

Values of Calcium, Phosphorus and Magnesium Concentrations in Blood Plasma of Cows in Dependence on the Reproductive Cycle and Season

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Geliş Tarihi /Received: 21.02.2011

ABSTRACT

The research was conducted on 240 clinically healthy Holstein-Friesian cows in various stages of the reproductive cycle (lactation, dry period, and up to 15 days from calving). Sampling was taken during the summer and winter periods. Concentrations of calcium, phosphorus and magnesium in blood plasma were determined by using the appropriate method with the “Beckmann DU-64 UV/VIS” spectrophotometer. Significant differences were determined in the values of concentrations of the examined minerals during various stages of the reproductive cycle but in dependence on the season of sample taking.

Key Words: Lactation, dry period, postpartum period, minerals

ÖZET

REPRODÜKTİF DÖNEM VE MEVSİME BAĞLI OLARAK İNEKLERDE KAN PLAZMASI KALSİYUM, FOSFOR VE MAGNEZYUM KONSANTRASYONU DÜZEYLERİ

Çalışma, farklı reproduktif dönemlerdeki (laktasyon, kuru period ve buzağılama sonrası ilk 15 günlük dönem) 240 baş sağlıklı Siyah Alaca inek ile yürütülmüştür. Örneklemeler yaz ve kış aylarında yapılmıştır. Kan plazmasındaki kalsiyum, fosfor ve magnezyum konsantrasyonları “Beckmann DU-64 UV/VIS” spektrofotometresi ile uygun metotlar kullanılarak belirlenmiştir. Örnekleme mevsiminin etkisine bağlı olarak, incelenen mineral konsantrasyonları bakımından çeşitli reproduktif dönemler arasında önemli farklılıklar belirlenmiştir.

Anahtar Kelimeler: Laktasyon, kuru dönem, doğum sonu dönem, mineraller

Introduction

Life processes take place within a closed space which is connected with its environment through a number of activities. The internal ambiance, milieu interior, as called by Claude Bernard, the founder of modern physiology, in

its communication with the external world, as well as its own metabolism, attempts to maintain the living organism's harmony, namely its homeostasis. Disruption of homeostasis provokes illness, and ultimately, death. Minerals play a vital role in maintaining

homeostasis. Minerals are involved in all living processes, either in the capacity of structural elements or as regulators of almost all metabolic processes (Bauman and Currie, 1980). Apart from the above indicated roles, minerals are important for milk production of high-yielding cows. Consequently, their role assumes an increasing importance. Homeostasis of calcium, phosphorus and magnesium is primarily affected by the very same homeostatic mechanisms, and as a result, the changes in their concentrations are in most cases mutually linked. Calcium is the most pervasive mineral in an organism. The maintenance need of calcium is approximately 15.4 mg Ca/kg of body mass (Hansard et al., 1954). The need in lactation is approximately 1.23 g Ca/kg of the produced milk. Calcium absorption depends on its intake, and it is reduced when the animal takes more calcium in its food than it is needed.

Puerperal paresis or milk fever of dairy herds is characterized by moderate or conspicuous hypocalcaemia, frequently accompanied by hypophosphatemia or hypoglycaemia, but magnesium concentration may be normal, reduced or increased (Littledike et al., 1998). Although milk fever is primarily a disease of recently periparturient cows, hypocalcaemic paresis can also occur in cattle and lactating cows which are not in the periparturient period (Nurmio et al., 1974). Milk fever is caused by the insufficient absorption of calcium at the beginning of the lactation period (Shultz, 1988). In the course of disease the concentration of calcium and phosphorus in the blood decreases while the magnesium concentration increases. The most probable cause of this disease is the imbalance in ratios of calcium, phosphorus, magnesium and calcium. Cattle with the increased incidence of hypocalcaemia are characterized by a higher calcium level in the dry period (Barnouin, 1991). This fact is explained by Phillipou et al. (1994). The explanation is that the feed with the calcium levels higher than those recommended (NRC, 2002) in the dry period, and with the increased needs in early lactation period will trigger a weaker response of the tissue to D vitamin and parathyroid hormones. In addition

cations, primarily calcium and sodium, create a metabolic alkalosis, which, in turn, affects the homeostatic mechanisms of calcium by weakening the tissue's response to parathyroid hormones. Prevention involves a careful balancing of particular minerals and other nutritious ingredients.

Considering the fact that phosphorus and calcium jointly participate in the bone building, these two minerals are often considered together. The effect of calcium and phosphorus ratios on the performances of ruminants is considered to be overstated. Alfaro et al. (1989) showed that the calcium and phosphorus ratios in feed in the range of 1:1 up to 7:1 resulted in the same performances. Phosphorus is indispensable for rumen's microorganisms for their growth and cellular metabolism. A great part of the overall phosphorus in cereals and oil crops, as well as its side products, is linked with the phytic acid, and as such, it is not resorptive. However, the rumen's micropopulation consists of the phytase enzyme which hydrolyses the organically linked phosphorus, thus making it accessible to resorption.

Dairy cows with abomasal displacement or abomasal volvulus may have phosphorus homeostasis disorder (Grunberg et al., 2005). In these conditions hypophosphatemia is accounted for by a decreased intake of feed and the liver disorder or its damage (Grunberg et al., 2005). The cows with the pronounced hypophosphatemia (<0.32 mmol/L) are exposed to postpartum hemoglobinuria, which is (poses) a higher risk than the emergence of a hemolytic disease. The development of this condition is also influenced by other factors. It has been established that the continuous intravenous application of a 50% dextrose solution causes the decrease of the phosphorus serum concentration in lactating cows. This hypophosphatemia is considered to be a result of insulin-provoked shift of the plasma phosphorus into the intracellular compartments (Grunberg et al., 2005). Hypophosphatemia is often connected with moderate hypocalcaemia (Kruger et al., 1996).

