

Determination of antioxidant indices in dairy cows during the periparturient period

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The aim of the present study was to evaluate indicators of antioxidant status – glutathione peroxidase (GPx); superoxide dismutase (SOD); total antioxidant status (TAS); vitamins A, E and beta carotene in 10 dairy cows of the Holstein breed from 15–20 days *ante partum* (a.p.) to 25–30 days *post partum* (p.p.). Blood samples were collected 5 × during this period: 15–20 days a.p., 1–3 days a.p., 2–3 days p.p., 10–15 days p.p. and 25–30 days p.p. The mean GPx activity was significantly ($P = 0.013$) lower in the 10–15 days p.p. compared to 15–20 days a.p. ($P < 0.05$) and 1–3 days a.p. ($P < 0.01$). The increase of SOD values was monitored throughout the whole experiment. The mean TAS concentration was significantly ($P < 0.01$) decreased 2–3 days p.p. compared to 25–30 days p.p. A significantly lower mean concentration of vitamin A was also found 2–3 days p.p. compared to the concentration 15–20 days a.p. ($P < 0.05$) and 25–30 days p.p. ($P < 0.001$). A significant ($P < 0.001$) decrease in vitamin E concentration was recorded in cows 2–3 days p.p. compared to cows 15–20 days a.p. and 25–30 days p.p. The mean concentration of beta carotene was also decreased immediately after calving. Significant changes in the concentration of antioxidant parameters during the periparturient period indicate the occurrence of oxidative stress in dairy cows which can contribute to increased incidence of metabolic diseases.

Oxidative stress, antioxidant status, glutathione peroxidase, superoxide dismutase, vitamins

Under normal physiological conditions, the production of reactive oxygen species (ROS) and the function of the antioxidant defence system (ADS) are in balance (Miller et al. 1993). The periparturient period, i.e. 3 weeks before to 3 weeks after parturition, is a challenging time for dairy cows when cows are more susceptible to many diseases (Spears and Weiss 2008). Changes in protein and lipid metabolism occur during this period. The energy demand is higher, particularly after parturition, for which reason a negative energy balance (NEB) arises. Energy deficiency causes increased lipid mobilization which leads to lipid peroxidation and further creation of ROS (Sordillo 2005; Sordillo and Aitken 2009; Pilarczyk et al. 2012). A larger amount of ROS causes oxidative stress and may contribute to a higher incidence of diseases after parturition (Miller et al. 1993). Common diseases include liver steatosis and ketosis linked to NEB and a disruption of energy metabolism and mastitis, metritis and retained placenta which are associated with a disturbed immune function (Esposito et al. 2014; Illek 2017). Antioxidants play a key role in immunity and health in transition cows. A deficiency of vitamins (important cellular antioxidants) can cause a weakening of immunity and lead to the above-mentioned diseases (Spears and Weiss 2008). Therefore, the antioxidant levels should be controlled and used for the monitoring of oxidative stress.

The aim of this study was to determine antioxidant indices such as glutathione peroxidase (GPx), superoxide dismutase (SOD), total antioxidant status (TAS), and vitamins A, E and beta carotene. The changes in the concentration of the determined indices during the periparturient period were subsequently analysed.

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Materials and Methods

Animals and diets

The study was carried out using 10 Holstein dairy cows at a farm located in the village of Uherčice (Břeclav, South Moravia). Blood samples were taken at five different stages: 15–20 days and 1–3 days *ante partum* (a.p.) and 2–3 days, 10–15 days and 25–30 days *post partum* (p.p.). Antioxidant indices were determined in a total of 50 blood samples (10 samples/cows for each group). Only cows with a single pregnancy were included in the experiment. All of the cows were fed a total mixed ration (TMR) according to the antepartum and the postpartum period (average body condition score – BCS of 3.6 a.p.) and had gone through two or more lactations (Table 1). The mean milk production was 11,161 litres per last lactation.

Table 1. Total mixed ration composition (kg/day/cow).

