

## DETERMINATION OF BLOOD SERUM CALCIUM, INORGANIC PHOSPHORUS AND MAGNESIUM IN DIFFERENT PRODUCTIVE STAGES OF HOLSTEIN DAIRY COWS

DJOKOVIĆ Radojica<sup>1</sup>, CINCOVIĆ Marko<sup>2</sup>, KURČUBIĆ Vladimir<sup>1</sup>, ILIĆ Zoran<sup>3</sup>, PETROVIĆ D. Milun<sup>1</sup>, PETROVIĆ Miloš<sup>1\*</sup>, ANDJELIĆ Biljana<sup>1</sup>

<sup>1</sup>University of Kragujevac, Faculty of Agronomy Čačak, Department of Animal Husbandry and Processing Technology, Serbia; <sup>2</sup>University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia; <sup>3</sup>University of Priština, Faculty of Agriculture Lešak, Department of Animal Science, Serbia

Received 26 February 2018; Accepted 10 April 2018

Published online: 15 June 2018

Copyright © 2018 Djokovic et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

*Introduction.* The aim of this study was to determine and compare levels of blood serum calcium, inorganic phosphorus and magnesium in the peripartal period and during mid lactation in Holstein dairy cattle.

*Materials and Methods.* Blood samples were collected from 12 cows in late pregnancy, 12 early lactation cows, and 12 mid lactation cows.

*Results and Conclusions.* Serum calcium and inorganic phosphorus in blood of dairy cows in early and mid lactation were significantly lower compared to the values in the blood serum of dairy cows in late pregnancy, probably indicating the increased use of these macro elements by the mammary gland at the early stages of lactation. When the cows were in the lactation period, blood serum magnesium levels were significantly lower compared to the values of dairy cows during late pregnancy, probably indicating the increased use of magnesium by the dairy cows during lactation. The results show that the homeostasis of the macro elements examined in the blood of transition and mid lactation dairy cows was maintained, suggesting their adequate supply from alimentary sources.

**Key Words:** Holstein cows, blood serum, calcium, phosphorus, magnesium

---

\*Corresponding author – e-mail: petrovic.milos87@kg.ac.rs

## INTRODUCTION

Macro elements are essential to maintain the normal function of vital biochemical processes in the dairy cow's body. Different degrees of deficiency of these inorganic substances can lead to clinical and subclinical symptoms, and significantly reduce productive and reproductive performance in dairy cows (Ivanov et al. 1993; Taylor, 2009). Lactating dairy cows use large quantities of calcium for milk synthesis (Thilising et al., 2007). Hypocalcemia in intensively managed dairy cows causes periparturient paresis and contributes to "downer cow syndrome", while during lactation it can also cause fertility disorders (Kalaitzakis et al., 2010). Deviation from the normal values of the calcium and inorganic blood parameters of cows in early lactation, as well as their deficiency in the diet can lead to subclinical or clinical manifestations (puerperal paresis, tetany), which have a negative impact on health and fertility (Roche & Berry, 2006; Holtenius et al., 2008; Kalaitzakis et al., 2010; Kamiya et al., 2010; Kronqvist et al., 2011). Late pregnancy and the beginning of lactation present a burden for the cow's physiology, as it has increased demands for calcium, which is necessary for the construction of the fetal skeleton. Veterinarians recognize the critical nature of the transition period, as it relates to periparturient disease (Van Saun, 2014). Phosphorous is the second most important macro element for bone tissue production. In addition, it participates in the cellular process of phosphorylation and energy production, affects acid-base balance and plays a role in the detoxification process (Sevinc et al., 1997; Reinhardt et al., 2011). Determination of calcium, magnesium and inorganic phosphorus in the blood serum is of important diagnostic value in preventing puerperal paresis and other diseases (Overton & Waldorn, 2004; Djokovic et al., 2009; Kamiya et al., 2010). Critical control points to prevent periparturient metabolic disease and improve lactational and reproductive performance were identified as: maximize dry matter intake; provide a properly balanced diet; maintain calcium status, and; minimize immune dysfunction (Van Saun, 2013). These nutrients play a critical role in physiological processes related to health, growth, and reproduction, and in the adequate function of the immune and endocrine systems. Blood levels of calcium, inorganic phosphorus, and magnesium in dairy cows during the periparturient period and lactation reflect metabolism of these macro elements or their supply through feed and their utilization by peripheral tissues, the mammary gland in particular (Ivanov et al., 1993; Taylor, 2009; Djokovic et al., 2014). However, their values can differ in cattle for many reasons, among which is the breed (Littledike et al., 1995). The aim of this study was to determine and compare levels of blood serum calcium, inorganic phosphorus and magnesium in periparturient period and during the mid of lactation in Holstein dairy cattle.

