

Effect of oxidative status on the occurrence of haemolactia in dairy cows after calving

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Abstract

Introduction: Dairy cows may infrequently give milk tinged with blood after calving, which is a condition termed haemolactia. Economic losses for dairy farmers are caused by cases of haemolactia because of the condemnation of such milk, potential contamination of good bulk tank milk with haemolactic milk, and need for veterinarian intervention. This study was performed to elucidate the oxidative status of dairy cows with haemolactia during the peripartum period. **Material and Methods:** Plasma glutathione peroxidase, malondialdehyde (MDA) and superoxide dismutase concentrations along with serum vitamin A, C and E concentrations were determined as indices of oxidative stress. The sampled dairy cows comprised two haemolactic (n = 11 and n = 6) and two non-haemolactic (n = 11 and n = 6) groups. **Results:** On the first day when haemolactia was identified in colostrum (at mean 2.1 days after parturition), a significantly increased concentration of plasma MDA was noted in the haemolactic group. During the prepartum period, low levels of serum vitamin E were continuously observed from prepartum week 4 to the parturition day but only in the haemolactic group. **Conclusion:** These results demonstrate that continuous low levels of serum vitamin E in the prepartum period may play a pivotal role as a requisite factor in the onset of haemolactia after calving.

Keywords: dairy cows, haemolactia, malondialdehyde, oxidative stress, vitamin E.

Introduction

After calving, dairy cows occasionally produce blood-tinged milk that is light pinkish to dark reddish in colour; haemolactia is the term for the condition these animals are diagnosed with (3, 5, 7, 11). Haemolactia causes serious economic losses because the discarding of all milk containing blood and protracted veterinary care are necessary and the elimination of risk of the mixture of this milk with acceptable bulk milk must be guaranteed. The pathophysiology of haemolactia involves local or systemic hyperaemia and/or increased blood vessel permeability due to mastitis, trauma or unknown causes (5, 11, 15). However, little information is available on the pathogenesis underlying the mechanism of haemolactia.

According to previous reports (3, 5, 15), haemolactia in dairy cows occurred within one week of parturition, and no marked changes were noted in

routine haematology, coagulation, serum chemistry or urinalysis. In humans, increased oxidative stress accompanied by reduced endogenous defences has been reported to play a role in the pathogenesis of several diseases during the pre- and perinatal period (12). Based on this information, we focused on oxidative status during the peripartum period because transition cows are exposed to complicated stress leading to immune dysregulation and inflammation associated with infection and metabolic disorders (10, 17, 19, 23). As plasma or serum indices of oxidative stress, the enzymatic antioxidants glutathione peroxidase (GPx) (8) and superoxide dismutase (SOD) (4), the degradation product malondialdehyde (MDA) (9) from peroxidised lipids and the nonenzymatic antioxidant vitamins A (14), C (21), and E (16, 22) have all been measured. These have been established in dairy cows as biomarkers for evaluating oxidative stress during late pregnancy and early lactation (1, 2, 10, 18, 20).

The aim of the study was to delineate the oxidative status of dairy cows with haemolactia and to detect the sequential changes in oxidative status at the peripartum stage.

Material and Methods

Animals. Dairy cows were reared in stalls on 10 commercial farms (Miyagi, Japan), where incidences of haemolactia occurred. Cows were twice daily fed 6 to 8 kg of feed on a dry matter basis and provided tap water *ad libitum*.

Criterion of haemolactia. The classification of haemolactia was conducted visually based on the findings of a previous report (11). The cows with haemolactia were divided into three grades by milk appearance as follows: slightly (pale pink milk with trivial to spot-like erythrocyte sediment in centrifugation), moderately (pink milk with thin-layered erythrocyte sediment) or severely (dark red milk with thick-layered erythrocyte sediment) tinged with blood.

Experiments. This study consisted of two experiments (Table 1). In the first experiment (Exp. 1), to delineate the oxidative status of dairy cows with haemolactia, two groups of 11 dairy cows were utilised, one haemolactia and the other non-haemolactia. Examinations were conducted using blood samples collected on the day when haemolactia was first identified in the colostrum, namely between parturition day and 5 days postpartum. Only the cows showing severe haemolactia were assigned to the haemolactia group in this study, because it was expected that there would be considerable fluctuations in oxidative indices. The cows in the non-haemolactia group were selected from the animals with the same parturition day, which was an average of 2 days after parturition, and served as the control group. There were no substantial differences in body weight (haemolactia: 658 ± 12.1 kg vs. non-haemolactia: 658 ± 13.9 kg), body condition score (BCS, haemolactia: 3.36 ± 0.05 vs. non-haemolactia: 3.41 ± 0.06), or parity (haemolactia: 2.6 ± 0.3 vs. non-haemolactia: 2.5 ± 0.3) between the two groups.

