Activities of N-acetyl-β-D-glucosaminidase and glutathione peroxidase in bovine colostrum and milk

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ABSTRACT: Parturition and *post partum* period are susceptible for antioxidative/oxidative imbalance as well as inflammatory processes related to either uterus or mammary gland. Fifteen Holstein-Friesian cows were used to examine the relationship between the antioxidant enzyme glutathione peroxidase (GSH-Px) and inflammatory enzyme N-acetyl-glucosaminidase (NAGase) in colostrum and milk during 12 days after parturition. Samples from each udder quarter were collected immediately after parturition, after 24 h and 48 h as well as after 6 and 12 days *post partum*. The activity of GSH-Px was measured spectrophotometrically and NAGase spectrofluorimetrically. NAGase activity generally remained constant during the examined period confirming no inflammation and healthy udder. Moreover, no significant differences in the activity among quarters were detected. The activity of GSH-Px rose significantly ($P < 0.05$) in the examined period suggesting the dynamic balance of antioxidative defence. No differences among quarters confirmed that in healthy quarters metabolic processes are on a similar level but positive correlations between the antioxidative and inflammatory enzyme in quarters and with regard to time may suggest a possible relationship during inflammation.

Keywords: glutathione peroxidase; N-acetyl-glucosaminidase; colostrum; cow

Milk synthesis starts in the epithelial cells of the mammary gland at the end of pregnancy to support the nutrition and to promote the health of the offspring (Hurley, 2001).

Milk is a food for neonates that meets the nutritional requirements for their growth and development. Changes in the contents of its major nutritive components during lactation are well known in humans (Allen et al., 1991) as well as in domestic animals such as cows (Rook and Campling, 1965; Hanuš et al., 2010). But colostrum contains also many biofunctional constituents, including growth factors, antipathogenic compounds and immune enhancing components, as well as antioxidants and their action during lactation still requires elucidation (Lindmark-Mansson and Akesson, 2000; Albera and Kankofer, 2009). The biological role of colostrum has therefore been suggested to provide not only nutrients but also anti-infection factors against pathogenic bacteria and viruses for the newborn whose immune system is not fully developed yet as well as antioxidative defence against reactive oxygen species (ROS). That is why any alterations in colostrum content and activity decrease its protective properties and may lead to higher susceptibility to the disease of not only the mammary gland itself but the newborn as well.

Oxidative stress is a result of an imbalance between ROS production and neutralizing capacity of

antioxidant mechanisms in living organisms (Sies, 1993; Halliwell, 2006). Oxidative stress leads to peroxidative damage to lipids and other macromolecules, with consequent alteration of cell membranes and other cellular components (Toyokuni, 1999). Moreover, it may cause the modification of important physiological and metabolic functions. The transition period is particularly important for the health and subsequent performance of dairy cows that are exposed to drastic physiological changes and metabolic stress (Grummer, 1993; Goff and Horst, 1997; Drackley, 1999).

The stress of parturition and the initiation of lactation in dairy cows are associated with marked immune suppression (Goff and Horst, 1997; Castillo et al., 2005), oxidative stress and increased susceptibility to diseases such as mastitis or Johne's disease (Chiodini et al., 1984) which may appear during the periparturient period.

The enzyme glutathione peroxidase (GSH-Px) is a selenocysteine-containing protein that plays an important role in the cellular defence against oxidative stress (Raes et al., 1987) by utilizing reduced glutathione (GSH) to reduce hydrogen peroxide (H_2O_2) and lipid peroxides to their corresponding alcohols (Ursini et al., 1995).

