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Relationships between trace elements, oxidative stress and subclinical ketosis during transition period in dairy cows

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Abstract

The possible relationships between trace elements, oxidative stress and subclinical ketosis during the transition period were evaluated in dairy cows. Blood samples were obtained by jugular venipuncture at four times of the transition period: 3 weeks and 1 week before and 1 week and 3 weeks after calving. The highest concentrations of malondialdehyde (MDA) and beta-hydroxybutyrate (BHB) were observed on the week 1 after calving. In contrast, the serum values of copper, zinc, albumin, uric acid and TIBC decreased after calving. After calving, zinc concentration showed a significant negative correlation with MDA concentration ($p<0.005$). The concentrations of MDA after calving showed a positive correlation with BHB ($P<0.005$), while zinc showed a negative correlation with BHB at this time ($p<0.05$). The results of logistic regression test showed that the decreased concentration of zinc in the weeks 1 before and week 1 after calving was associated with the probability of occurrence of subclinical ketosis. Moreover, an increase in MDA concentration at the week 1 after calving was associated with the risk of subclinical ketosis. The results of the present study showed that cattle with insufficient concentrations of trace elements are prone to oxidative stress and cattle with increased oxidative stress may be subclinical Ketosis-prone.

Keywords: oxidative stress, trace elements, subclinical ketosis, transition period, dairy cow

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Introduction

The period from three weeks before to three weeks after calving is considered the transition period (Quiroz-Rocha *et al*., 2009a). During the dairy cows' production cycle, the transition period is critical due to the substantial metabolic and physiological adaptations that accompany parturition and the initiation of lactation (Quiroz-Rocha e*t al*., 2009b). Negative energy balance (NEB) and suboptimal mineral levels are common in the transition period in cows (Bell, 1995; Goff, 2006). Negative energy balance is partially caused by reduced feed intake around the time of calving, and the fast-growing demand for energy resulting from increased milk production (Grummer *et al*., 2004; Gehrke and Markiewicz, 2009). Elevated levels of circulating ketone bodies occur in early lactation in response to the homeorhetic drive to sustain high levels of milk production at a time when dry matter intake is reduced. Subclinical ketosis is a condition marked by increased levels of the circulating ketone bodies without the presence of the clinical signs of ketosis (Duffield, 2000). Coordinated shifts in nutrient partitioning must occur in order to meet the increased demand for energy and other nutrients necessary for fetal growth and lactation (Sordillo and Aitken, 2009). This mainly triggers catabolic pathways which, at the cellular level, increase the production of reactive oxygen species (ROS) (Pedernera *et al*., 2010). Although ROS are unavoidable products of normal metabolic processes and are not always harmful, when produced in excessive quantities, or at rates faster than they can be removed by antioxidant mechanisms, ROS may lead to the development of oxidative stress (Nordberg and Arnér, 2001).

Recent studies have reported variable degrees of oxidative stress in the transition period of dairy cows (Bernabucci *et al*., 2005; Castillo *et al*., 2005; Sordillo, 2005; Lykkesfeldt and Svendsen, 2007; Sordillo and Aitken, 2009). Although involvement of oxidative stress in etiologies of certain disorders of dairy cows is suggested by reductions in incidence of retained placenta and mastitis when the antioxidants, vitamin E and Se, are supplemented (Miller *et al*., 1993), its direct effects on the animal performance or health remain unclear.

Several defense mechanisms are available to prevent oxidative damage including antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, low molecular weight antioxidants such as glutathione, vitamin E, ubiquinone, β-carotene, ascorbic acid, transfer in and uric acid, (Lykkesfeldt and Svendsen, 2007) and thiol (SH) groups on proteins such as albumin, which have strong reducing properties (Soriani *et al*., 1994; Moran *et al*., 2001). Trace elements are also essential components of the antioxidant defense of the body that play an important role in the prevention of free radical-induced damages to tissues (Evans and Halliwell, 2001). A number of trace minerals such as iron, copper and zinc are involved in the antioxidant defense system and changes of any of these nutrients may cause oxidative stress in transition cows (Miller *et al*., 1993). Trace elements also play a vital role in augmenting production and reproduction and in prevention of disorders such as mastitis, lameness and retained placenta in the transition period (Wilde, 2006). However, there is no information available concerning the relationships between trace elements and oxidative stress in dairy cows during transition period.

In the present study, we assessed the influence of oxidative stress [malondialdehyde (MDA) as a marker of lipid peroxidation and antioxidants including albumin, uric acid and total iron binding capacity (TIBC)] and trace element changes (zinc, copper and iron) on the occurrence of subclinical ketosis. In addition, the possible relationships between trace elements and oxidative stress during transition period were evaluated.

