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The influence of dietary clinoptilolite on blood serum mineral profile in dairy cows

Ivan Folnožić[1]*, Dražen Đuričić[2], Ivona Žura Žaja[3], Silvijo Vince[1], Sonja Perkov[4], Romana Turk[5], Hrvoje Valpotić[6], Damjan Gračner[7], Nino Maćešić[1], Martina Lojkić[1], Mislav Kovačić[8], and Marko Samardžija[1]

[1]Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[2]Veterinary Practice Đurđevac, Đurđevac, Croatia

[3]Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[4]Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia

[5]Department of Pathophysiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[6]Department for Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[7]Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[8]Mount trade d.o.o., Garešnica, Croatia

FOLNOŽIĆ, I., D. ĐURIČIĆ, I. ŽURA ŽAJA, S. VINCE, S. PERKOV, R. TURK, H. VALPOTIĆ, D. GRAČNER, N. MAĆEŠIĆ, M. LOJKIĆ, M. KOVAČIĆ, M. SAMARDŽIJA: The influence of dietary clinoptilolite on blood serum mineral profile in dairy cows. Vet. arhiv 89, 447-462, 2019.

ABSTRACT

The objectives of this study were to determine whether or not dietary clinoptilolite (CPL) has an influence on the levels of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and sodium (Na) in the blood serum of dairy cows during gravidity and early lactation. The study was conducted on 78 dairy cows of Holstein-Friesian breed. The cows were randomly assigned into two groups: the CPL- fed treated group (n = 38) which received 50 g of natural powdered zeolite CPL twice a day from day 180 days before to 60 days after parturition, and the control non-treated group (n = 40). Blood samples were taken on days 180, 90, 60, 30 and 10 before parturition, on the day of calving and on days 5, 12, 19, 26, 33, 40 and 60 following parturition. There were no significant differences in the Ca concentrations between the CPL-fed and the control group. However, after parturition it was noticeable that the Ca concentration was higher in the CPL-fed group, especially on

*Corresponding author:

Assist. Prof. Ivan Folnožić, PhD, DVM, Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 322; Fax: +385 1 244 1390; E-mail: folnozic@vef.hr

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day 33 (P = 0.06). The concentration of P was significantly lower ($P<0.05$) in the CPL-fed group on day 0 and day 5 in comparison to the control group. A significantly higher ratio of Ca:P was calculated in the CPL- fed group *vs*. the control group on days 0, and 12. There were no significant differences observed in Mg, K, and Na concentrations between the cows in the CPL-fed group and the control group throughout the duration of the study. The results of the study suggest that dietary CPL influenced the blood levels of Ca and P in dairy cows, and improved the serum Ca:P ratio of dairy cows during the early postpartal period. The CPL applied did not produce any clinically visible disorders in the metabolism of the tested minerals. In addition, the blood levels of all tested minerals were within physiological ranges, which indicates that CPL did not alter their homeostasis in dairy cows.

Key words: zeolites; macromineral level; blood serum; dairy cows

Introduction

During the peripartal period nutritional needs increase rapidly due to the fetal growth and milk synthesis (GRUMMER et al., 2004; SAMARDŽIJA et al., 2008; GRUMMER et al., 2010). Along with the selection of dairy cows intended for high milk production, a negative impact simultaneously occurred on their ability for hormonal regulation of metabolic processes (KOČILA et al., 2013). The regulation of the metabolic processes during the peripartal period is extremely complex due to the interrelationship between nutrition and the metabolic and endocrine systems (GRUMMER et al., 2004; KOČILA et al., 2009). During that period, the health and productivity of dairy cows are highly related to the metabolism of minerals (GOFF, 2006). Minerals are essential chemical elements that play important roles in numerous physiological functions. The most important macroelements that can influence homeostasis in dairy cows are: total calcium (Ca), total magnesium (Mg), inorganic phosphorus, potassium (K) and sodium (Na) (DEGARIS and LEAN, 2008). The imbalances in mineral metabolism during the peripartal period are a precondition for the development of disorders such as: hypocalcaemia, ketosis, displacement of abomasum, retention of fetal membranes, grass tetany, mastitis and lameness (GOFF, 2006; MULLIGAN and DOHERTY, 2008; ĐURIČIĆ et al., 2011; VINCE et al., 2017).