The lysosomal enzyme β-N-acetyl-glucosaminidase (NAGase; EC 3.2.1.30) is found in milk, serum and other body fluids. It hydrolyses terminal, non-reducing N-acetyl-β-D-glucosamine residues from N-acetyl-β-D-glucosaminides. Milk NAGase activity increases during mastitis. Most of this increase is thought to be due to leakage from damaged secretory epithelial cells (Kitchen et al., 1978; Fox et al., 1987). By the time clinical mastitis is detected, NAGase has risen to or near its peak value (Fitz-Gerald et al., 1981; Kunkel et al., 1987). It has been speculated that NAGase assay may be developed as a rapid test for predicting the severity of clinical mastitis at the time of onset (Kitchen et al., 1984; Chaqunda et al., 2006).

The relationship between NAGase and GSH-Px activity should be considered in terms of inflammatory processes where both enzymes are involved. Although the clear role of GSH-Px in colostrum and milk has not been defined yet (Fox and Kelly, 2006), it can surely protect from ROS not only the newborns but also the mammary gland tissue itself. Moreover, the antioxidative status represented by antioxidative vitamins and microelements may influence resistance to diseases and development of inflammation.

The hypothesis was formulated that any ROS imbalance or immunosuppression related subclinical mastitis which may occur in the peripartum period may result in the increase of GSH-Px and/or NAGase activity, which can be detected in colostrum and milk. These possible alterations would influence not only the mammary gland itself but also the content of colostrum/milk. Whether these processes would be limited to one of the mammary gland quarters or to the whole gland requires elucidation.

The aim of the study was to investigate and to describe a dynamic relationship between GSH-Px and NAGase activity detected separately in 4 quarters of the clinically healthy mammary gland in cows immediately after physiological parturition and during 12 days *post partum* in order to find any connection among the quarters. Moreover, a relationship between the antioxidative and inflammatory enzyme was supposed to be detected.

MATERIAL AND METHODS

The experimental procedures were approved by the Local Ethical Committee for the Permission of Animal Experimentation and followed the actual law of animal protection.

Colostrum and milk were collected from each udder quarter (FR-front right, RR-rear right, FL-front left, RL-rear left) of healthy HF cows (n = 15), aged between 2 and 6 years from a local dairy farm. The selection of animals was based on clinical examination and uncomplicated parturition. Mammary glands did not show any clinically relevant symptoms of inflammation.

Cow colostrum was collected three times: immediately after parturition (0 h), 24 h and 48 h *post partum*. Milk samples were collected 6 and 12 days *post partum*. The time of sample collection was selected on the basis of literature where no data on several time points for sample collection was found and of our own previous studies and the possibility to register dynamic changes in the activity of examined parameters after parturition (Kankofer and Lipko, 2008; Albera and Kankofer, 2009).

Colostrum and milk samples were collected by hand milking and they were frozen immediately after collection and stored at –80°C until analysis. Samples were defatted by centrifugation at 2 500 × g for 15 min and the supernatant was used for further analysis.

Determination of β-N-acetyl-glucosaminidase

Determination of NAGase activity was carried out according to the method of Kitchen et al. (1978).

Colostrum and milk were diluted in 0.01 mol/l Tris-HCl buffer pH 7.5 containing 0.250 mol/l saccharose. 50 µl of sample was mixed with 200 µl of substrate solution (0.002 mol/l 4-4-methyl-umbelliferyl-N-acetyl-β-D-glucosaminide (Sigma, Poznań, Poland) in 0.25 mol/l citrate buffer pH 4.6 and incubated at 37°C for 10 min. The reaction was terminated with 5.5 ml of 0.1 mol/l carbonate buffer pH 10.0 and the released 4-methylumbelliferone was measured with a spectrofluorometer using the excitation and emission wavelength of 365 nm and 450 nm, respectively. The standardization of the spectrofluorometer (Jasco FP-6300, Tokyo, Japan) to 100 deflection was performed with 5 µmol/l 4-methylumbelliferone (Sigma, Poznan, Poland) in 0.1 mol/l carbonate buffer pH 10.0. Enzyme activity was expressed in SI units – nanokatals (nkat) per gram protein.