Materials and methods

Animals, Housing, and Feeding

The trial was carried out in a commercial dairy herd consisting of 850 lactating cows. This herd consisted of pure bred animals of Holstein breed. The herd was totally confined in free-stall housing without access to pasture. The rolling herd average for milk production was between 10750 and 11800 kg. Cows were dried two months before the expected time of parturition and were transferred to a separate stall. Dry cows' fad diet consisted of 19 kg of corn silage, 1 kg of Alfalfa and 7 kg of concentrate composed of corn (50%), wheat bran (8%), barley (16%), soybean meal (26%) and vitamin and mineral supplement (0.5%). The lactating cows fad diet consisted of 25 kg of corn silage, 2.1 kg of Alfalfa and 15 kg of concentrate composed of wheat bran (2.4%), corn (36%), barley (9.5%), cotton seed meal (13%), cotton seed (11%), soybean meal (19.5%), fish meal (2.5%), fat supplement (2.5%), sodium bicarbonate (1%), vitamin and mineral supplement (1%), oyster shell powder (1.4%) and salt (0.2%). Fifty-two pregnant Holstein cows were selected based on their expected calving date. The animals were monitored during the last 30 days of pregnancy and the first 30 days in milk (DIM). All cows had a normal easy calving and no clinical abnormalities were seen during the postpartum period. During the study period all of the animals were kept under identical conditions.

Sample collection and analytical procedures

Blood samples were obtained by jugular venipuncture at four times: 3 weeks and 1 week before and 1 week and 3 weeks after calving. Samples were collected into evacuated tubes without anticoagulant. All tubes were immediately placed on ice and were transferred to the laboratory. Plane tubes were centrifuged at 1,800×g for 10 min followed by removal of serum. Serum was stored at −20°C until analysis.

Biochemical analysis

The amounts of iron, zinc, copper, beta-hydroxybutyrate (BHB), uric acid, albumin and total iron binding capacity (TIBC) in serum samples were measured by commercial kits [Pars Azmoon, Iran for iron, uric acid, albumin and TIBC; Giesse Diagnostics, Italy for zinc; EliTech diagnostics, France for copper; Randox, Antrim, UK for BHB] using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy.

The concentration of MDA was estimated in serum according to the method of Placer *et al*. (1966). The reaction mixture consisted of 0.2 ml of serum, 1.3 ml of 0.2 MTris–0.16 MKCl buffer (pH 7.4) and 1.5 ml of Thiobarbituric acid reagent. The mixture was heated in a boiling water bath for 10 min. After cooling, 3 ml of pyridine/n butanol (3:1, v/v) and 1 ml of 1N Sodium hydroxide were added and mixed by vigorous shaking. A blank was run simultaneously by incorporating 0.2 ml distilled water instead of the serum. The absorbance of the test sample was read at 548 nm. The concentration of MDA (nmol/ml of serum) was calculated using 1.56×10^5 as the extinction coefficient.

Diagnosis of subclinical ketosis

According to the concentration of serum BHB at 1-3 weeks after calving, the animals were divided into two groups. The first group comprised of healthy cows with serum BHB concentrations less than 1.4 mmol/L (n=37), and the second group comprised of cows with subclinical ketosis (BHB concentrations more than 1.4 mmol/L at week 1 and/or week 3 after calving, n=15).

Statistical analysis

To evaluate the trend of each biochemical parameter during the transition period, the repeated measure ANOVA was used. We also used Pearson's correlation analysis to identify significant correlations between oxidative

stress markers and trace elements. The relationship of trace elements and oxidative stress markers with occurrence of subclinical ketosis was evaluated using the logistic regression test. All analyses were performed with the statistical Package SPSS (release 16, SPSS Inc, Chicago, II). Statistical significance was taken to be indicated by $p< 0.05$.

Results

Oxidative stress markers, trace elements and BHB concentrations during the transition period

Table 1 shows mean values of the oxidative status markers, trace elements and other parameters during the transition period. Serum zinc showed lower ($p<0.05$) values at weeks 1 and 3 after calving compared with data registered at week 3 before calving. Serum concentration of copper decreased after calving. This change resulted in lower values at weeks 1 and 3 after calving compared with those observed before calving ($p<0.05$). Mean iron levels did not show any clear trend within the transition period.