In particular, hypocalcaemia is a frequent and important health and economic problem in dairy cows. It develops due to the inability of animals to maintain optimal Ca concentrations in their blood following parturition. Hypocalcaemia may be manifested in either a clinical or subclinical form depending on the Ca concentration in the blood and the occurrence of clinical symptoms. The incidence of clinical hypocalcaemia or milk fever is usually within 24-48 hours after parturition. Cows with milk fever are recumbent and unable to rise, muscle tremor is present, while the cows with subclinical hypocalcaemia usually do not exhibit pronounced clinical symptoms (GOFF, 2008; HESAM and SAMUEL, 2018). Ca homeostasis is regulated by the interaction of hormones calcitonin, parathormone (PTH) and by $1{,}25(OH)_2D_3$ (GOFF and HORST, 1997). The predisposing factors which can influence the incidence of hypocalcaemia are: metabolic alkalosis, rations high in Ca before parturition, hypomagnesaemia and a high level of dietary P. Metabolic alkalosis occurs due to an excessive quantity of cations, primarily of K and Na from feed during their dry period (HESAM and SAMUEL, 2018). Further, in older cows, milk fever is more frequent with more severe symptoms in comparison to younger cows (REINHARDT et al., 2011). In addition, the age of cows increases the risk of hypocalcaemia development by approximately 9% per lactation (DEGARIS and LEAN, 2008). Thus, the proper management of cows during the peripartal period is of extreme importance for prevention of metabolic and reproductive disorders which usually lead to significant health issues and economic losses (OVERTON and WALDRON, 2004).

One of the most common control procedures for hypocalcaemia in dairy cows is the restriction of the quantity of Ca in the ration in order to maintain negative Ca balance in the last weeks of gravidity. However, this restriction is difficult to attain due to the fact that many forms of dairy cow forage have a relatively high Ca concentration (SANTOS et al., 2016). In earlier studies, the supplementation of zeolite in the feed was suggested as an approach which may decrease the incidence of milk fever as well as subclinical hypocalcaemia (THILSING-HANSEN et al., 2002, 2003; KATSOULOS et al., 2005; THILSING-HANSEN et al., 2007; GRABHERR et al., 2009; KHACHLOUF et al., 2019). Zeolites are complex mineral compounds comprising molecules of SiO_4 and AlO_4 arranged in the form of sieves which are interconnected. The AlO_4 in this structure has a negative charge and thus must be balanced by cations such as: K^+, NH_4, Ca^{2+} and Mg^{2+}, which may remain reversible bonded or may be released depending on the microenvironment (COOMBS et al., 1997; BOSI et al., 2002). Over recent decades a type of natural zeolite, clinoptilolite, has been tested in many studies as an alternative feed additive, particularly for farm animals, which may bind mycotoxins and thus improve health and production (PAPAIOANNOU et al., 2005). Also, clinoptilolite is able to facilitate ruminal fermentation and enable the regulation of pH value by buffering against hydrogen ions derived from organic acids (VALPOTIC et al., 2017). The aim of this study was to determine whether or not dietary supplementation of clinoptilolite has an influence on the levels and dynamics of the tested minerals in the blood of dairy cows during gravidity and early lactation.

Materials and methods

Animals and housing. This study was conducted on 78 Holstein-Friesian cows, aged between 2 and 7 years. The dairy cows were housed and kept on the „OPG Pleško“ farm, located in the Koprivnica-Križevci County, Đurđevac, Croatia (coordinates 45°59’ N, 17°03’ E). The cows were randomly assigned into two groups: the CPL in-feed treated group of cows (n = 38) which received 50 g of natural powdered zeolite CPL twice a day, modified by vibroactivation and micronization (Vibrosorb®, Viridisfarm, Podpićan, Croatia) from the 3rd month of gravidity (day 180 prior to parturition) to the end of monitoring, and the control non-treated group of cows (n = 40). Prior to assignment to the groups the cows were randomly selected according to body condition score (BSC). Namely, they were evaluated according to the BCS method, numerically from 1 to 5 with a precision score of 0.25 points, as described earlier by EDMONSON et. al. (1989), and all the cows had a BCS from 2.75 to 3.5. No significant differences in milk yield were found between the groups of cows.