Determination of glutathione peroxidase

Determination of GSH-Px was determined as described by Paglia and Valentine (1967). The reaction mixture contained 100 µl of 8.4 mmol/l NADPH (Sigma, Poznan, Poland), 10 µl glutathione reductase (GSSG-R, 100 U/mg protein/ml, Sigma, Poznan, Poland), 10 µl 1.125 mol/l sodium azide (Sigma, Poznan, Poland), 100 µl 0.02 mol/l glutathione (GSH, Sigma, Poznań, Poland) to which phosphate buffer (0.05 mol/l, pH 7.0) was added up to the volume of 2.5 ml, 100 µl of 0.022 mol/l H_2O_2 and 100 µl sample. The rate of GSSG formation was measured by a subsequent decrease in absorbance of the reaction mixture at 340 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden) as NADPH was converted to $NADP^+$, between the 2^{nd} and 4^{th} min after the initiation of the reaction employing a cuvette with a 1 cm light path. Calculations were based on a standard curve prepared with different concentrations of NADPH. Enzyme activity was expressed in SI units – nanokatals (nkat) per gram protein.

Protein determination

The colostrum and milk protein content was determined using the Total Protein kit (Cormay, Lublin, Poland).

Statistical analysis

The determinations of enzyme activities in duplicate were averaged and subjected to statistical

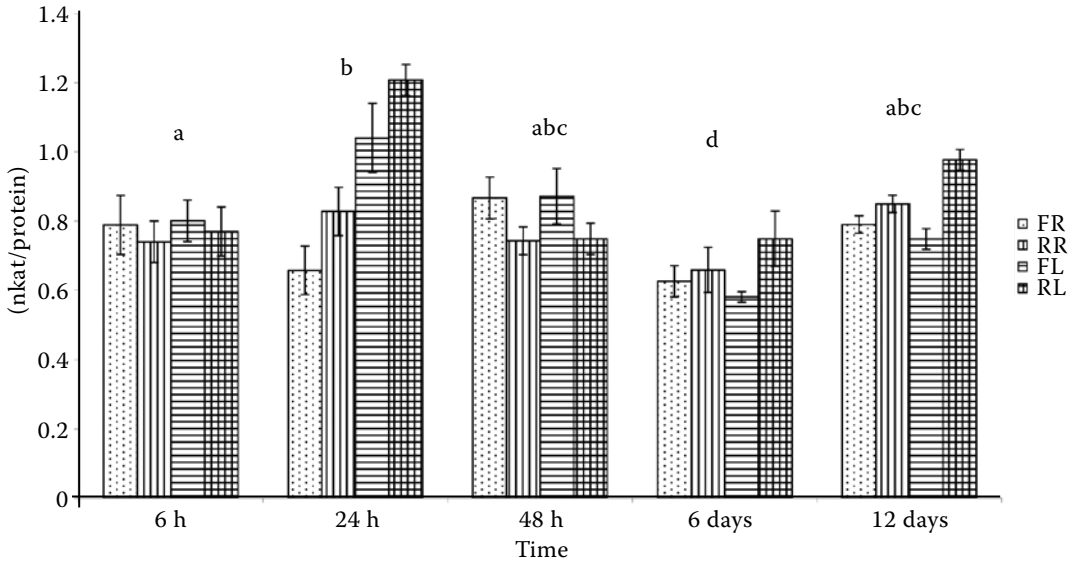

Figure 1. The activity of N-acetyl-β-D-glucosaminidase in cow colostrum and milk;
homogeneous groups (within these groups there are no significant differences in the enzyme activity among measurements) are designated by the same small letter; mean values of each quarter were compared among the examined times of collection

analysis for significance of differences using SAS 9.1 software, repeated measures analysis by Mixed Models option and Akaike's information criterion or Schwarz's Bayesian criterion to choose the best covariance matrix (Littell et al., 1998). The Kołmogorow-Smirnow test did not show the normal distribution of experimental data. Applying data transformation in the form of natural logarithm allowed to obtain normal distribution consistent with the assumption of analysis of variance. Analysis of correlation was also performed.