In the second experiment (Exp. 2), to detect the sequential changes in oxidative status at the peripartum

stage, 20 pregnant dairy cows were registered in prepartum week 5. The expected delivery date was determined from the date of artificial insemination. Their milk after calving was graded as non- ($n = 9$), slightly ($n = 3$), moderately ($n = 6$) or severely ($n = 2$) haemolactia. The six cows with moderate haemolactia were selected for this study because of the limited number of cows with severe haemolactia. Six of the nine cows in the non-haemolactia group were selected randomly as the corresponding control. There were no large differences in body weight (haemolactia: 700 ± 7.3 kg vs. non-haemolactia: 708 ± 9.8 kg), BCS (haemolactia: 3.50 ± 0.06 vs. non-haemolactia: 3.46 ± 0.08) or parity (haemolactia: 2.3 ± 0.3 vs. non-haemolactia: 1.8 ± 0.4) between the two groups. The same oxidative indices as in Exp. 1 were measured five times (in prepartum weeks 4 and 2, on the parturition day and at postpartum weeks 2 and 4) during the peripartum period.

Cows showing mastitis or trauma were excluded from the study based on the results of a modified California mastitis test (PLT; ZENOAQ, Fukushima, Japan) and macroscopic examination of milk, teats and udder. Cows diagnosed with hypocalcaemia, ketosis, fatty liver or abomasal displacement were also excluded.

Blood collection and analysis of oxidative indices. Individual blood samples were collected from the caudal vein and transported to the laboratory in ice boxes shortly after collection. The plasma levels of GPx, MDA (assay wavelength: 532 nm) and SOD were determined using commercially available kits (Northwest Life Science Specialties, Vancouver, WA, USA). Retinol (vitamin A), α -tocopherol (vitamin E) and ascorbic acid (vitamin C) serum levels were quantified using reversed-phase high performance liquid chromatography and ion pair reversed phase high performance liquid chromatography methods and a Prominence chromatograph (Shimadzu, Kyoto, Japan).

Statistical analysis. Numerical data are expressed as the mean \pm standard error of the mean (SEM). Statistical evaluation was performed using Student's *t*-test or repeated-measures analysis of variance with Free EZR version 1.40 (Saitama Medical Centre, Jichi Medical University, Saitama, Japan). Statistical significance was set at $P < 0.05$.

Table 1. Group composition and blood sampling time

Experiment	Study group	Haemolactia grade	n	Prepartum week			Postpartum week	
				-4	-2	0	2	4
Exp. 1	Haemolactia	Severe	11	-	-	○ ^{a)}	-	-
	Non-haemolactia	-	11	-	-	○ ^{a)}	-	-
Exp. 2	Haemolactia	Moderate	6	○	○	○ ^{b)}	○	○
	Non-haemolactia	-	6	○	○	○ ^{b)}	○	○

Moderate grade: pinkish milk with thin-layered erythrocyte sediment

Severe grade: dark reddish milk with thick-layered erythrocyte sediment

○ – blood collection; – – no blood collection; ^{a)} – on the day when haemolactia was found; ^{b)} – on parturition day

Results

In Exp. 1, in which the experimental cows were severely haemolactic, the time identified as the first incidence of haemolactia in the colostrum was 2.1 ± 0.5 days after parturition. The average plasma MDA value on the day of onset significantly increased in the haemolactic group ($4.63 \pm 0.28 \mu\text{mol/L}$) compared to that in the non-haemolactic group ($3.12 \pm 0.23 \mu\text{mol/L}$) without any changes in GPx, SOD or vitamin levels (A, C and E) in plasma or serum (Fig. 1).

In Exp. 2, in which the experimental cows were moderately haemolactic, low levels of serum vitamin E were observed continuously from prepartum week 4 to the parturition day in the haemolactic group (mean range: $150\text{--}200 \mu\text{g/dL}$ vs. non-haemolactic group: $290\text{--}310 \mu\text{g/dL}$) without any changes in plasma MDA value. Alterations in plasma GPx, SOD and vitamin A serum were noted sporadically throughout the observation period (Fig. 2).