There were no significant differences in milk yield between the groups (control group 8058 and CPL-fed group 8394). The cows were fed a ration composed of haylage, corn silage, hay and a complete feed mixture for dry and lactating cows, with 19% crude protein. The cows were housed in a free stall barn with straw bedding, fed a 50:50 forage to concentrate ration, and milked twice a day at 6 o'clock a.m. and 4 o'clock p.m.

Blood sampling. Blood samples were taken after the morning milking by the 'Vacutainer' system from the tail vein (v. coccygea) into tubes without anticoagulant but with clot activator. After clotting at room temperature for 1 h, blood samples were centrifuged at 1500 g for 15 min. Sera were separated and stored at -70 °C until analysis. For determination of blood mineral concentration, samples were taken 13 times as follows: on days 180, 90, 60, 30 and 10 before parturition, on the day of calving, and on days 5, 12, 19, 26, 33, 40 and 60 after parturition. Values before parturition correspond to the real day (± 1 day).

Mineral profile. Serum Ca, P, Mg, K and Na concentrations were assayed using standard commercial kits (Beckman Coulter Biomedical Ltd., O'Callaghan's Mills, Co. Clare, Ireland) on an automatic Beckman Coulter AU 680 analyzer (Beckman Coulter Biomedical Ltd, Ireland). Total calcium was measured by photometric o-cresolphthalein method (OSR 6113). Inorganic phosphorous was measured with the photometric UV-test (OSR 6122). Total magnesium was measured by the photometric xylidyl blue method (OSR 6189). Sodium and potassium were measured by indirect potentiometry.

Statistical analysis. Statistical analyses were conducted using the SAS 9.4 program package (Statistical Analysis Software 2002-2012 by SAS Institute Inc., Cary, USA). The normal distribution of data was tested by the module PROC TRANSREG. When the presumptions of normal distribution of the analyzed dependent variables were disturbed and in the case of heteroscedasticity of the variances, variables were logged on base 10 (Mg, K and Na) or exponentially transformed (Ca) before analysis. The quick test of dependent variables (Ca, P, Mg, K, Na and ratio between Ca and P) was performed by multivariance analysis of variance, based on the criterion of Wilks' lambda by the GLM procedure. The main model was created by the mixed module (PROC MIXED) and comprised the fixed effect of the group, period and their interactions. The random effect of the animal with repeating samplings/observations (animal identification number) over time was incorporated in the model. Decisions regarding the type of variance-covariance structure to be used in the model were based on the SAS criteria for evaluating model fit (AIC and BIC). The results were presented as the least squares means and the standard error of the mean. The multiple comparison test of the least-square means with Tukey's correction was conducted by the SLICE option, to compare each group level within the period and the level of statistical significance was set at values of P<0.05 and lower. Following analysis, the data were back transformed to the original values (if transformation was used) and are presented in Figs 1 to 6.

Results

In both groups of cows Ca concentration was significantly decreased (P<0.05) around parturition in comparison to the values recorded at the start and at the end of blood sampling. There were no significant differences in the Ca concentration between the cows in the CPL-fed group and the control group. However, after parturition it was noticeable that the Ca concentration was higher in the CPL-fed group, especially on day 33 (P = 0.06), but not significantly (P>0.05) (Fig. 1).