In this statistical analysis time was the most important parameter for the activity of enzymes (significant differences in enzyme activity over time were found on the basis of the *P*-value for NAGase 0.0038, which was lower than the nominal level 0.05, and for GSH-Px – 0.0001, which was lower, similarly like NAGase, than the nominal level 0.05). Post-hoc tests allowed to isolate the following homogeneous groups which were designated by the same small letter.

RESULTS AND DISCUSSION

In the present study the dynamics of changes in the activity of the antioxidative enzyme GSH-Px and inflammatory enzyme NAGase in colostrum and milk obtained from each quarter of the cow mammary gland was analyzed in physiological conditions during 12 *post partum* days. Moreover, this study provided the first information about the relationship between GSH-Px and NAGase measured in each quarter during the *post partum* period.

The activity of NAGase in each quarter varied slightly during the examined period (Figure 1). In FR quarter immediately after parturition the value reached 0.789 nkat/g protein ± 0.19 and in RR quarter the value 0.74 nkat/g protein ± 0.12 was similar. No significant differences among the examined times were detected.

In FL quarter immediately after parturition the value reached 0.801 nkat/g protein ± 0.12 and tended to rise to 1.04 nkat/g protein ± 0.2 after 24 h. Fortyeight hours after parturition the activity decreased significantly ($P < 0.05$) to 0.872 nkat/g protein ± 0.16 reaching the lowest value of 0.581 nkat per g protein ± 0.03 six days after parturition. 12 days after parturition the value of the enzyme increased to 0.748 nkat/g protein ± 0.06.

In RL quarter the lowest NAGase activity was immediately after parturition – 0.77 nkat/g protein ± 0.14. After 24 h the activity of the enzyme was significantly ($P < 0.05$) higher and reached 1.207 nkat/g protein ± 0.09. It tended to decrease to the value of 0.749 nkat/g protein ± 0.09 after 48 h. After 6 days the activity of NAGase remained constant – 0.749 nkat/g protein ± 0.16. 12 days after parturition the activity reached the value of 0.977 nkat/g protein ± 0.06. Significant differences were also detected between samples collected after 24 h and 6 days.

In accordance with literature the stage of lactation had a highly significant effect upon the NAGase activity. The general trend of NAGase activity during lactation is similar to that reported by Mattila and Sandholm (1985) and by Miller and Paape (1988): relatively high immediately after parturition and in early lactation, low in mid-lactation, and upward in late lactation and in dry secretion samples. The effects of quarter position, year and month of sampling were not significant (Miller and Paape, 1988).

In the present study the NAGase activity generally remained constant during the examined period confirming no subclinical inflammation and healthy udder. Moreover, no significant differences ($0.7911 > 0.05$) in the activity among quarters at the same time were detected suggesting a similar metabolic activity of healthy quarters. The parallel examination of enzyme activity in all udder quarters presented here was aimed to elucidate the presence of inter-quarter dependence in the healthy gland and to highlight the knowledge of the mammary gland functioning.

Whether udder quarters are functionally and morphologically independent or not is not clear yet (Sol et al., 2000; Berry and Meaney, 2006). Rendel and Sundberg (1962) suggested that the morphological structure can partly be responsible for the higher prevalence of infection in the rear udder quarters. Higher milk yield is attributed to rear quarters (Lancelot et al., 1997), which in turn increases mastitis prevalence because it increases along with milk production (Houben et al., 1993).

Mellors (1968) was the first to report the presence of NAGase in bovine milk and suggested that this enzyme is derived wholly from leukocytes and its level in milk may be a convenient measure of the mammary gland infection.