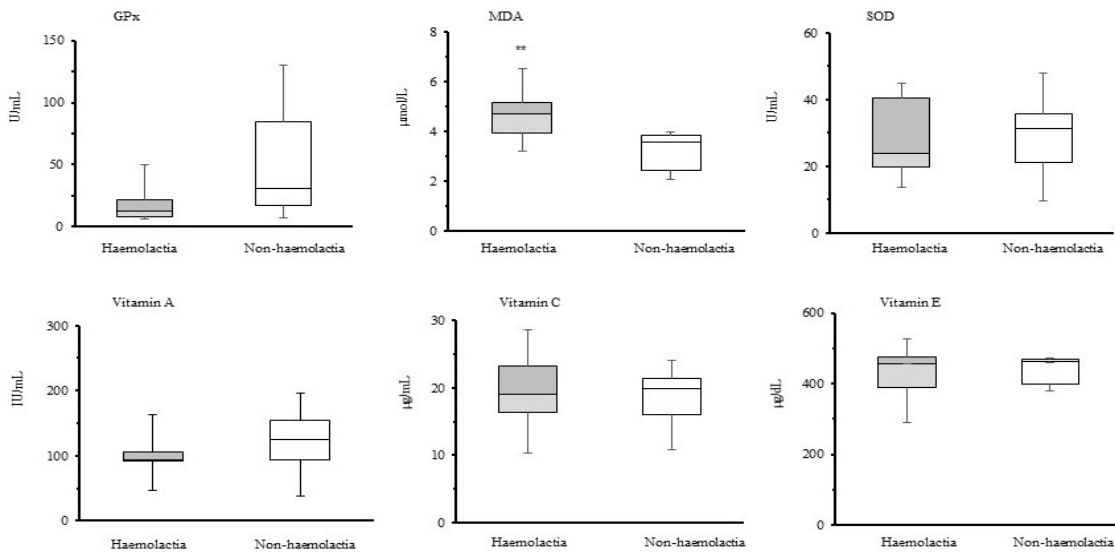


Fig. 1. Changes in plasma or serum indices of oxidative stress in dairy cows with haemolactia (solid grey column, n = 11) or without haemolactia (void column, n = 11) after calving. Upper bars – maxima; lower bars – minima; horizontal bars through boxes – medians; GPx – glutathione peroxidase; MDA – malondialdehyde; SOD – superoxide dismutase. ** – P < 0.01 vs. non-haemolactic group