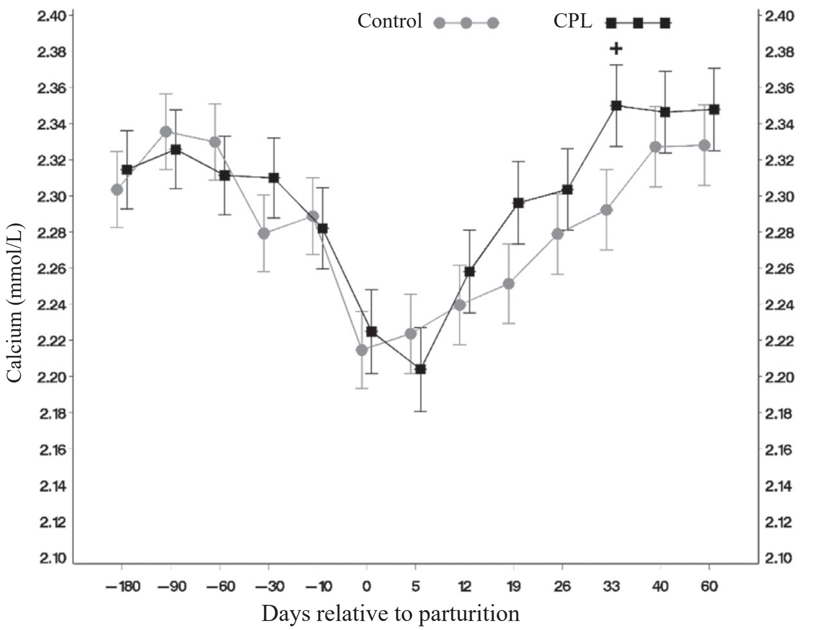

Fig. 1. Mean values and standard error of calcium (Ca) in the clinoptilolite in-feed treated group (CPL) and the control group of cows from 3 months gravidity (day 180 prior to parturition) until 60 days postpartum. + Values with a plus within the column indicate that the differences between the groups are near to statistical significance (P<0.10).

The concentration of P was significantly lower (P<0.05) in the CPL-fed group on day 0 and day 5, with higher tendency to differ on day 12 (P = 0.07) than in the control group. Furthermore, the P concentration was significantly decreased (P<0.05) around parturition in the CPL-fed group compared to the values obtained at the start of the study. However, in the control group, the P concentration was not significantly decreased around parturition in comparison to that observed at the start of the study (Fig. 2).

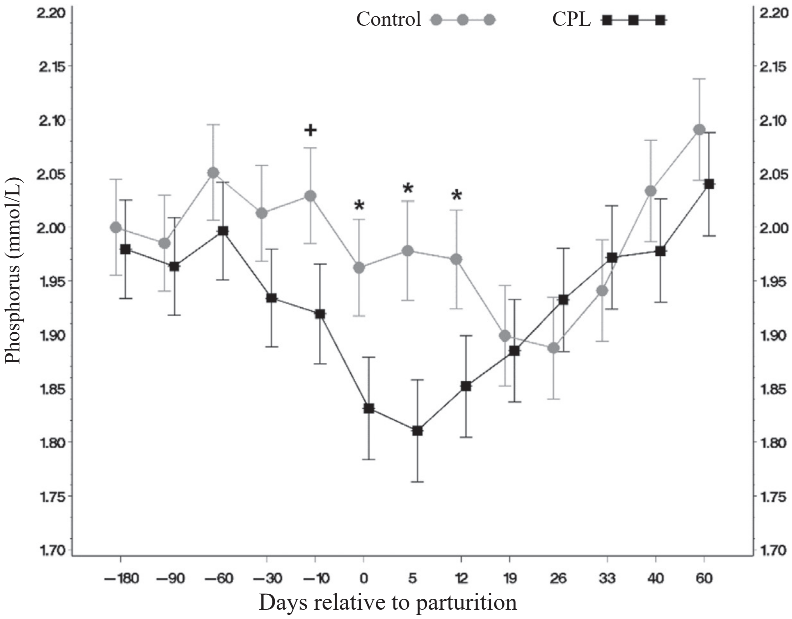

Fig. 2. Mean values and standard error of phosphorus (P) in the clinoptilolite in-feed treated group (CPL) and the control group of cows from 3 months gravidity (day 180 prior to parturition) until 60 days postpartum. * Values with an asterisk within the column indicate a statistically significant difference between the groups (P<0.05). + Values with a plus within the column indicate that differences between the groups are near to statistical significance (P<0.10).

There were no significant differences observed in the Mg concentration between the cows in the CPL-fed group and the control group. Furthermore, in both groups of cows the Mg concentration was significantly decreased ($P<0.05$) around parturition in comparison to the values at the start and at the end of sampling (Fig. 3).