NAGase has been found to originate also from white blood cells that increase in milk as a result of inflammation in mastitis (Kaarhm et al., 1988). It seems possible that the quarters suffering from chronic mastitis could elevate basal NAGase due to the increased infiltration of SCC (somatic cell

count) into milk, even when clinical signs are absent. If so, the use of milk NAGase as a prognostic test for new clinical episodes of chronic cases might be less accurate than for new infections.

Colostrum is characterized by a higher number of somatic cells that can be responsible for the activity of NAGase during the first few days after parturition in healthy udder.

The relationship between the antioxidative and inflammatory enzyme in the bovine mammary gland might be based on the mechanisms underlying the development of mastitis when peroxidative processes related to oxidative burst take place. Hamed et al. (2008) investigated the relationship between milk somatic cell counts, macrophages, lymphocytes, polymorphonuclear leukocytes and antioxidative enzymes and described some correlations which, among others, were based on the generation of oxidative stress by polymorphonuclear leukocytes. On the other hand, oxidative stress which is related to cell damage may be responsible for the increase in NAGase activity.

In the present study the activity of GSH-Px rose significantly in the examined period suggesting the dynamic balance of antioxidative defence which can adjust to the current challenge of either newborn or mammary gland.

The lowest activity of GSH-Px was detected in each quarter immediately after parturition (Figure 2). In FR quarter the activity of the enzyme at parturition was 0.067 nkat/g protein ± 0.017 and increased during the whole period of observation. The significantly ($P < 0.05$) highest value, as compared to parturition and 24 h, was recorded 12 days after parturition, reaching 0.159 nkat/g protein ± 0.032.

The lowest activity of the enzyme in RR quarter was observed immediately after parturition – 0.049 nkat per g protein ± 0.012. Then the activity significantly ($P < 0.05$) increased to 0.104 nkat/g protein ± 0.026 after 24 h *post partum* and remained constant after 48 h and 6 days *post partum*.

The highest activity was detected 12 days after parturition – 0.151 nkat/g protein ± 0.03 and was significantly different from the time of parturition.

The same pattern was found in FL quarter where the lowest activity was detected immediately after parturition, reaching the value of 0.057 nkat/g protein ± 0.013. The activity significantly ($P < 0.05$) increased to 0.102 nkat/g protein ± 0.031 in 24 h after parturition and to 0.119 nkat/g protein ± 0.032 in 48 h after parturition. Six days after parturition the activity of GSH-Px remained constant while the highest activity was reached 12 days after parturition – 0.156 nkat/g protein ± 0.036, which was significantly ($P < 0.05$) different from the time of parturition and 24 h.

In RL quarter the lowest activity of the enzyme immediately after parturition reached the value of

