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Blood selenium, copper, and zinc in dairy heifers during the transition period and effects of clinoptilolite administration

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ABSTRACT: Selenium (Se), copper (Cu), and zinc (Zn) play important antioxidant role during the transition period of dairy cattle. However**,** there is limited information about their blood fluctuations during the entire transition period, especially in heifers. Furthermore, it is questionable whether the use of clinoptilolite, a natural zeolite, affects the availability of these trace elements during this period. The objective of the present study was to monitor the blood concentrations of Se, Cu, and Zn during the transition period of dairy heifers and to investigate whether the dietary inclusion of clinoptilolite has any effect on them. Forty clinically healthy Holstein heifers were used in the experiment. They were randomly allocated in two equal groups (n = 20) formed according to their body condition score. The control group was fed only the basal ration whereas the daily feed of treatment group was supplemented with 200 g clinoptilolite. The experiment started 28 days before the expected day of calving and lasted until day 21 after parturition. Blood samples were taken at the onset of the experiment and then at weekly intervals until parturition, at the day of calving, and on days 7, 14, and 21. All samples were analyzed for blood Se and plasma Cu and Zn concentrations. The results indicate that the levels of Se, Cu, and Zn in blood change significantly ($P < 0.05$) throughout the transition period in dairy heifers and increase significantly ($P < 0.05$) immediately after calving. Furthermore, the dietary administration of clinoptilolite does not significantly affect their blood concentration ($P > 0.05$). Blood levels of Se, Cu, and Zn, although undergoing significant changes throughout the transition period in dairy heifers, remain practically stable until parturition and increase significantly immediately after calving. Clinoptilolite does not impair the dietary availability of the trace elements evaluated when added in heifers' rations during this period.

Keywords: trace elements; zeolite; parturition; dairy cattle

INTRODUCTION

The transition period, defined as the period from three weeks before to three weeks after calving, is extremely stressful for dairy cows. During this period, the animals go through physiological stress due to their preparation and recovery from parturition (Drackley 1999) and their metabolism is dramatically altered in order to supply the mammary gland with nutrients necessary for milk synthesis (Goff et al. 2002). Along with the reduced dry matter intake that normally occurs around calving, cows are subjected to negative energy balance (Roche et al. 2009) and oxidative stress (Sordillo and Aitken 2009) at this stage. As a result, immune function is weakened and dairy cows have a decreased capacity to fight disease.

A number of trace minerals play important role in dairy cows' immune function (Spears 2000), especially those involved in the antioxidant defence system by decreasing oxidative stress levels. Selenium (Se) is an essential component of the

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family of glutathione peroxidase enzymes which destroy hydrogen peroxide and lipid hydroperoxides. Thioredoxin reductase is another selenoenzyme that may function towards the prevention of oxidative stress (Mustacich and Powis 2000). Copper (Cu) is involved in the antioxidant enzymes Cu–Zn superoxide dismutase (SOD) and ceruloplasmin. The former is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Halliwell and Gutteridge 1999), whereas the latter, among others, is an acute phase protein that increases during disease and may be important in scavenging superoxide radicals (Broadley and Hoover 1989). Zinc (Zn) is another component of Cu–Zn SOD. Zinc also induces synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals (Prasad et al. 2004). In addition to its antioxidant role, Zn may affect immunity via its important role in cell replication and proliferation (Weiss and Spears 2006). So, ensuring adequate levels of these minerals during the transition period is an important factor for the maintenance of cow health and productivity.

Evidence in literature supports that blood levels of Se, Cu, and Zn vary at different production stages of dairy cows but there is limited information about their fluctuations during the entire transition period, especially in heifers. This information is considered important in order to identify possible time-points at risk for extra supplementation of these trace elements during this critical period. Weiss and Hogan (2005) proved that blood serum Se concentrations decrease from dry-off to calving by 23–45%; Piccioli Cappelli et al. (2007) observed that blood Se levels decrease in the close-up period and increase or keep decreasing after calving, depending on the selenium intake. Hussein and Staufenbiel (2012) recorded increased blood levels of Cu in dairy cows at the first week post calving compared to the close-up period (3–1 weeks before calving). Similar results were obtained for Cu by Noaman et al. (2012) who observed lower blood Cu levels in dry cows than in the lactating ones. At the same study, the opposite trend was recorded for blood Zn, which was higher in dry cows.