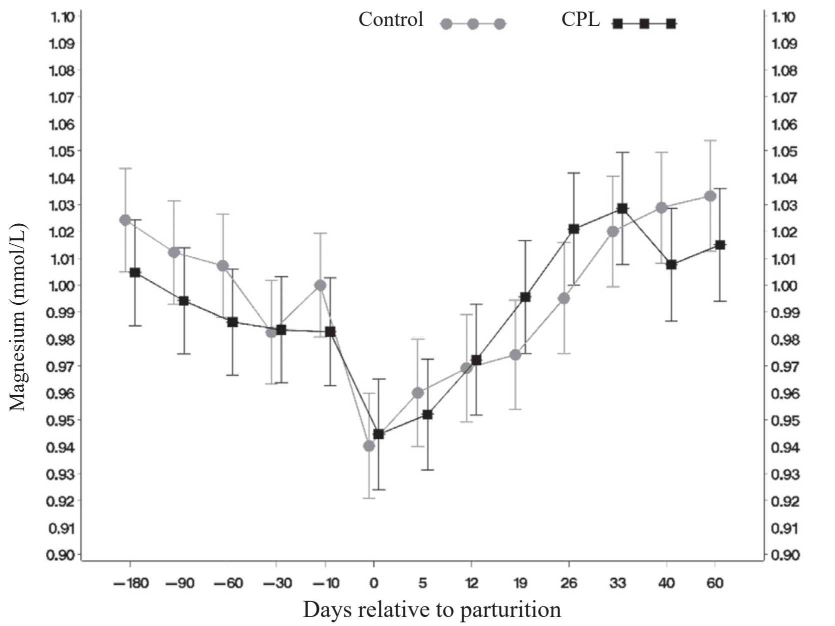

Fig. 3. Mean values and standard error of magnesium in the clinoptilolite in-feed treated group (CPL) and the control group of cows from 3 months gravidity (day 180 prior to parturition) until 60 days postpartum.

There were no significant differences found in the K concentrations between the cows in the CPL-fed group and the control group during duration of the study (Fig. 4).

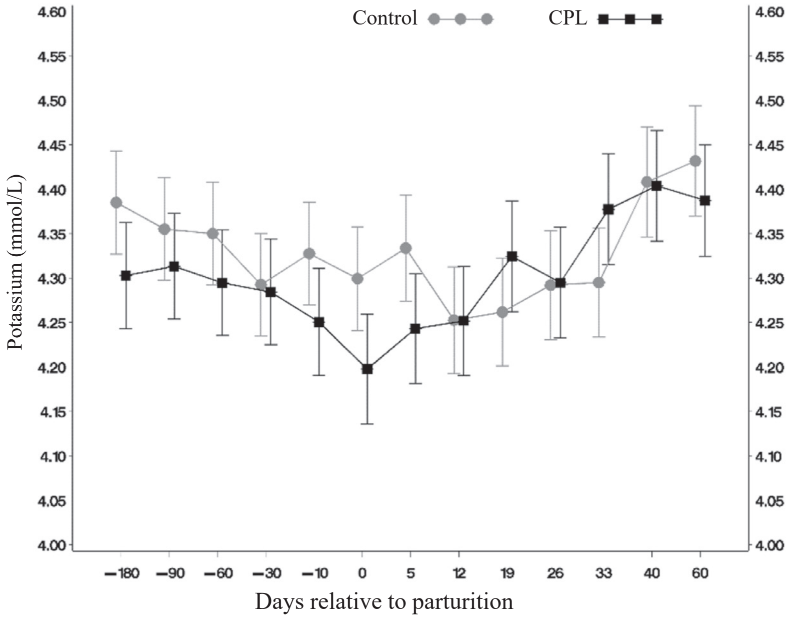

Fig. 4. Mean values and standard error potassium (K) in the clinoptilolite in-feed treated group (CPL) and the control group of cows from 3 months gravidity (Day 180 prior to parturition) until 60 days postpartum.

There were no significant differences observed in the Na concentrations between the cows in the CPL-fed group and the control group, but the concentration of the Na was lower (P = 0.07) on day 5 after parturition in the CPL-fed group of cows in comparison to the control group (Fig. 5).

