezinska-Slebodzinska, F.C. Madsen, 1993, Oxidative stress, antioxidants, and animal function. J. Dairy Sci., 76: 2812–2823.

[15] Sharma N., N.K. Singh, O.P. Singh, V. Pandey, P. K. Verma (2011). Oxidative stress and antioxidant status during transition period in dairy period. Asian-Aust. J. Anim. Sci., 24(4): 479-484.

[16] Sies H., 1985, Oxidative stress: Introductory remarks. In: Sies H, ed. Academic Press, London, 1–8.

[17] Sies H., 1986, Biochemistry of oxidative stress. Angew. Chem. Int. Ed. Eng., 25:1058–107.

[18] Sordillo L.M., S. Aitken, 2009, Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol, doi:10.1016/j.vetimm.2008; 10.305.

[19] Sordillo L.M., N. O'Boyle, J.C. Gandy, C.M. Corl, E. Hamilton, 2007, Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J. Dairy Sci., 90: 1186–1192.

[20] Stefanon B., S. Sgorlon, G. Gabai, 2005, Usefulness of nutraceutics in controlling oxidative stress in dairy cows around parturition. Vet. Res. Commun., 29(2): 387–390.

[21] Valko M., D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, 2007, Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell. Biol., 39: 44–84.

[22] Wilde D., 2006, Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. Anim. Reprod. Sci. 96: 240–249.