## **MATERIALS AND METHODS**

### **Animals, diets and protocol design**

A total of 36 dairy cows were randomly selected from the same Holstein herd containing 445 cows (Farm: Šarulja, Knić, Central Serbia). The cows were high-yielding with a preceding lactation of about 8500 l. Three groups of clinically healthy cows were chosen from the herd. Group 1 consisted of cows in late pregnancy ( $n=12$ ) from 30 to 1 day ( $28\pm 15$ ) to partus; Group 2 consisted of early lactation cows ( $n=12$ ) in the first month of lactation ( $22\pm 15$  days); Group 3 included mid lactation cows ( $n=15$ ) with between 90 to 150 days of lactation ( $133\pm 75$  days). The experimental cows were free in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of late pregnancy, early and mid lactation cows. Diet for cows in late pregnancy consisted of 7 kg grass hay, 5 kg corn silage (30% Dry Matter (DM)), 4 kg sweet corn silage, 6 kg beet noodle silage, 5 kg straw, 1 kg concentrate (18% crude protein (CP)). Diet for early lactation cows consisted of 4 kg grass hay, 10 kg corn silage (30% DM), 20 kg sweet corn silage, 12 kg beet noodle silage, 4 kg concentrate (18% CP) and 1 kg molasses. Diet for mid lactation cows consisted of 4.5 kg lucerne hay, 19 kg corn silage (30% DM), 16 kg beet noodle silage, 9 kg concentrate (18% CP) and 1.2 kg soybean expeller.

### **Sample collection**

Blood samples were collected at 9 a.m. about 3 h after milking and feeding. Blood samples (10 ml) were taken by jugular venipuncture into a sterile tube from each animal, and the blood serum was separated by centrifugation at room temperature ( $1.800\times g$ , 15 min). Blood serum was stored at  $-20^{\circ}\text{C}$  until biochemical analyses.

### **Biochemical analyses of blood serum**

The concentrations of calcium and magnesium in serum were determined by atomic absorption spectrophotometry (AAS), method QP-11.09 (SRPS ISO 8070:2013) and inorganic phosphorus with method QP-11.55 (SRPS EN 15621:2017), using the spectrophotometer BT 1000, Biotechnica Italia.

### **Statistical analysis**

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphics Centurion, Statpoint Technologies Inc., Warrenton, Virginia, USA). Analysis of variance was used to evaluate the probability of the significance of the differences between mean parameter values in each group. Values of  $p<0.05$  were considered significant.

## RESULTS

Results for the blood macro elements studied for all groups of cows are shown in Table 1.

**Table 1.** Blood macro elements in late pregnant, early and mid lactation dairy cows (n=12 in each group). Results are expressed as mean  $\pm$  standard deviation (SD)\*

Variables	Cows in Late pregnancy	Early lactation cows	Mid lactation cows
Ca (mmol/l)	2.07 $\pm$ 0.17 <sup>a</sup>	1.85 $\pm$ 0.15 <sup>b</sup>	1.92 $\pm$ 0.16 <sup>b</sup>
P (mmol/l)	2.33 $\pm$ 0.30 <sup>a</sup>	2.13 $\pm$ 0.16 <sup>b</sup>	2.22 $\pm$ 0.13 <sup>a</sup>
Mg (mmol/l)	1.20 $\pm$ 0.17 <sup>a</sup>	1.03 $\pm$ 0.29 <sup>b</sup>	1.04 $\pm$ 0.14 <sup>a</sup>

\*Different letters in the same row indicate a significant difference at  $p < 0.05^{(a-b)}$

The results show that among this group of Holstein dairy cows, lowest calcium levels were found in early and mid lactation, and these values were significantly lower ( $p < 0.05$ ) than the level of calcium in the blood of dairy cows in late pregnancy. Similarly to serum calcium, inorganic phosphorus values were significantly lower ( $p < 0.05$ ) in cows in puerperium compared to the values of inorganic phosphorus in the blood of cows in late pregnancy. The highest mean magnesium concentration was found in cows in late pregnancy (1.20 $\pm$ 0.17 mmol/l), and this level was significantly higher ( $p < 0.05$ ) than in early and mid lactation cows.