Serum concentration of MDA is relatively steady before calving ($p>0.05$). One week after calving, MDA increased and reached levels higher than those observed at weeks 1 and 3 before calving ($p <0.05$), followed by a subsequent decrease at week 3 after calving ($p<0.05$). Mean serum albumin content dropped after calving and weeks 1 and 3 after calving showed a significant difference ($p<0.05$) compared with week 3 before calving. The lowest value of uric acid was observed at week 1 after calving and there was a significant difference ($p<0.05$) between this time and week 1 before calving. The concentration of TIBC showed a significant decline ($p<0.05$) at weeks 1 and 3 after calving compared with the week 3 before calving. BHB concentration showed a clear and statistically significant increase ($p<0.05$) at week 1 after calving when compared with weeks 1 and 3 before calving.

Correlations of oxidative stress markers and trace elements with the occurrence of subclinical ketosis

The results of logistic regression test showed that the concentrations of zinc, uric acid and MDA influenced the occurrence of subclinical ketosis. For every 1 unit decrease in zinc and uric acid concentrations at week 1 before calving, the risk of subclinical ketosis increased by 1.04 and 40 times, respectively. At week 1 after calving, a 1 unit decrease in zinc concentration was associated with the risk of subclinical ketosis by 1.07 times. In addition, a 1 unit increase in MDA concentration at week 1 after calving was associated with the risk of subclinical ketosis by 1.4 times.

Stage groupings and correlations between trace elements, oxidative stress markers and BHB concentration

We next grouped the sampling times into two periods [late pregnancy (LP), weeks 1 and 3 before calving and early lactation (EL), weeks 1 and 3 after calving], and evaluated the correlation between trace elements, oxidative stress markers and BHB concentration in each period. In the LP period, zinc showed a positive correlation with albumin ($r = 0.210$, $p = 0.032$), while iron showed a positive correlation with TIBC ($r = 0.742$, $p = 0.000$). In the EL period, zinc showed a significant negative correlation with MDA ($r = -0.301$, $p = 0.002$). In the EL period, MDA showed a positive correlation with BHB ($r = 0.321$, $p = 0.001$), while zinc showed a negative correlation with BHB ($r = -0.248$, $p = 0.011$).

Discussion

The condition of oxidative stress is the result of an excessive exposure to oxidants and an inadequate availability of antioxidants, or a combination of both. The results of the present study showed that the postpartum period (particularly the first week after calving) is characterized by a depleted antioxidant status (i.e. during the transition period, the lowest

values of albumin, uric acid and TIBC were observed at this time.). This physiological phase can impose oxidative stress as indicated by the increase of MDA concentration in week 1 after calving. In fact, the antioxidant system cannot cope efficiently with lipoperoxide production during the first week after calving and thus it cannot protect against oxidative stress. Lipid peroxidation is a complex phenomenon involving the generation of numerous products. Serum MDA levels observed in the lactating period suggest that the body presents high levels of free radicals which cause lipid peroxidation in the first week after calving . This effect is related to the intensity of the metabolic changes, under endocrine regulation, that occur in the onset of lactation. Similar findings have been reported by Bernabucci *et al*. (2005); Castillo *et al*. (2006); Adela *et al*. (2006); Sharma *et al*. (2011); Turk *et al*. (2004, 2008). In addition, after calving, cows showed a decrease of plasma and erythrocyte antioxidants, and an increase of oxidative markers. Metabolic and endocrine adjustments, related to metabolism of mammary gland might be responsible for some variations of the oxidative status in early postpartum period (Bernabucci *et al*., 2005). Like the studies performed by Adela *et al*. (2006) and Mudron *et al*. (2006), the MDA concentration decreased significantly at week 3 after calving which suggests that the lipid peroxidation increase is maintained only for a short period of time. However, there might be some variations in cows' antioxidant system efficiency. Turk *et al*. (2008) reported low serum paraoxonase and high MDA level in both late pregnancy and early lactation periods. They concluded that antioxidative capacity was not adequate to remove the resultant lipid peroxidation products; thus, oxidative stress occurred throughout the transition period. Kankofer *et al*. (2010) observed that total antioxidative capacity increased in the prepartum period with a sharp decrease at parturition. The values increased again at postpartum with another decrease after 3 weeks postpartum. The intensity of NEB could be responsible for some differences in oxidative/antioxidative imbalance and in correlations between lipid peroxidation and antioxidants in different studies (Turk *et al*., 2008).