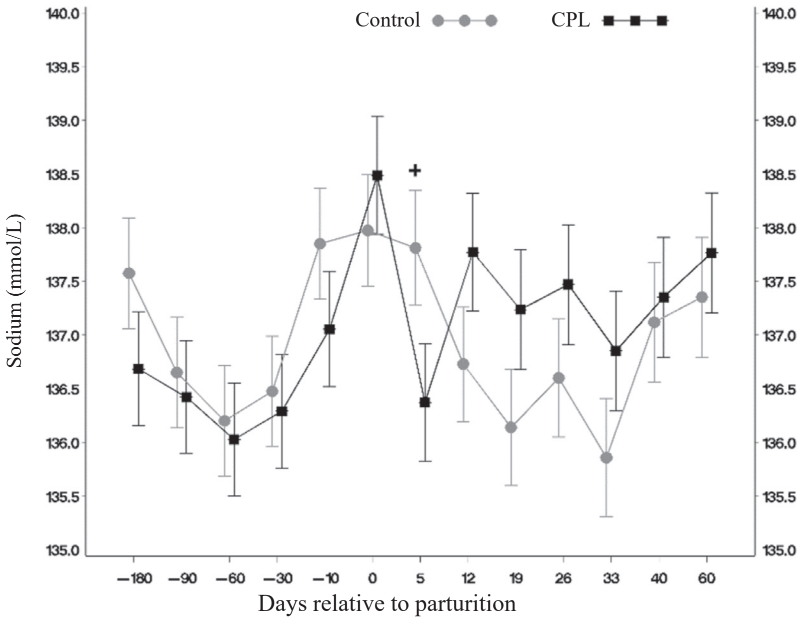

Fig. 5. Mean values and standard error of sodium (Na) the in-feed treated group (CPL) and the control group of cows from 3 months gravidity (day 180 prior to parturition) until 60 days postpartum. + Values with a plus within the column indicate that the differences between the groups are near to statistical significance ($P<0.10$).

We established a significantly improved ratio between Ca and P in the CPL-fed group in comparison to the control group on days 0 and 12 (Fig. 6).

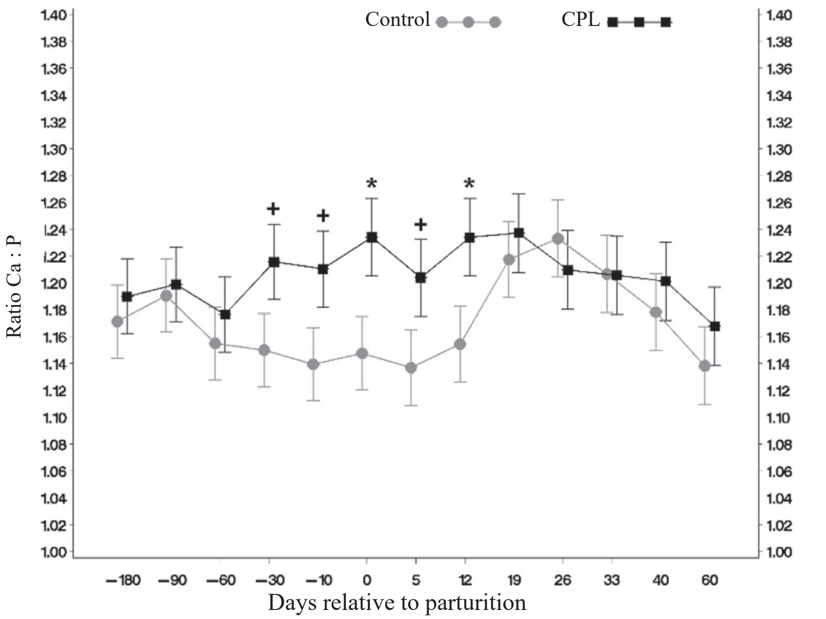

Fig. 6. Mean values and standard error of calcium *vs.* phosphorus (Ca : P) ratio in the clinoptilolite in-feed treated group (CPL) and the control group of cows from 3 months gravidity (day 180 prior to parturition) until 60 days postpartum. * Values with an asterisk within the column indicate statistically significant differences between the groups ($P<0.05$). + Values with a plus within the column indicate that the differences between the groups are near to statistical significance ($P<0.10$).