Ingredients	<i>Ante partum</i>	<i>Post partum</i>
Alfalfa hay	0	1
Barley straw	2.2	0
Concentrate (DOVP)	0	5
Concentrate (DOVP – a.p.)	2.5	0
Pamitate	0	0.25
mp iont	0.5	0
High moisture corn	0	3.8
Brewer's grains	6	10
Wholecrop rye	4	8
Alfalfa haylage	0	6
Maize silage	14	9

DOVP – complementary feed for lactating dairy cows;
DOVP – a.p. - complementary feed for dairy cows *ante partum*

of these two indices. Vitamin A, E and beta carotene concentrations in serum were determined using the HPLC system Ultimate 3000 (Dionex, USA).

Statistical analysis

The obtained results were tested for homogeneity of variances (Hartley-Cochran-Bartlett test) and normality of distribution (Shapiro-Wilk test). The data were analysed statistically by one-way analysis of variance (ANOVA) followed by the Fisher's LSD *post hoc* test. All results were expressed as mean value (\bar{x}) \pm standard deviation (SD).

Results

The mean GPx activity was significantly lower (866.1 μ kat/l) in the fourth blood sampling (10–15 days p.p.) compared to the first (15–20 days a.p.; $P < 0.05$) and second (1–3 days a.p.; $P < 0.01$) blood samplings. A significant decrease (747.9 μ kat/l) in the GPx activity was also recorded 25–30 days p.p. compared to the blood collections before parturition ($P < 0.001$). Increased SOD values were recorded during the whole periparturient period. The lowest mean TAS concentration (0.78 mmol/l) was recorded in the third blood sampling (2–3 days p.p.) and was significantly lower ($P < 0.01$) compared to the fifth blood sampling (25–30 days p.p.). A significantly decreased TAS concentration (0.84 mmol/l) was also found at 10–15 days p.p. compared to the TAS concentration at 25–30 days p.p. ($P < 0.05$). A significantly lower mean concentration of vitamin A (0.48 μ mol/l) was found in the third blood sampling (2–3 days p.p.) compared to the concentration in the first (15–20 days a.p.; $P < 0.05$), second (1–3 days a.p.; $P < 0.01$) and fifth (25–30 days p.p.; $P < 0.001$) blood samplings. A significant decrease in vitamin E concentration (4.10 μ mol/l) was also recorded in the cows at 2–3 days p.p. compared to the cows at 15–20 days a.p. ($P < 0.001$), 1–3 days a.p. ($P < 0.01$) and 25–30 days p.p. ($P < 0.001$). The lowest mean beta carotene concentration (3.96 μ mol/l) was observed 2–3 days p.p. as with vitamins A and E (Table 2).

Sampling and analysis

Blood samples were collected from vena coccygea media into Hemos sampling tubes without an anticoagulant for serum determination and into Hemos sampling tubes with a heparin anticoagulant for determination of whole blood. After the sampling, blood samples were delivered to the laboratory and processed according to standard procedures. For serum samples, blood was allowed to coagulate at room temperature and centrifuged at 1467 g for 10 min. Blood samples were used for analysis or stored at -70 °C immediately after collection. GPx activity in whole blood was measured with a RANSEL kit (Randox Laboratories Ltd., UK) using a UV method based on that of Paglia and Valentine (1967). A Liasys automatic biochemical analyser (AMS, Italy) was used for determination of GPx activity. The activity of SOD in erythrocyte lysate and serum TAS concentration were measured using standardized kits supplied by Randox Laboratories Ltd. An automatic Cobas Mira Plus biochemical analyser (Roche, Switzerland) was used for the measurement

Table 2. Antioxidant indices in dairy cows in the period from 15–20 days *ante partum* to 25–30 days *post partum*.

Sampling no.		GPx $\mu\text{kat/l}$	SOD U/ml	TAS mmol/l	vit A $\mu\text{mol/l}$	vit E $\mu\text{mol/l}$	$\beta\text{-Car}$ $\mu\text{mol/l}$
1	x	1035.6 ^{a,a}	331.2	0.87	0.76 ^a	8.06 ^{a,a}	5.30
15–20 days a.p.	SD	150.46	28.95	0.06	0.12	1.39	1.66
2	x	1067.2 ^{A,b}	348.8	0.81 ^A	0.79 ^A	7.29 ^A	4.87
1–3 days a.p.	SD	115.33	26.16	0.10	0.17	1.38	1.16
3	x	982.6 ^{β,c}	368.5	0.78 ^B	0.48 ^{a,β,A,a}	4.10 ^{A,a,b}	3.96
2–3 days p.p.	SD	147.18	80.00	0.12	0.13	2.08	1.35
4	x	866.1 ^{a,β,A}	347.8	0.84 ^a	0.71 ^{β,γ}	5.91 ^a	4.42
10–15 days p.p.	SD	120.69	89.36	0.08	0.22	1.30	1.78
5	x	747.9 ^{a,b,c}	371.5	0.95 ^{a,A,B}	0.93 ^{γ,a}	7.83 ^b	4.36
25–30 days p.p.	SD	85.10	42.11	0.09	0.29	3.24	2.09