The novel findings of the present study are the associations between trace elements, oxidative stress, and subclinical ketosis. Our results demonstrated an association between trace elements and alteration of oxidative status in transition dairy cows. This is highlighted by the lower zinc values observed in cows with higher oxidative stress (based on MDA concentration). Cows with lower trace elements and higher oxidative stress were particularly more sensitive to subclinical ketosis. An important feature of metabolic response to NEB is using fatty acids and ketone bodies as energy sources, which would be accompanied with increased mitochondrial capacity for fatty acids oxidation in tissues with high oxidative energy demand such as liver. (Azab *et al*., 1999; McCarthy *et al*., 2010). NEFA oxidation in liver leads to higher production of ketone bodies and ROS (Azab *et al*., 1999; Turk *et al*., 2008, Zhang *et al*., 2011). Therefore, NEB may cause oxidative stress, which could result in metabolic dysfunction and disturbance in normal physiology of cows (McCarthy *et al*. 2010, Turk *et al*. 2011).

In the EL period, MDA showed a positive correlation ($p < 0.05$) with BHB. MDA has been found to increase significantly in human beings (Jain e*t al*., 2006; Shen *et al*., 2009), cows (Sahoo *et al*., 2009), buffalos (Youssef *et al*., 2010) and ewes (Al-Qudah, 2011) with hyperketonemia. Bernabucci *et al*. (2005) demonstrated an association between metabolic status and alteration of oxidative status in transition dairy cows. They stated that cows with higher body condition score (BCS) and greater BCS loss were particularly sensitive to oxidative stress. Castillo *et al*. (2005) also reported a positive correlation between MDA and triglycerides. The possibility that metabolic activity might affect the oxidant status was supported by various

correlations detected in the different physiological conditions. In the LP cows, MDA was not correlated with BHB while in EL cows, with higher metabolic burden in comparison with the LP cows, MDA concentrations showed strong positive correlations with BHB. The results of the logistic regression test showed that the increased concentration of MDA at the first week after calving influenced the occurrence of subclinical ketosis. This finding showed that oxidative stress might have a role in subclinical ketosis and cattle with high lipid peroxidation are subclinical ketosis-prone. A role for reactive oxygen species in diabetes has been widely discussed in humans (West, 2000). Oxidative stress could cause initial β cell damage in type I diabetes or impaired insulin production, release, or function in type II diabetes (West, 2000; Bonnefont Rousselot, 2000).

Damage can occur if the antioxidants that prevent the accumulation of free radicals are absent, or present at suboptimal levels within the cell, or not available at the precise place within the cell where free radicals are formed. Cows that are under stress at calving or experiencing the peak demands of lactation have a heavier ROS loading and therefore require a greater supply of antioxidants (Bernabucci *et al*., 2002). Trace elements are an integral part of cellular antioxidant system and elements like copper and zinc participate in cellular defense against oxidants (Evans and Halliwell, 2001). Zinc and copper are also essential elements required for the growth and reproduction of dairy cows and are primarily involved in carbohydrate metabolism, physiological processes, and many other biochemical reactions (Ceylan *et al*., 2008). In the present study, serum zinc and copper decreased after calving. Similarly in the study performed by Meliga *et al*. (2004), serum zinc changed significantly over time and serum zinc was low at 2 days after calving when compared with one month before calving. However opposite of the present study, Akhtar *et al*. (2009) reported a significant increasing trend for copper in the postpartum period. In the present study, the lowest values of zinc and copper were observed along with the increased MDA concentration at week 1 after calving. In addition, a significant negative correlation was observed between zinc and MDA in the EL period. These findings suggest that zinc and copper have important roles in the antioxidant defense during transition period and reduction in zinc and copper availability in the early postpartum period of dairy cows might incorporate in the occurrence of oxidative stress and lipid peroxidation. Copper is involved in the antioxidant system via its involvement in the enzymes Cu–Zn superoxide dismutase (SOD) and Ceruloplasmin. Cu–Zn SOD is responsible for dismutation of superoxide radicals to hydrogen peroxide in the Cytosol. Ceruloplasmin is a copper transport protein that also exhibits oxidase activity. It oxidizes ferric iron (Fe+3) to ferrous iron (Fe+2) without the production of free Fe+3 that can cause oxidation and peroxidation to tissues (Spears and Weiss, 2008). In the antioxidant system Zn is a component of Cu–Zn SOD. Zinc also induces synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals (Spears and Weiss, 2008). Duzguner and Kaya (2007) and Faure *et al*. (1995) reported that oral zinc treatment reduced lipid peroxidation in diabetic rabbits and type I diabetic patients.