Discussion

Besides energy needs in dairy cows during the peripartal period, their health is greatly influenced by minerals. Insufficient concentrations and imbalanced ratios of minerals predispose the development of various disorders, characterized by altered muscular and neural functions.

In the current study, there were no significant differences in Ca concentrations between the CPL-fed group and the control group of dairy cows. It should be mentioned that the Ca concentration was higher in the CPL-fed group after the second week of lactation, but not significantly. Further, in the current study the concentrations of Ca in both groups of cows were over 2 mmol/L, which is considered by ROCHE and BERRY (2006) as a lower value in the physiological range of Ca concentration in the blood, and none of the cows developed milk fever symptoms. Similar results were reported by BOSI et al. (2002) and KATSOULUS et al. (2005) who did not establish significant differences in Ca values between cows fed different quantities of clinoptilolite and the control cows. However, in the later study the authors obtained the opposite results to ours two months after parturition. Namely, KATSOULUS et al. (2005) reported that the concentration of Ca was significantly higher in the control group in comparison to the cows that received 2.5% of dietary clinoptilolite. In contrast to our results, KHACHLOUF et al. (2019) obtained significantly higher values of Ca around parturition in cows fed a zeolite supplement in relation to the control group. Similar results were obtained with Zeolite A by THILSING-HANSEN and JØRGENSEN (2001) and THILSING-HANSEN et al. (2002, 2003). The later results could be explained by the fact that the cows in these studies were fed with much higher doses of Zeolite A and, also on day of calving and next two days postpartum, the cows from both groups were additionally fed per os with the Ca preparation.

THILSING-HANSEN et al. (2002) stated that increased concentrations of Ca in plasma around calving, in cows treated with zeolite, induced the activation of Ca homeostasis mechanisms. Namely, the decrease in the concentration of Ca prior to partus stimulates the release of PTH, which results in an increase in Ca reabsorption in the kidneys, resorption from bones, and stimulates vitamin D synthesis, that improves resorption of Ca in the intestines (GOFF, 2006). In addition, GRABHERR et al. (2009) observed that cows with a higher number of lactations had significantly lower concentrations of Ca when fed lower doses of zeolite, or without dietary zeolite, in comparison to the cows fed with higher quantities of zeolites. These authors stated that although a higher quantity of dietary zeolite could increase the concentration of Ca and prevent the development of hypocalcaemia, these cows had a significantly lower feed intake, probably due to the changed palatability of feed with a higher amount of zeolite.

In the current study, the concentration of P was significantly lower around parturition in the CPL-fed group than in the control group, although, the P concentration was within the reference range, from 1.3 to 2.6 mmol/L in dairy cows (GOFF, 1999). Similar to our results THILSING-HANSEN et al. (2002, 2003) recorded lower values of blood P after parturition in cows fed with Zeolite A. These authors explained that their results, of decreased P resorption, were due to P binding to the ions of aluminum within the zeolite structure and forming insoluble complexes. Conversely to our results KATSOULUS et al. (2005) and KHACHLOUF et al. (2019) did not establish significant differences in P concentrations between groups of cows fed with a zeolite supplement and the control group. Further, KHACHLOUF et al. (2019) reported that both groups of cows had significantly decreased P concentration immediately around parturition, but within the 3 weeks after partus, P concentrations were again at the level observed before parturition. Similar dynamics of P concentrations were also established in our study in the CPL-fed group of cows. HORST (1986) suggested that the hypophosphatemia that developed in cows around parturition could be explained by higher concentrations of PTH, which stimulates an increase in excretion of P by the kidneys and salivation.

In our study no significant differences in Mg concentrations were established between the CPL-fed group and the control group of cows. Also, similar results were obtained in studies by other authors (KATSOULUS et al., 2005; KHACHLOUF et al., 2019). Moreover, it is important to mention that both groups of cows in the current study had significantly lower Mg concentrations around parturition. RERAT et al. (2009) also observed decreased Mg concentrations in the blood following parturition, whereas this concentration was higher on the day of parturition in relation to the period before parturition.