x – mean value; SD – standard deviation; significant differences between groups are indicated by using the same indices in a column: ^{a, β} – $P < 0.05$; ^{A, B} – $P < 0.01$; ^{a, b, c} – $P < 0.001$; a.p. – *ante partum*; p.p. – *post partum*; GPx – glutathione peroxidase; SOD – superoxide dismutase; TAS – total antioxidant status; vit A – vitamin A; vit E – vitamin E; $\beta\text{-Car}$ – beta carotene

Discussion

The results demonstrate a decrease in antioxidant indices associated with the incidence of oxidative stress in the early postpartum period in dairy cows.

In the study, decreased GPx activity was recorded in all postpartum blood collections which is in accordance with our other study Píšťková et al. (2018) in which a significant difference in the GPx activity was recorded immediately after parturition and at 1 week p.p. Konvičná et al. (2015) also reported a decreased GPx activity at 1 week p.p. as a reason for increasing postpartum oxidative stress. The selenium-dependent antioxidant enzyme GPx, as one of the main components of the ADS, is extremely important in protecting the organism against ROS (Pilarczyk et al. 2012). In view of this fact, it is widely used in evaluating the antioxidant status. It is also used in the indirect determination of selenium status and indicates long-term selenium supplementation (Pavlatá et al. 2000; Illek et al. 2017).

Another enzyme involved in the ADS is Mn, Cu and Zn-dependent SOD. These enzymes are considered the first line of defence against pro-oxidants (converting the superoxide to hydrogen peroxide) and are also among the main intracellular antioxidants (Bernabucci et al. 2005; Machado et al. 2014). In the present study, the highest level of SOD was found 25–30 days p.p. The value increased throughout the entire experiment, except for the fourth blood collection when a slight decrease was recorded. The trend for increasing SOD was also observed in the study by Konvičná et al. (2015). Gaál et al. (2006) also described SOD activity in cows, and found a significant increase of SOD activity at calving as compared to a control group (pre- and post-calving). Bernabucci et al. (2005) recorded the highest SOD activity at 4 days before calving with a subsequent decline to pre-natal values. As reported by Barja de Quiroga et al. (1992), the antioxidant system seems to be under homeostatic control. The increase in GPx and SOD activity before parturition could be the result of an effort to cope with the increasing amount of ROS (Bernabucci et al. 2005). However, the insufficient amount of antioxidants and continually increasing amount of ROS led to a decrease in the GPx activity. The increased SOD activity during the entire experiment was not significant and is probably not sufficient to prevent the occurrence of oxidative stress (Fig. 1).

The TAS concentration expresses the antioxidant capacity of the organism, provides information about the concentration of antioxidants in blood and provides information