In the present study, zinc showed a negative correlation with BHB in the EL period. Moreover, the results of logistic regression test showed that the declined concentrations of zinc in week 1 before and week 1 after calving was associated with the occurrence of subclinical ketosis. Zinc plays an important role in the synthesis, storage, and secretion of insulin as well as conformational integrity of insulin in the hexameric form which affects the ability of islet cells to produce and secret insulin (Duzguner and Kaya, 2007). Zinc-deficient animals have reduced serum insulin content (Duzguner and Kaya, 2007). Therefore, insufficient zinc may influence the generation and metabolism of BHB by

effecting the secretion of insulin in dairy cows.

Conclusion

The results of the present study showed that (a) the dairy cows with weak antioxidant defenses and insufficient concentrations of trace elements are prone to oxidative stress and (b) the cattle with increased oxidative stress may be subclinical ketosis-prone. The new concept of oxidation stress and trace elements that are important triggers in the onset and progression of subclinical ketosis may offer a unique preventive and therapeutic option for subclinical ketosis by using antioxidants or trace elements with high antioxidant capacity.

Table 1. Mean ± SD of trace elements, oxidative stress markers and BHB in cows during transition period

Parameter	week 3 before calving	week 1 before calving	week 1 after calving	week 3 after calving
Zinc (µg/dL)	39.71±18.05^{a}	35.54±16.23^{ab}	28.14±17.86^{b}	30.42±13.80^{b}
Copper (µg/dL)	100.48±20.97^{ac}	104.45±27.11^{a}	87.18±21.17^{b}	89.59±23.04^{bc}
Iron (µg/dL)	131.76±26.45	120.82±22.77	122.93±26.18	125.68±26.51
MDA (µm/L)	18.31±7.34^{a}	17.21±5.50^{a}	28.72±12.68^{b}	18.68±5.14^{a}
Albumin (g/dL)	3.91±0.50^{a}	3.67±0.57^{ab}	3.56±0.52^{b}	3.65±0.52^{b}
Uric acid (mg/dL)	1.19±0.33^{ab}	1.33±0.47^{a}	1.09±0.38^{b}	1.22±0.40^{ab}
TIBC (µg/dL)	330.86±32.73^{a}	317.84±32.96^{ab}	312.28±28.65^{b}	310.78±33.69^{b}
BHB (mmol/L)	0.44±0.23^{a}	0.44±0.36^{a}	0.79±0.50^{b}	0.63±0.91^{ab}

Within each row, means with the same letter do not differ significantly at the 5% level.

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Conflict of interest statement

This study was supported by research fund of Ferdowsi University of Mashhad (project no. 3/15206). None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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همبستگی بین عناصرکمیاب،استرس اکسیداتیو و کتوز تحت بالینی طی دوره انتقال درگاوهای شیری

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چکیده

در مطالعه حاضر، همبستگی بین عناصر کمیاب، استرس اکسیداتیو و کتوز تحت بالینی طی دوره انتقال در گاوهای شیری مورد ارزیابی قرار گرفت. نمونه های خون از ورید وداج و در زمان های سه هفته و یک هفته قبل از زایش و یک هفته و سه هفته بعد از زایش اخذ گردید. بالاترین مقدار مالون دی آلدهید و بتاهیدروکسی بوتیرات در هفته اول پعد از زایش مشاهده گردید. بر عکس، غلظت سرمی مس، روی، آلبومین، اسید اوریک و ظرفیت تام اتصال به آهن (TIBC) بعد از زایش کاهش یافت. پس از زایش، غلظت روی همبستگی منفی معنی داری را با مقدار مالون دی آلدهید نشان داد ($p<0.005$). همبستگی مثبت معنی داری بین مقادیر مالون دی آلدهید و بتاهیدروکسی بوتیرات پس از زایش مشاهده گردید ($p<0.005$)، در حالی که همبستگی بین روی و بتا هیدروکسی بوتیرات در این زمان منفی بود ($p<0.05$). نتایج آزمون رگرسیون لوجستیک نشان داد که کاهش غلظت روی در یک هفته قبل و یک هفته بعد از زایش، احتمال رخداد کتوز تحت بالینی را افزایش می دهد. به علاوه افزایش غلظت مالون دی آلدهید در هفته اول بعد از زایش، خطر کتوز تحت بالینی را افزایش می دهد. نتایج حاصل از مطالعه حاضر نشلن می دهد که گاوهای شیری دارای مقادیر ناکافی از عناصر کمیاب مستعد به استرس اکسیداتیو و کتوز تحت بالینی هستند. از طرف دیگر گاوهای با افزایش استرس اکسیداتیو مستعد به کتوز تحت بالینی خواهند بود.

واژگان کلیدی: استرس اکسیداتیو، عناصر کمیاب، کتوز تحت بالینی، دوره انتقال، گاو