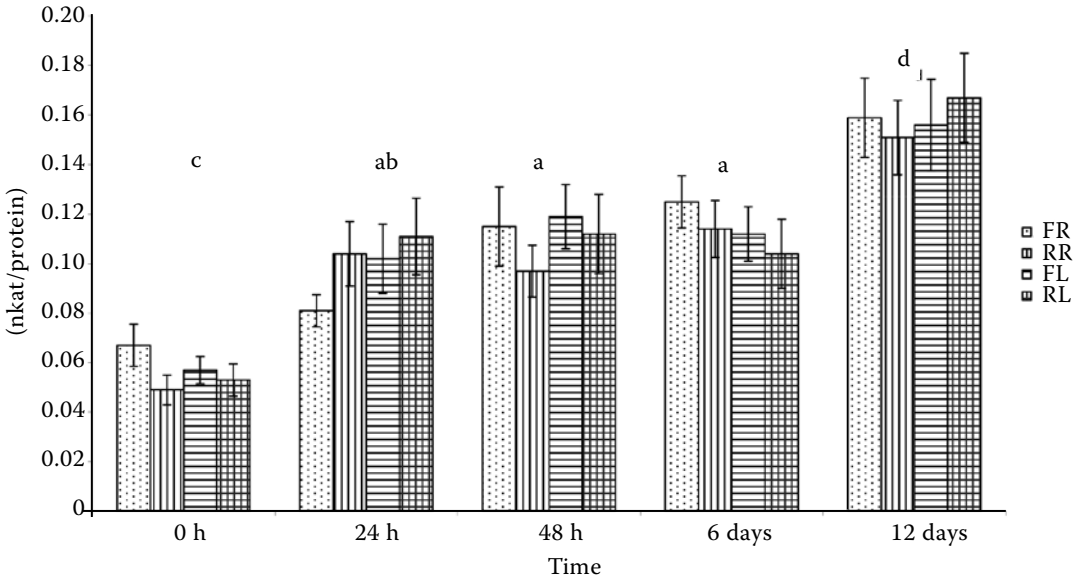

Figure 2. The activity of glutathione peroxidase in cow colostrum and milk;
homogeneous groups (within these groups there are no significant differences in the enzyme activity among measurements) are designated by the same small letter; mean values of each quarter were compared among the examined times of collection

0.053 nkat/g protein ± 0.011. The activity of GSH-Px significantly ($P < 0.05$) increased to 0.111 nkat/g protein ± 0.028 after 24 h *post partum* and to 0.112 nkat per g protein ± 0.026 after 48 h *post partum*. Six days after parturition the activity reached 0.104 nkat/g protein ± 0.022. Similarly like in the other quarters, the highest activity of the enzyme was detected 12 days after parturition, reaching the value of 0.167 nkat/g protein ± 0.037 and being significantly ($P < 0.05$) different from the time of parturition and 6 days.

GSH-Px is one of the biological active forms of selenium in milk (Hojo, 1982; Debski et al., 1987). Both GSH-Px activity and selenium content of milk have been shown to decrease during lactation (Hojo, 1986).

GSH-Px plays a key role in defending against ROS, its deficiency should lead to an increase in the oxidative stress intensity. GSH-Px has much higher Michaelis-Menten constant for H_2O_2 than catalase (Jones et al., 1981), another important enzyme involved in the detoxification of H_2O_2.

In dairy cows, the peripartum period is especially critical and requires considerable physiological mechanisms to maintain homeostasis and to avoid significant metabolic stressors that may contribute to the onset of diverse disorders (Miller et al., 1993; Goff and Horst, 1997).

No significant differences in GSH-Px activity among the examined quarters at the same time (0.9605 > 0.05) confirmed that in healthy quarters metabolic processes are on a similar level but positive Pearson's correlations between the antioxidative and inflammatory enzyme either in quarters and with regard to time may suggest a possible relationship between the activities of examined enzymes.

Positive Pearson's correlations between the examined enzymes in particular quarters were detected:

in quarter FR 48 h after parturition – 0.77;
in quarter RR 48 h after parturition – 0.89;
in quarter FL 24 h after parturition – 0.81 and 48 h after parturition – 0.69;
in quarter RL 24 h after parturition – 0.88, 48 h after parturition – 0.79, 6 days after parturition – 0.69 and 12 days after parturition – 0.56.

Positive Pearson's correlations between NAGase and GSH-Px with regard to time were detected:

in 24 h after parturition – 0.71;
in 48 h after parturition – 0.79;
in 12 days after parturition – 0.46.

Buescher and Mcilherhan (1988) suggested that colostrum has a heterogeneous antioxidant capability that may aid the immature antioxidant defence system of the premature infant.

The examination of lactoperoxidase, lactoferrin and ceruloplasmin, which have also antioxidative properties, in colostrum and milk of cows showed dynamic profiles of these biologically active molecules during 12 days *post partum*. Antioxidative activity of ceruloplasmin decreased, antioxidative activity of lactoferrin increased while the activity of lactoperoxidase fluctuated with a tendency to increase during the examined period (Albera and Kankofer, 2009). It can confirm the present results suggesting that the antioxidative system reacts in accordance with the current situation.