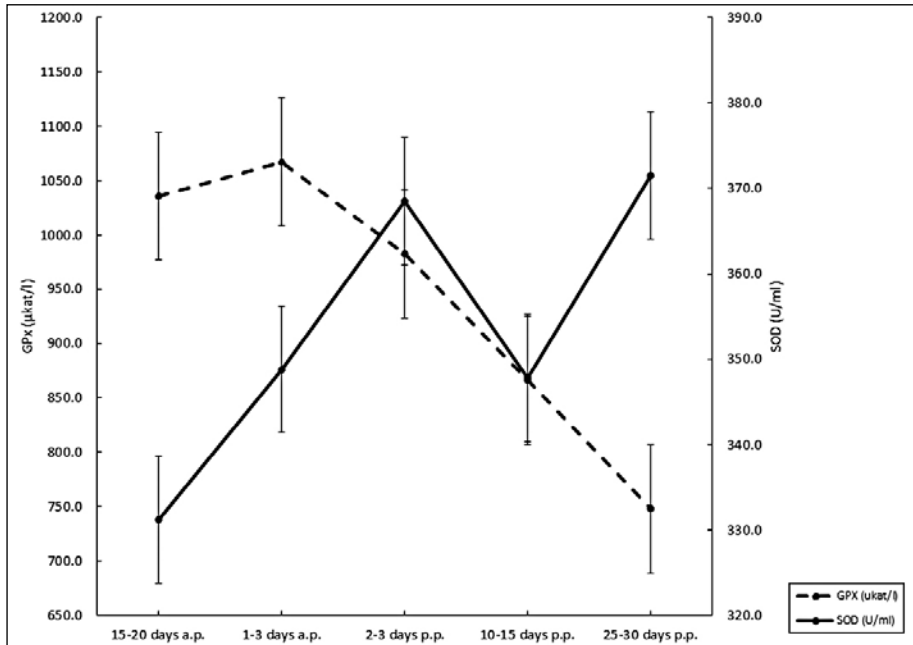


Fig. 1. Mean glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities in dairy cows during the periparturient period

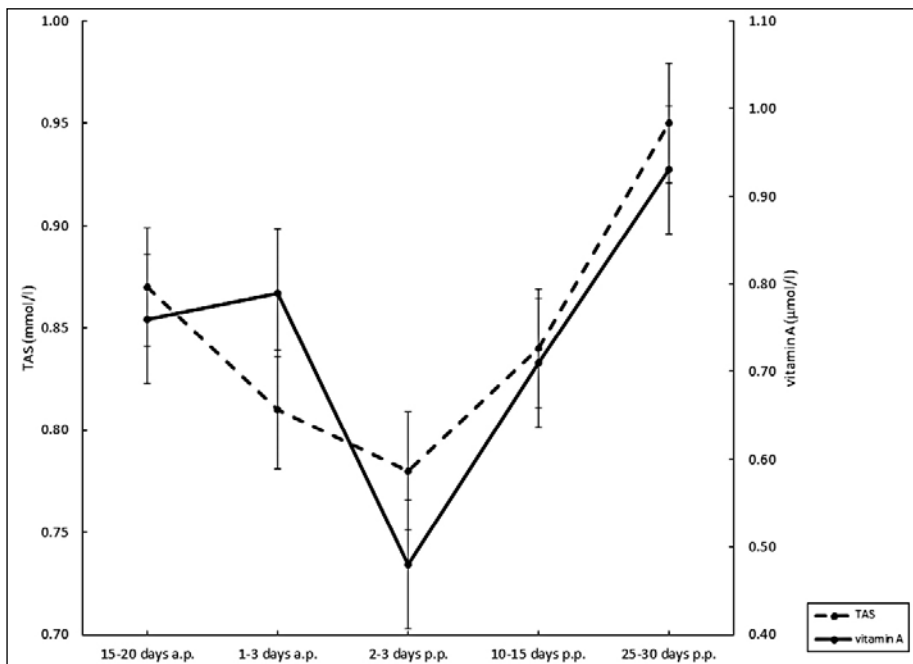


Fig. 2. Mean total antioxidant status (TAS) and vitamin A concentrations in dairy cows during the periparturient period