In recent years there is an increased interest for using clinoptilolite, a natural zeolite, as feed additive in dairy rations during the transition period due to its beneficial effects on the incidence of metabolic disorders (Katsoulos et al. 2005a, 2006) and on the immune response after vaccination against *E. coli* (Karatzia 2010). Because of its high cation exchange capacity, clinoptilolite may interfere with trace elements in the gastrointestinal tract and impair their absorption. To the best of our knowledge, the effects of clinoptilolite administration on the blood Se concentration in dairy cattle or other animal species have not been studied yet. Although Se is an anionic element, a recent *in vitro* study provides evidence for sorption of Se ions by natural zeolites under certain conditions (Zonchoeva and Sanzhanova 2011). The effects of zeolites on blood levels of the cationic elements Cu and Zn in heifers during the transition period have not been evaluated in detail yet; Katsoulos et al. (2005b) concluded that the long-term clinoptilolite administration in multiparous dairy cows does not significantly affect their blood concentration.

The objective of the present study was to monitor the fluctuations of blood concentrations of Se, Cu, and Zn during the transition period of clinically healthy dairy heifers and to investigate whether the dietary inclusion of clinoptilolite affects their blood concentrations.

MATERIAL AND METHODS

Animals and experimental design. The study was conducted on a commercial freestall dairy farm in northern Greece. Prior to the onset of the study, a pilot trial with 10 animals was run for obtaining average values and standard deviations (SD) of the blood Se, Cu, and Zn concentrations in order to determine by power analysis the number of cows that should be used in the experiment. The values obtained were 2.45 ± 0.50 μmol/l for Se, 15.3 ± 2.6 μmol/l for Cu, and 15.9 ± 2.5 μmol/l for Zn. The critical values of 1.27 μmol/l for Se (Backall and Scholz 1979; Gerloff 1992), 8.97 μmol/l for Cu (Underwood and Suttle 1999), and 9.18 μmol/l for Zn (Underwood and Suttle 1999) were used for the determination of the effect size, i.e. the minimum difference between two groups that can be considered as clinically significant (Charan and Katharia 2013). The sample size was calculated using Univariate Approach to Repeated Measures with Geisser-Greenhouse Correction procedure at the GLIMMPSE software (http://glimmpse.samplesizeshop.org/). The desired power was set at 0.8, the type I error rate at 0.05, the means

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scale factor and the variability scale factor at 2. The results of the analysis revealed that for the evaluation of clinoptilolite supplementation the required sample size for Se was 17 cows (power = 0.830), for Cu 17 cows (power = 0.853), and for Zn 14 cows (power = 0.841). For the evaluation of the time effect and the interaction time × clinoptilolite supplementation a sample size of 21 cows (power = 1) was necessary for all trace elements. Taking into consideration that about 10% of the animals could be excluded for some reason from the study and the very high power for the effect of time and the interaction, the number of 20 cows per group was considered adequate for the purposes of the study. Forty pregnant Holstein heifers that were judged to be healthy based on the results of clinical examination and the farm health records were finally used in the study. All animals were artificially inseminated at the age of 15–16 months and the pregnancy was confirmed with rectal palpation on days 60 and 210 of gestation. The experiment started 28 days before the expected day of parturition (day 0 of the experiment) and lasted until day 21 after calving. The forty heifers were randomly assigned to one of the two groups according to their body condition score (BCS) in order to be equal: the animals of the first group (treatment group; n = 20, BCS = 3.60 ± 0.31) were fed the basal ration (described below) supplemented with 200 g clinoptilolite per day; the heifers of the second group (control group; n = 20, BCS = 3.57 ± 0.32) were fed the basal ration.

The rations provided to the animals were formulated according to the recommendations by the National Research Council (2001). The ration offered daily to the animals in both groups during the dry period consisted of 10 kg corn silage, 3 kg wheat straw, and 3 kg concentrate feed. The concentrates were divided into equal portions and offered to the animals twice daily in the milking parlour supplemented or not with clinoptilolite, according to the group assignment. The concentrate portion was comprised of 12.6% soybean meal, 16.0% maize grains, 22.0% wheat bran, 18.0% sunflower meal, 8.0% rape cake, 21.0% carob fruits, 0.2% vitamins and trace minerals premix, 1.3% sea salt, 0.5% calcium carbonate, and 0.4% dicalcium phosphate. The composition of the mixture of trace minerals was (in g/kg): Mg 100, Mn 50, Fe 75, Cu 20, Zn 100, I 1.5, Co 0.3, Se 0.4, and Mo 0.6.