In conclusion, this preliminary study clearly shows that experiments on the antioxidative profile in colostrum and milk require further research not only in physiological but also in pathological conditions in order to describe the influence on cows, their newborns and on the quality of milk for human consumption.

REFERENCES

Albera E., Kankofer M. (2009): Antioxidants in colostrum and milk of sows and cows. Reproduction in Domestic Animals, 44, 606–611.

Allen J.C., Keller R.P., Archer P., Neville M.C. (1991): Studies in human lactation: milk composition and dairy secretion rates of macronutrients in the first year of lactation. American Journal of Clinical Nutrition, 54, 69–80.

Berry D.P., Meaney W.J. (2006): Interdependence and distribution of subclinical mastitis and intramammary infection among udder quarters in dairy cattle. Preventive Veterinary Medicine, 75, 81–91.

Buescher E.S., Mcilherhan S.M. (1988): Antioxidant properties of human colostrum. Pediatric Research, 24, 14–19.

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

Chaquanda M.G., Larsen T., Bjerrinq M., Inqvartsen K.L. (2006): L-lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. Journal of Dairy Research, 73, 431–440.

Chiodini R.J., Van Kruiningen H.J., Merkal R.S. (1984):

Ruminant paratuberculosis (Johne's disease): The current status and future prospects. Cornell Veterinary, 74, 218–262.

Debski B., Picciano M.F., Milner J.A. (1987): Selenium content and distribution of human, cow and goat milk. Journal of Nutrition, 117, 1091–1097.

Drackley J.K. (1999): Biology of dairy cows during the transition period: The final frontier? Journal of Dairy Science, 82, 2259–2273.

Fitz-Gerald C.M., Deeth H.C., Kitchen B.J. (1981): The relationship between the levels of free fatty acids, lipoprotein lipase, carboxylesterase, N-acetylbeta-D-glucosaminidase, somatic cell count and other mastitis indices in milk. Journal of Dairy Research, 48, 52–57.

Fox P.F., Kelly A.L. (2006): Indigenous enzymes in milk: Overview and historical aspects – Part 2. International Dairy Journal, 16, 517–532.

Fox L.K., Shook G.E., Schulk L.H. (1987): Factors related to milk loss in quarters with low somatic cell counts. Journal of Dairy Science, 68, 2100–2104.

Goff J.P., Horst R.L. (1997): Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science, 80, 1260–1268.

Grummer R.R. (1993): Etiology of lipid-related metabolic disorders in periparturient dairy cows. Journal of Dairy Science, 76, 3882–3896.

Halliwell B. (2006): Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology, 141, 312–322.

Hamed H., El Feki A., Gargouri A. (2008): Total and differential bulk cow milk somatic cell counts and their relation with antioxidant factors. Comptes Rendus Biologies, 331, 144–151.

Hanuš O., Frelich J., Tomáška M., Vyletělová M., Genčurová V., Kučera J., Třináctý J. (2010): The analysis of relationships between chemical composition, physical, technological and health indicators and freezing point in raw cow milk. Czech Journal of Animal Science, 55 11–29.

Hojo Y. (1982): Selenium concentration and glutathione peroxidase activity in cows milk. Biology of Trace Element Research, 4, 233–239.

Hojo Y. (1986): Sequential study of glutathione peroxidase and selenium contents of human milk. Science of the Total Environment, 52, 83–91.

Houben E.H., Dijkhuizen A.A., Van Arendonk J.A., Huirne R.B. (1993): Short- and long-term production losses and repeatability of clinical mastitis in dairy cattle. Journal of Dairy Science, 76, 2561–2578.

Hurley W.L. (2001): Mammary gland growth in the lactating sow. Livestock Production Science, 70, 149–157.

Jones D.P., Eklow L., Thor H., Orrenius S. (1981): Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. Archives of Biochemistry and Biophysics, 210, 505–516.