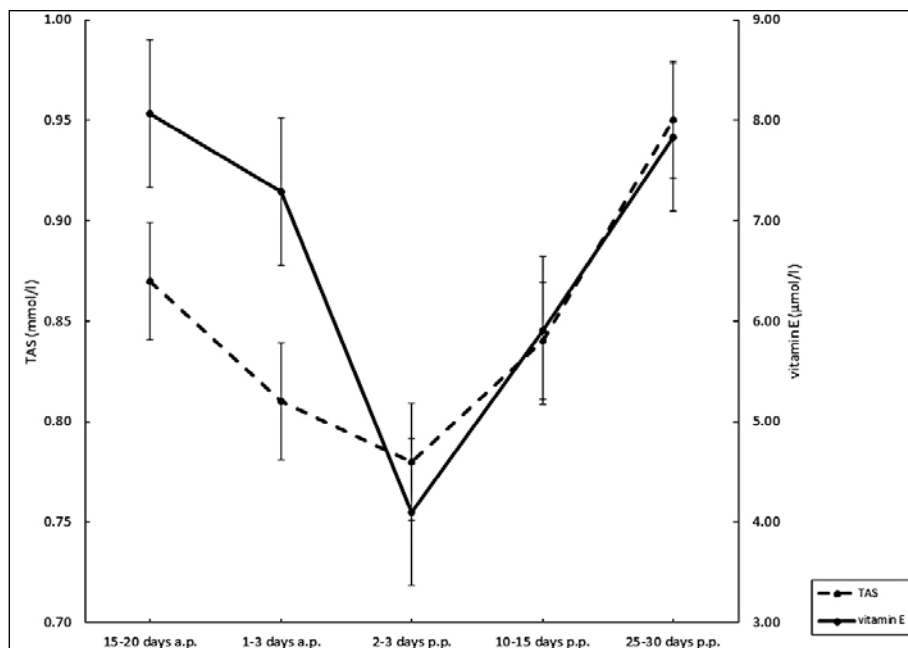


Fig. 3. Mean total antioxidant status (TAS) and vitamin E concentrations in dairy cows during the periparturient period

about the pro-oxidants to antioxidants balance (Ghiselli et al. 2000). In our study, the lowest TAS was found at 2–3 days p.p., as were the lowest vitamin A, E and beta-carotene concentrations which correspond to the total antioxidant capacity of the organism (Figs 2, 3). A decreased TAS level in the early lactation period was also found by Gong and Xiao (2016) and Píšťková et al. (2018).

Vitamins as non-enzymatic antioxidants are important free radical scavengers and are therefore an important part of the ADS (Sordillo and Aitken 2009). Their deficiency is typical for the periparturient period mainly because of their increase in the colostrum (Rizzo et al. 2013). In our study, a decrease in vitamin concentrations around parturition was also observed, as well as in the study by Píšťková et al. (2018) in which a significantly decreased vitamin A concentration was found immediately after parturition and the vitamin E concentration was significantly lower at 1 week p.p. This could be due to the fore-mentioned release of vitamins from blood into the colostrum and an insufficient intake in the diet. Konvičná et al. (2015) recorded the lowest vitamin E value at 1 week p.p., followed by an increase in concentration until 9 week p.p. This is in agreement with our findings which also recorded an increase in all determined vitamins from 2–3 days p.p. until the last blood collection (25–30 days p.p.).

According to the study by Bernabucci et al. (2005), the cows in our experiment could be considered cows with a higher BCS (3.6 a.p.) which could be more sensitive to the occurrence of oxidative stress in the transition period. This fact may also contribute to explaining the decreased antioxidant concentrations in dairy cows after parturition.

There are several studies dealing with the relationship between oxidative stress and the incidence of periparturient diseases in dairy cows. One of the important predisposing factors for retained foetal membranes is the antioxidant status in dairy cows before parturition. According to the study by Pontes et al. (2015), supplementation with vitamin E during

the prepartum period resulted in a reduced incidence of stillbirth and retained foetal membranes in dairy cows. The study by Kizil et al. (2010) focused on oxidative stress in dairy cows with acute puerperal metritis and determined plasma malondialdehyde (MDA) concentrations as a secondary product of lipid peroxidation. An increased mean MDA concentration and significantly decreased vitamin A, E, C and beta-carotene concentrations were found in cows with puerperal metritis compared to the control group. In addition to these diseases, dairy cows are also prone to mastitis during the periparturient period. Production of ROS followed by oxidative stress, which may be one of the reasons for the disease, is increased because of the high energy demand caused by rapid differentiation of secretory parenchyma, intense mammary gland growth and onset of milk synthesis (Sordillo 2005). Oxidative stress can also contribute to bovine pneumonia in dairy cows. In some cases, neutrophils secrete reactive nitrogen intermediates (NO⁻) in the lungs and alter acute or chronic inflammatory reactions. Tissue damage is caused by reaction with O₂⁻ (Lykkesfeldt and Svendsen 2007).

In conclusion, our results confirm that the periparturient period is critical for the occurrence of oxidative stress, especially early *post partum*. Dairy cows are then more susceptible to the diseases listed above. Determination of antioxidant levels can be used to monitor the oxidative stress in order to prevent the incidence of the said diseases in dairy cows.

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