During lactation the animals were offered 25 kg corn silage, 2 kg molasse, and 300 g concentrates per litre of milk produced per day. The concentrates with or without clinoptilolite supplementation were divided into equal portions and offered twice a day during milking. All animals were monitored for incidence of any disease. Scheduled farm visits were made at weekly intervals and extra visits were made when a heifer was suspected of having any disease. Based on the health records kept, only the cows that remained clinically healthy throughout the study period were finally included in the experiment.

The procedures and the experiment done respected the ethical standards of the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

Zeolitic material. The zeolitic material used in the experiment had particle size <.80 mm and contained approximately 92% clinoptilolite and the admixture was 8% opal ($SiO_2 \times nH_2O$), as determined by X-ray powder diffraction. The cation exchange capacity of the material was 220 cmol/kg and its chemical composition was (in %): SiO_2 69.9, Al_2O_3 11.27, CaO 3.02, MgO 0.6, Na_2O 0.75, K_2O 2.23, Fe_2O_3 0.11, and loss on ignition (LOI) 13.05.

Blood samplings and analysis. Two sets of samples were taken after afternoon feeding at the onset of the experiment and then at weekly intervals until parturition, at the day of calving and on days 7, 14, and 21 after calving. Blood was sampled from each animal after afternoon feeding by jugular vein puncture with an 18-gauge needle, using two vacuum glass tubes with heparin as anticoagulant. The first set of samples was used for the Se analyses and the second one for the determination of Cu and Zn concentrations in plasma. The plasma was separated by low speed centrifugation, transferred in plastic vials, and subsequently forwarded for analysis.

Selenium was determined in whole blood by the fluorometric method described by Ayiannidis and Voulgaropoulos (1990), using a Hitachi F2000 fluorometer (Hitachi Instruments Inc., Tokyo, Japan). Blood plasma concentrations of Cu and Zn were determined by means of flame atomic absorption spectrophotometry, using a Perkin-Elmer A Analyst 100 instrument (Perkin-Elmer Corp., Norwalk, USA), according to Perkin-Elmer analytical methods (Perkin-Elmer Co. 1996).

Statistical analysis. The statistical analysis of the experiment results was conducted using the

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SPSS statistical software (Version 21.0, 2012). Normality of the data was tested with Kolmogorov-Smirnov test and the homogeneity of variances with Leven's test. Repeated Measures Analysis was run to identify the effects of time, clinoptilolite supplementation (treatment), and their interactions (time × treatment) on the blood concentrations of the trace elements evaluated. Values obtained at day 0 of the experiment were used as covariates. The data at each individual sampling were also analyzed by Univariate Analysis of Variance, using values on day 0 as covariates as well. In both models, Bonferoni test was used for the comparison of the main effects of time and treatment; the results are presented as means ± SEM. A significance level of $P \leq 0.05$ was used in all comparisons.

RESULTS

In accord with the health records kept, data of one animal from the treatment group and of two animals from the control group were excluded from the analysis because of retained placenta (2 cases, one in each group) and of left abomasal displacement and ketosis (1 case, control group).

Repeated measures analysis revealed that blood Se levels were significantly affected by the time of sampling ($P < 0.05$). As shown in Table 1, the Se concentration in blood three weeks after calving (day 21) was significantly higher than one week before (day –7) and at the calving day ($P < 0.05$). However, blood Se was significantly affected neither by the supplementation of clinoptilolite (means: 2.09 and 1.90 μmol/l; SEM = 0.05 for treatment and control group, respectively, $P > 0.05$; Figure 1A), nor the interaction time × treatment ($P > 0.05$).

The average plasma Cu concentration was also significantly ($P < 0.05$) affected by the time of sampling. Plasma Cu levels three weeks before calving were significantly ($P < 0.05$) lower compared to those one, two, and three weeks after the calving day (Table 1). The dietary inclusion of clinoptilolite had no effect on plasma Cu concentration (means: 17.67 and 17.39 μmol/l; SEM = 0.41 for treatment and control group, respectively, $P > 0.05$; Figure 1B). Also, the interaction time × treatment was not significant ($P > 0.05$).