## DISCUSSION

These findings are similar to the findings of other researchers, who suggest that a sudden loss of calcium from the body occurs in puerperal cows, as for every liter of colostrum, 1.0-2.0 g of calcium is consumed (Sevinc et al., 1997; Bojkovski et al., 2005). Furthermore, a physiological decline of calcium in the blood holds steady for several days, and on account of intensive milk production, serum calcium decrease is correlated with the appearance of puerperal paresis (Sevinc et al., 1997). Calcium metabolism is closely related with phosphorus metabolism and is responsible for the proper metabolism of vitamin D (Green et al., 1981; Overton & Waldorn, 2004). Inorganic phosphorus levels in the blood of cows are at low concentrations, in the range of 2.1-3.0 mmol/l, while significantly more phosphorus is bound to organic compounds such as phospholipids (Puls, 1998; Bojkovski et al., 2005). Similarly to serum calcium, inorganic phosphorus was lower in cows in puerperium than in cows in late pregnancy. Hypophosphatemia is present in the blood of highly productive Holstein cows throughout the postpartum period. This condition is associated with the entry of glucose in the glycolytic path of peripheral tissues, as well as the phosphorus being used to supply the necessary quantities of mammary gland phosphorus (Grunberg et al., 2009; Djokovic et al., 2009; 2014). Some authors believe that hypophosphatemia

allows proper glucose metabolism which prevents ketosis immediately after calving (Reinhardt et al., 2011). The sudden loss of phosphorus from the body occurs in puerperal cows when 0.8-1.9 g phosphorus per liter of colostrum is spent (Bojkovski et al. 2005). The current study shows a decrease in serum calcium and inorganic phosphorus in the blood of cows in early lactation, suggesting increased use of these macro elements by the mammary glands. The results show that blood calcium and phosphorus levels were within the physiological limits (adequate supply) in all experimental cows, with calcium levels approaching the limit values in puerperium cows due to a sudden increase in mammary gland activity and increase in calcium mobilization from both the blood and the body depots. Similar results have been reported elsewhere (Puls, 1988; Ivanov et al., 1993; Littledike et al., 1995; Overton & Waldorn, 2004; Djokovic et al., 2014). Magnesium, like calcium reduces neuromuscular irritability and a drop in its concentration in the blood results in spontaneous muscle contractions or tetany (Holtenius et al. 2008). The results obtained in our study show that the magnesemia was within the normal range (Puls, 1988) in all studied cows, and lower values were found in lactating cows. This probably points to the increased use of magnesium during negative energy balance in early lactation.

## CONCLUSION

Serum calcium, inorganic phosphorus and magnesium in the blood of Holstein dairy cows in puerperium and during mid lactation were significantly lower than levels determined in cows in late pregnancy, indicating the increased use of these macro elements by the mammary gland of cows in early lactation. The results show that the homeostasis of the macro elements tested in the blood of transition and mid lactation dairy cows was maintained, suggesting their adequate supply from alimentary sources.

## REFERENCES

- Bojkovski J., Borozan S., Jozef I., Samanc H. 2005. Colostrum ingredients before and after calving in Holstein-Friesian Cows. *Veterinary Record*, 156:744-745. DOI: 10.1136/vr.156.23.744
- Djokovic R., Samanc H., Ilic Z., Kurcubic V. 2009. Changes in blood values of glucose, insulin and inorganic phosphorus in healthy and ketotic cows after intravenous infusion of glucose solution. *Acta Veterinaria Brno*, 78:385-389. DOI: 10.2298/BAH1404571D
- Djokovic R., Kurcubic V., Ilic Z. 2014. Blood serum levels of macro and micronutrients in transition and full lactation cows. *Bulgarian Journal of Agricultural Science*, 20(3):715-720.
- Green H. B., Horst R. L., Beitz D. C., Littledike E. T. 1981. Vitamin D metabolites in plasma of cows fed parturient low calcium diet for prevention of parturient hypocalcemia. *Journal of Dairy Science*, 64:217-2019.
- Grunberg W., Staufenbiel R., Constable P. D., Dann H. M., Morin D. E., Drackley J. K. 2009. Liver phosphorus content in Holstein-Friesian cows during the transition period. *Journal of Animal Science*, 92:2106-2117. DOI: <https://doi.org/10.3168/jds.2008-1897>