In contrast to our results KHACHLOUF et al. (2019) detected decreased Mg concentrations in the blood of both groups of cows after parturition. THILSING-HANSEN et al. (2002) recorded lower Mg concentration only in the group of cows fed with a zeolite supplement on the day of parturition, while the control group of cows had significantly higher Mg concentrations on day of partus and for two days after. Temporarily increased Mg blood concentrations following parturition were also recorded in other studies (GOFF and HORST, 1997; KRONQVIST, 2011). GOFF (2008) and TAYLOR et al. (2008) suggested that increased Mg concentrations may induce a decrease in Ca concentrations around parturition, which consequently leads to an increased release of PTH and increased reabsorption of Ca and Mg in the kidneys. Moreover, we may point out that the decreased Mg concentrations observed in both groups of cows in our study were only temporarily exhibited and they normalized within a few days following parturition.

BOSI et al. (2002) and KATSOULUS et al. (2005) reported that there were no significant differences in K and Na concentrations between CPL-fed and the control groups of cows, similar to what we found in our study. In addition, GRABHER et al. (2009) did not establish any influence of different Zeolite A doses on K serum concentrations in dairy cows.

Conclusion

It may be concluded that dietary clinoptilolite influences the blood levels of Ca and P in dairy cows, depending on management and farm technology, primarily nutrition. Furthermore, the beneficial effect of dietary CPL on Ca:P ratio could increase resorption of Ca from the intestines in dairy cows during the early postpartal period. Namely, if nutrition is not appropriate to the specific needs of dairy cows, dietary clinoptilolite could improve their blood mineral profiles. The clinoptilolite supplement did not produce any clinically visible disorders in the metabolism of the tested minerals. In addition, the blood levels of all tested minerals were within the physiological ranges, which indicates that clinoptilolite did not alter their homeostasis in dairy cows.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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SAŽETAK

Cilj je ovoga istraživanja bio ustvrditi utjecaj klinoptilolita (KPL) dodanog u hranu na razine kalcija (Ca), magnezija (Mg), fosfora (P), kalija (K) i natrija (Na) u krvnom serumu mliječnih krava tijekom gravidnosti i rane laktacije. Istraživanje je provedeno na ukupno 78 krava holštajnsko-frizijske pasmine. Krave su nasumično podijeljene u dvije skupine: krave s dodatkom KPL-a u hranu (pokusna skupina) (n = 38) koje su primale dva puta dnevno po 50 g prirodnoga praškastog zeolita KPL-a, počevši 180. dan prije porođaja do 60 dana nakon porođaja, i na kontrolnu skupinu (n = 40). Uzorci krvi uzeti su 180., 90., 60., 30. i 10. dan prije teljenja, na dan teljenja te 5., 12., 19., 26., 33., 40. i 60. dana nakon porođaja. Nisu ustanovljene znakovite razlike u koncentraciji Ca između krava pokusne i kontrolne skupine. No nakon porođaja bilo je vidljivo da je koncentracija kalcija bila viša u pokusnoj KPL skupini, posebice 33. dan (P = 0,06). Koncentracija P bila je znakovito niža (P<0,05) u pokusnoj skupini nulti i 5. dan u odnosu na kontrolnu skupinu. Znakovito viši omjer Ca i P izračunan je u pokusnoj skupini u odnosu na kontrolnu skupinu nulti i 12. dan. Nisu ustanovljene znakovite razlike u koncentraciji Mg, K i Na u krava iz pokusne i kontrolne skupine tijekom istraživanja. Rezultati ovog istraživanja upućuju na to da je KPL dodan u hranu utjecao na razine Ca i P u krvi mliječnih krava te da je poboljšao omjer Ca i P u serumu tijekom ranog puerperija. Primijenjeni pripravak KPL-a nije prouzročio klinički vidljive poremećaje metabolizma istraživanih minerala Osim toga razine svih istraživanih minerala u krvi bile su unutar fizioloških raspona što pokazuje da KPL nije promijenio njihovu homeostazu u mliječnih krava.

Ključne riječi: zeolit; razina makrominerala; krvni serum; mliječne krave

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