Kaarhm L., Kuosa P.L., Veijalainen K., Sandholm M. (1988): Compartmentalization of milk N acetyl-D-glucosaminidase (NAGase). Release of NAGase from the cellular compartment by storage, freezing and thawing, detergent and using cell stimulants. Journal of Veterinary Medicine B, 35, 408–414.

Kankofer M., Lipko J. (2008): Physiological antioxidative/oxidative balance in bovine colostrum and mature milk. Acta Veterinaria Beograd, 58, 231–239.

Kitchen B.J., Middleton G., Salmon M. (1978): Bovine milk N-acetyl-beta-D-glucosaminidase and its significance in the detection of abnormal udder secretions. Journal of Dairy Research, 45, 15–19.

Kitchen B.J., Kwee W.S., Middleton G., Andrews R.J. (1984): Relationship between the level of N acetyl-beta-D-glucosaminidase (NAGase) in bovine milk and the presence of mastitis pathogens. Journal of Dairy Research, 51, 11–16.

Kunkel J.R., Bushnell R.B., Cullor J., Aleong J. (1987): Studies on induced Klebsiella mastitis with relationships among N-acetyl-D-glucosaminidase, bacterial and somatic cell counts. Cornell Veterinary, 77, 225–231.

Lancelot R., Faye B., Le Scourret F. (1997): Factors affecting the distribution of clinical mastitis among udder quarters in French dairy cows. Veterinary Research, 28, 45–53.

Lindmark-Mansson H., Akesson B. (2000): Antioxidative factors in milk. British Journal of Nutrition, 84, 103–110.

Littell R.C., Henry P.R., Ammerman C.B. (1998): Statistical analysis of repeated measures data using SAS procedures. American Society of Animal Science, 76, 1216–1231.

Mattila T., Sandholm M. (1985): Antitrypsin and N-acetyl-β-D-glucosaminidase as markers of mastitis in a herd of Ayrshire cows. American Journal of Veterinary Research, 46, 2453–2457.

Mellors A. (1968): B-N-acetylglucosaminidase in bovine milk. Canadian Journal of Biochemistry, 46, 451–455.

Miller J.K., Brzezinska-Slebodzinska E., Madsen F.C. (1993): Oxidative stress, antioxidants, and animal function. Journal of Dairy Science, 76, 2812–2823.

Miller R.H., Paape M.J. (1988): Effects of Parity, Bacteriological Status, Stage of Lactation, and Dry Period on N-Acetyl-beta-D-Glucosaminidase Activity of Milk and Dry Secretion. Journal of Dairy Science, 71, 9–14.

Paglia D.E., Valentine W.N. (1967): Studies on the quan-

titative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70, 158–69.

Raes M., Michiels C., Remacle J. (1987): Comparative study of the enzymatic defense systems against oxygen-derived free radicals: the key role of glutathione peroxidase. Free Radical Biology and Medicine, 3, 3–7.

Rendel J., Sundberg T. (1962): Factors influencing the type and incidence of mastitis in Swedish dairy cattle. Acta Veterinaria Scandinavica, 3, 13–21.

Rook J.A., Campling R.C. (1965): Effect of stage and number lactation on the yield and composition cow's milk. Journal of Dairy Research, 32, 45–55.

Sies H. (1993): Strategies of antioxidant defence. European Journal of Biochemistry, 215, 213–219.

Sol J., Sampimon O.C., Barkema H.W., Schukken Y.H. (2000): Factors associated with the cure after therapy of clinical mastitis caused by *Staphylococcus aureus*. Journal of Dairy Sciences, 83, 278–284.

Toyokuni S. (1999): Reactive oxygen species-induced molecular damage and its application in pathology. Pathology International, 49, 91–102.

Ursini F., Maiorino M., Brigelius-Flohe R., Aumann K. D., Roveri A., Schomburg D. (1995): Diversity of glutathione peroxidases. Methods in Enzymology, 252, 38–53.

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