- Holtenius K., Kronqvist C., Briland E., Sprondly R. 2008. Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *Journal of Dairy Science*, 91:743-748. DOI: 10.3168/jds.2007-0309
- Ivanov I., Damnjanovic Z., Radojic S. 1993. Metabolic disorders of macroelements in late pregnancy and during the early lactation. *Veterinary Bulletin*, 47(4-5):319-329.
- Kalaitzakis E., Panousis N., Roubies N., Giadinis N., Kaldrymidou E., Georgiadis M., Karatzia S. H. 2010. Clinicopathological evaluation of downer dairy cows with fatty liver. *Canadian Veterinary Journal*, 51:615-622.
- Kamiya Y., Kamiya M., Tanaka M. 2010. The effect of high ambient temperature on Ca, P and Mg balance and bone turnover in high yielding dairy cows. *Animal Science Journal*, 81:482-486.
- Kronqvist T. C., Emanuelson U., Sprondly R., Holtenius K. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. *Journal of Dairy Science*, 94:1365-1373. doi: 10.3168/jds.2009-3025.
- Littledike E. T., Wittum T. E., Jenkins T. G. 1995. Effect of breed, intake and carcass composition on the status of several macro and trace minerals of adult beef cattle. *Journal of Animal Science*, 73:2113-2119.
- Overton T.R., Waldorn M. R. 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science*, 87:E105-E119. DOI: [https://doi.org/10.3168/jds.S0022-0302\(04\)70066-1](https://doi.org/10.3168/jds.S0022-0302(04)70066-1)
- Puls R. 1988. Mineral levels in animal health. Diagnostic data. Sherpa international, Clearbrook, British Columbia V2T 4X2, pp.240, Canada.
- Reinhardt T. A., Lippolis J. D., McCluskey B. J., Goff J. P., Horst R. L. 2011. Prevalence of subclinical hypocalcemia in dairy herds. *Veterinary Journal*, 188:122-124. doi: 10.1016/j.tvjl.2010.03.025.
- Van Saun, R. J. 2014. Transition Cow Nutrition and Management: The Key to Herd Reproductive Performance. Department of Veterinary Science, Pennsylvania State University. Available at: <https://www.researchgate.net/publication/242724156>
- Van Saun, R. J. 2013. Transition Cow Management. Department of Veterinary & Biomedical Sciences. Pennsylvania State University
- Roche J. R., Berry D. P. 2006. Periparturient climatic, animal, and management factors influencing the incidence of milk fever in grazing systems. *Journal of Dairy Science*, 89:2775-2783. DOI: 10.3168/jds.S0022-0302(06)72354-2
- Sevinc M., Basaglu A., Birdane F., Gokcen M., Kucukfindik M. 1997. The changes of metabolic profile in dairy cows during dry period and after. *Turkish Journal of Veterinary and Animal Science*, 3:475-478.
- SRPS ISO 8070:2013 Milk and milk products -- Determination of calcium, sodium, potassium and magnesium contents -- Atomic absorption spectrometric method
- SRPS EN 15621:2017 Animal feeding stuffs: Methods of sampling and analysis - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES
- Taylor M. S., 2009. Calcium and phosphorus metabolism in Jersey and Holstein cows during early lactation, PhD Thesis, Virginia State University.
- Thilising T., Larsen, T., Jorgensen R. J., Houe H. 2007. The effect in zeolite A treated dry cows on periparturient calcium and phosphorus homeostasis. *Journal of Veterinary Medicine*, 54:82-91. DOI: 10.1111/j.1439-0442.2007.00887.x

## **ODREĐIVANJE KALCIJUMA, NEORGANSKOG FOSFORA I MAGNEZIJUMA U KRVNOM SERUMU RAZLIČITIH PROIZVODNIH GRUPA HOLŠTAJN MLEČNIH KRAVA**

DJOKOVIĆ Radojica, CINCOVIĆ Marko, KURČUBIĆ Vladimir, ILIĆ Zoran, PETROVIĆ D. Milun, PETROVIĆ Miloš, ANDJELIĆ Biljana

### **Kratak sadržaj**

*Uvod.* Cilj ovog rada bio je utvrđivanje i upoređivanje nivoa kalcijuma, neorganskog fosfora i magnezijuma u krvnom serumu u peripartalnom periodu i tokom sredine laktacije kod holštajn mlečnih krava.

*Materijal i metode.* Uzorci krvi su sakupljeni od 12 krava u visokoj bremenitosti, 12 krava u ranoj laktaciji i 12 krava u sredini laktacije.

*Rezultati i zaključak.* Kalcijum u serumu i neorganski fosfor u krvi mlečnih krava u ranoj i srednjoj laktaciji bili su značajno niži u poređenju sa vrednostima u krvnom serumu mlečnih krava u visokoj bremenitosti, verovatno ukazujući na povećanu upotrebu ovih makroelemenata od strane mlečne žlezde u ranim fazama laktacije. Kada su krave bile u toku laktacije, nivo magnezijuma u krvnom serumu bio je značajno manji u poređenju sa vrednostima kod krava u toku visoke bremenitosti, verovatno ukazujući na povećanu upotrebu magnezijuma kod mlečnih krava u toku laktacije. Rezultati pokazuju da se homeostaza testiranih makroelemenata mlečnih krava u krvi održava tokom tranzicionog perioda i u sredini laktacije, što ukazuje na njihovo adekvatno snabdevanje iz hranljivih izvora.

**Ključne reči:** holštajn krave, krvni serum, kalcijum, fosfor, magnezijum.