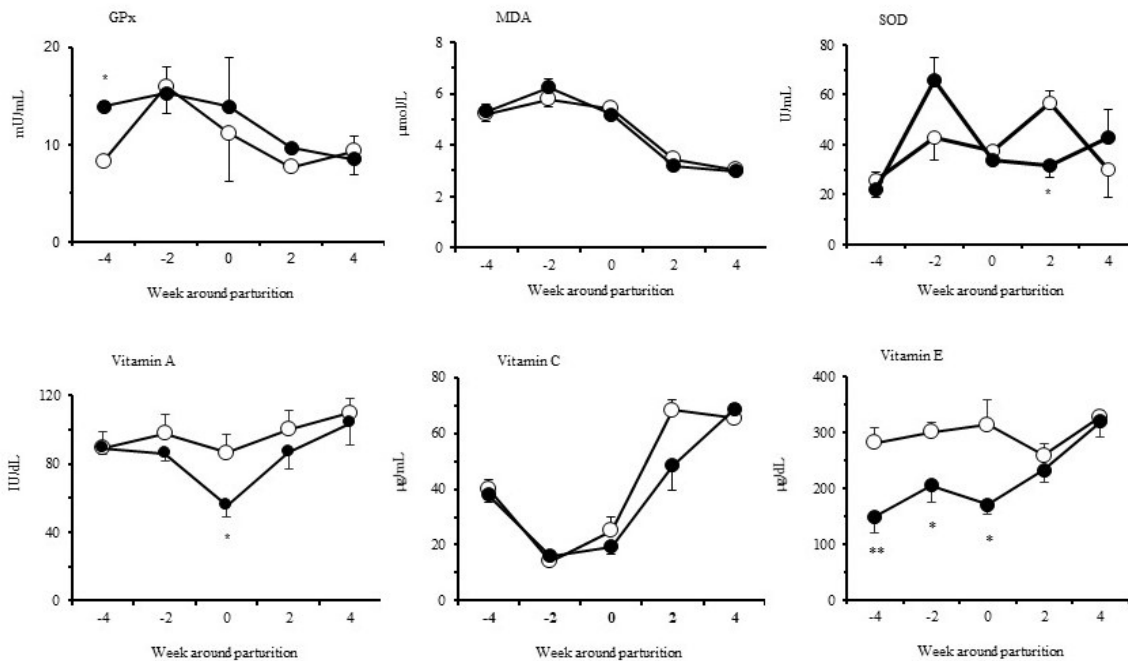


Fig. 2. Changes in plasma or serum indices of oxidative stress in dairy cows with haemolactia (solid circles, n = 6) or without haemolactia (void circles, n = 6) during the peripartum period. Values are expressed as the mean \pm standard error of the mean. * – P < 0.05 and ** – P < 0.01 vs. non-haemolactic group; GPx – glutathione peroxidase; MDA – malondialdehyde; SOD – superoxide dismutase

Discussion

The onset day of haemolactia after calving was highly consistent with the day stated in previous reports (5, 15). Differences in fluctuations of MDA and vitamin E levels were found between Exp. 1 and Exp. 2; however, these may relate to some extent to dietary provision. Vitamin E serum levels decreased only in the prepartum period of Exp. 2 but did not during the postpartum period of this experiment or Exp. 1. This may be partially explained by the reduced intake of dietary vitamin E in the prepartum period (3), followed by adaptive response of vitamin E (recovery from a deficient level to the control level with increased feed intake) to MDA overproduction immediately after calving in Exp. 1. However, further studies are required to determine this difference. The clinical importance of the sporadic changes in plasma GPx and SOD levels and serum vitamin A in Exp. 2 remains unknown.

The present data and data from previous reports (3, 9, 15, 16, 22) imply the same underlying pathophysiological process. Vitamin E is a requisite element of the nutritional regimens that meet the antioxidant requirements of dairy cows, especially in the peripartum period (3, 16, 22). Several reports suggest vitamin E at the recommended level enhances the host's defence against metabolic diseases and confers protection against them to transition cows (1, 6, 16, 22). Considering that gestation or parturition is accompanied by the high energy demand of various bodily functions with an increased oxygen requirement (13), deteriorated levels (*i.e.* continuously decreased vitamin E) of antioxidant capacity during the prepartum period followed by additional oxidative stress due to parturition may contribute to the development of lipid peroxidation (increased MDA). The overproduction of MDA has been reported to increase the risk of oxidative stress (1, 3, 9), which may be a mediator of endothelial dysfunction. Retrospective surveillance of dairy cows with haemolactia in Japan (15) by microscopy found that of two indicia, namely increases in mammary epithelial cells (including their cell debris) and neutrophils and blood clots in the milk sediment, the former was more extensive in severe cases, presumably because of epithelial damage to the mammary gland. Therefore, continuously low levels of serum vitamin E in the prepartum period appear to play a key role in the onset of haemolactia.

Recently, it was reported that the calculation of the relative ratio of blood oxidants (MDA) to antioxidants can provide a more accurate representation of the redox status (balance) in the transition period of dairy cows (1, 2). Among the ratios calculated in this study, only the MDA/vitamin E ratio ($\times 100$) significantly increased from the prepartum period (Exp. 2 haemolactia group: mean range 3.21–3.88 *vs.* non-haemolactia group: 1.88–1.95, $P < 0.05$) to the day when haemolactia was identified (Exp. 1 haemolactia group: 1.18 ± 0.10 *vs.* non-haemolactia group: $0.74 \pm$

0.07 , $P < 0.01$). Observable high MDA/vitamin E ratios during the peripartum period may be useful in the profiling of the pathophysiological process (such as continual redox imbalance) in haemolactia after calving.

In conclusion, it is suggested that continuously low levels of serum vitamin E in the prepartum period may play a pivotal role as a requisite factor in the onset of haemolactia, and that high MDA levels in plasma after calving may reflect at least in part the degraded physiological status of the mammary gland. Further studies are necessary to determine whether an appropriate vitamin E-supplemented diet during the prepartum period would prevent the onset of haemolactia after calving.

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