Plasma Zn concentrations were significantly ($P < 0.05$) affected only by the time of sampling. Mean plasma Zn was significantly ($P < 0.05$) higher two and three weeks after the calving day than

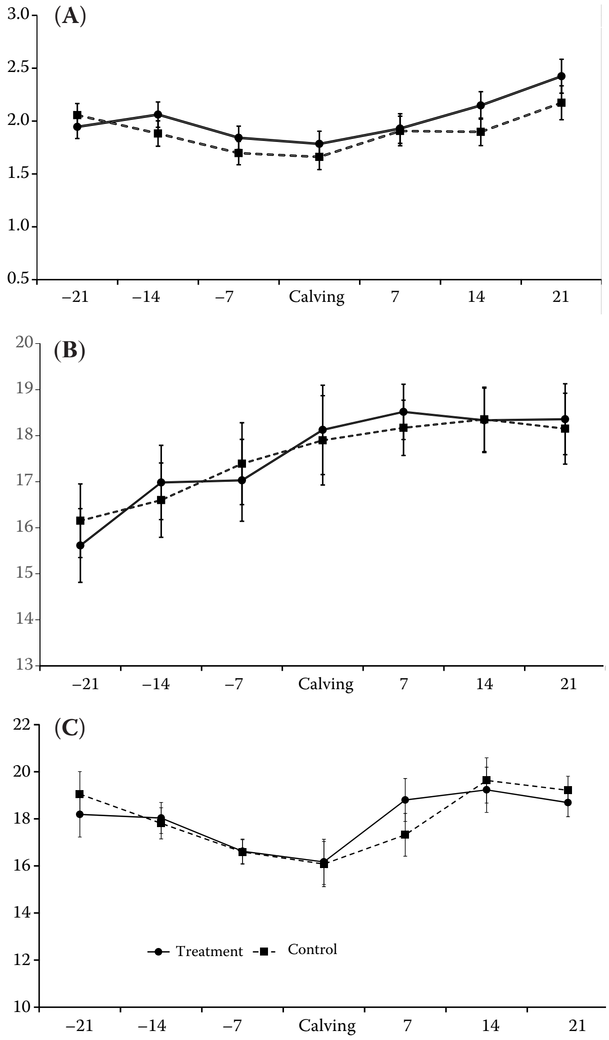

Figure 1. Means ± SEM of blood concentrations of selenium (Se) (**A**), copper (Cu) (**B**), and zinc (Zn) (**C**) of the 19 heifers of the treatment group receiving daily 200 g clinoptilolite and the 18 heifers of the control group finally included in the study as related to the day of calving

one week before parturition. Clinoptilolite feeding (means: 17.89 and 18.03 μmol/l; SEM = 0.40 for treatment and control group, respectively, $P > 0.05$; Figure 1C) and the interaction time × treatment had no significant effect on plasma Zn levels ($P < 0.05$).

DISCUSSION

The main objective of the present study was to investigate how the blood levels of Se, Cu, and Zn modify during the transition period of clinically healthy dairy heifers in order to identify possible time-points at risk for extra supplementation of

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Table 1. Means ± SEM of blood concentrations of selenium (Se), copper (Cu), and zinc (Zn) of the 37 heifers finally included in the study as related to the day of calving

Days relative to calving	Se (μmol/l)	Cu (μmol/l)	Zn (μmol/l)
–21	2.00 ± 0.082^{ab}	15.9 ± 0.40^{a}	18.6 ± 0.47^{ab}
–14	1.97 ± 0.074^{ab}	16.8 ± 0.56^{ab}	17.9 ± 0.68^{ab}
–7	1.77 ± 0.081^{a}	17.2 ± 0.62^{ab}	16.6 ± 0.37^{a}
Calving day	1.72 ± 0.082^{a}	17.8 ± 0.68^{ab}	16.1 ± 0.76^{a}
+7	1.92 ± 0.099^{ab}	18.3 ± 0.42^{b}	18.1 ± 0.64^{ab}
+14	2.02 ± 0.093^{ab}	18.3 ± 0.49^{b}	19.4 ± 0.68^{b}
+21	2.30 ± 0.106^{b}	18.3 ± 0.54^{b}	19.0 ± 0.43^{b}

^{a,b}different superscripts in the same column denote a significant difference ($P < 0.05$)

these trace elements during this critical period. In order to be close to the practices followed in many farms, at least in Greece, the rations offered to the animals were formulated according to NRC (2001), a commercial mixture of trace elements and vitamins was used, and no trace element analysis was performed to the feedstuffs in order to correct potential imbalances of the trace elements. The other objective was to evaluate the effects of dietary inclusion of clinoptilolite on their concentrations in blood. To the best of the authors' knowledge, there has been no information about the fluctuations of blood trace elements concentrations in heifers during the transition period. Furthermore, heifers might be more adequate to study trace element changes at this period than older cows as they are less affected by oxidative stress around calving compared to the older ones due to the extremely low incidence of metabolic disorders in these animals and the lower risk of udder infections from the previous milking period.

Blood Se concentration remained above the critical value of 1.27 μmol/l throughout the experimental period. The average blood Se values were numerically reduced until parturition and started to increase immediately after calving reaching significantly higher levels 28 days after calving compared to the day of parturition. The significant increase of blood Se after calving is attributed to the higher Se intake during the lactation compared to the dry period, as there is a linear relationship between Se intake and blood Se levels (Maus et al. 1980). In accordance with our findings, Piccioli Cappelli et al. (2007) observed a slight decrease before parturition, and increase of blood Se concentration after calving in multiparous dairy cattle that were fed adequate levels of Se. However, at the same study, a decrease in blood Se concentration continued throughout the lactation period in the group of animals receiving lower than the recommended Se levels.

The dietary inclusion of clinoptilolite had no significant effect on blood Se concentration at the present study. This is the first evidence that the in-feed administration of clinoptilolite has had no adverse effects on the dietary availability of Se. Recently, Zonchoeva and Sanzhanova (2011) have proved *in vitro* that clinoptilolite binds Se but this sorption occurs at pH 9, which is much higher than attain the pH levels in the main parts of the gastrointestinal tract.

It was decided to determine Cu in blood plasma instead of blood serum because a proportion of Cu is trapped during the fibrin clot formation and leads to underestimation of the true blood Cu status (Kincaid et al. 1986, Hussein and Staufenbiel 2012). The values recorded throughout the experimental period were higher than the critical concentration of 8.97 μmol/l suggested by Underwood and Suttle (1999). The results obtained here indicate that plasma Cu concentration increases during the transition period and reaches significantly higher levels the first weeks after calving in comparison with the onset of the transition period. In accordance with these findings, Hussein and Staufenbiel (2012) observed higher plasma Cu levels in fresh and early lactation cows than in those in the close-up period. They suggested that this increase of plasma Cu is secondary and is attributed to the increased ceruloplasmin activity at this stage, as ceruloplasmin represents more than 95% of plasma Cu.

Plasma Cu concentrations remained unaffected by the supplementation of clinoptilolite throughout

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the transition period, indicating that clinoptilolite does not impair the dietary availability of Cu at this critical stage. This is probably due to the low chemisorption index of clinoptilolite for Cu in the gastric fluid (Tomasevic-Canovic et al. 2001). The results obtained here are in agreement with the findings of Katsoulos et al. (2005b) who observed that the long-term in-feed inclusion of clinoptilolite at the levels of 1.25% and 2.5% of concentrates has no significant effect on blood serum concentration of Cu in multiparous dairy cows.

Plasma Zn levels at the current study started to increase immediately after calving and the highest levels were recorded during the last two weeks of the transition period. In addition, all the values recorded exceeded the critical limit of 9.18 μmol/l (Underwood and Suttle 1999). Although there is no evidence in the available literature concerning the variations of Zn during the transition period, it is possible that this increase is due to the increased dry matter intake during the first weeks of lactation. Opposite to the results of the present study, Noaman et al. (2012) observed that dry cows had significantly higher blood Zn levels than those during lactation. However, these cattle were not in the close-up period and were not first-calf heifers.

The results of the present study indicate that the use of clinoptilolite during the transition period has no significant effect on plasma Zn concentration. This is probably due to the low selectivity of clinoptilolite for Zn (Tomasevic-Canovic et al. 2001). In accordance with this finding, previous studies also indicated that blood serum concentrations of Zn are not significantly affected either by the short-term (Bosi et al. 2002) or the prolonged (Katsoulos et al. 2005b) administration of clinoptilolite in multiparous lactating dairy cows.

CONCLUSION

Although the blood levels of Se, Cu, and Zn undergo significant changes throughout the transition period in dairy heifers, their values remain practically stable until parturition and increase significantly immediately after calving. Based on this observation we may conclude that there is no critical time-point that necessitates an extra supplementation of these trace elements in the transition period of heifers. Another conclusion arising from the present study is that clinoptilolite does not impair the dietary availability of the trace elements evaluated when added in heifers' rations during the transition period.

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