The focal place of magnesium absorption in ruminants is in the rumen (Greene et al., 1983). In many respects magnesium metabolism is specific in relation to other microelements. The regulatory mechanisms of magnesium flow have not been discovered up to now. Inside the cell magnesium acts as a catalyst to all reactions that use ATP. In this manner magnesium affects all endergonic and exergonic processes in the organism (Anastasiadis et al., 1981). In case of magnesium deficit insulin partly increases the concentration of intracellular Mg, and this reflects the equilibrium of enzyme regulation. Simultaneously, taurin is mobilised with the effect similar to that of Mg. In this way the organism compensates for the intracellular hypomagnesaemia to some extent, but at the expense of the extracellular liquid (Stojević et al., 1993). In general, Mg acts as an antagonist to Ca, while its deficiency emphasizes the effect of Ca. At this the release of acetylcholine onto the neuromuscular plates is more pronounced. In addition to this, extracellular hypomagnesaemia weakens the activity of cholinesterase which, in turn, additionally prolongs the effect of acetylcholine. The consequence of the above is tetany, one of the basic symptoms of hypomagnesaemia (Rosol and Capen, 1997). Along with hypocalcaemia hypomagnesaemia can cause pasture tetany in cows and goats. Hypocalcaemia may be a major cause for the emergence of hypomagnesaemia (Roussel et al., 1982). Although there is no strong correlation between clinical signs and the serum magnesium, the cows in which the magnesium concentration in serum is lower than 0.4 mmol/L run a higher risk of developing pasture tetany. A more important indicator of magnesium status lies in determining the value of magnesium in urine. Thus, in case of a significant magnesium deficiency, magnesium will not be present in urine (Hoffis et al., 1989).

The purpose of this study was to determine serum Ca, P and Mg levels during lactation period, dry period and postpartum period of cattle and to determine the relationships between periods.

Material and Methods

The research was conducted on the total of 240 cows of Holstein-Friesian breed, in the 3–5 age range. The cows were in different stages of lactation (1–8), but the greatest number of cows was in the second, third and fourth stage of lactation. Samples were taken during summer of 2008 (n = 120), and in the winter period of 2008/2009 (n = 120). The examined animals were divided into two groups according to the respective stages of the reproductive cycle. Group A was composed of lactating cows (n = 120), while B group involved cows in dry period (n = 60) and group C was composed of cows up to 15 days from calving (n = 60).

Blood samples were taken by punctuating *venae coccigaeae* and were stored in 5 ml vacuum blood containers. After that the blood was transported in the portable fridges at temperature of 4 °C to the Veterinary Faculty in Sarajevo. The blood was immediately centrifuged (LC 320, 3000 turns/10 min) in order to extract plasma. Concentrations of calcium, phosphorus and magnesium in blood plasma were determined with spectrophotometer (Beckmann DU-64 UV/VIS) using commercial kits manufactured by the "Human" (Max-Planck-Ring 21, D-65205 Wiesbaden, Germany), Calcium liquicolor, REF. 10011; Phosphorus liquirapid, REF. 10027 and Magnesium, REF. 10010.

The SPSS 10.00 software program was used for the statistical elaboration of data. Mean values of the examined parameters between different animal groups in dependence of the sampling season, were compared by using t-test. The differences were considered statistically significant at $P < 0.05$ significance level.

Results and Discussion

Results are presented in figures.

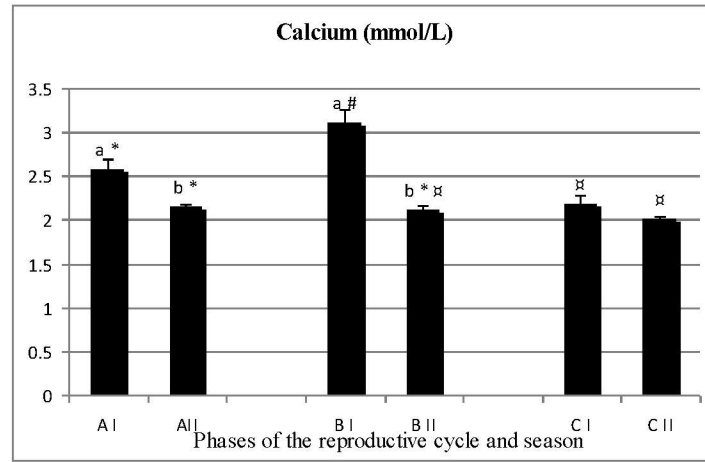


Figure 1. Mean value of calcium concentration (mmol/L) in blood plasma depending on the reproductive cycle of cows and sampling season. Values are mean±S.E. Marks I, II represent different seasons, where: I = summer period, II = winter period. A, B, C = different group of animals within different reproductive cycle, where A = lactating cows, B = dry period, C = cows up to 15 days from calving. a, b = values with different letters are statistically significant within the group ($P<0.05$). Marks *, α, # = values with different labels are statistically significant between groups.

Şekil 1. Reprodüktif dönem ve örnekleme mevsimine bağlı olarak kan plazması kalsiyum konsantrasyonu (mmol/L) ortalamaları (Ortalama değer ± standart hata). I= yaz dönemi, II = kış dönemi; A= laktasyondaki inekler, B= kuru dönem, C=buzağılamadan sonraki ilk 15 gün. a, b = grup içerisinde farklı harf taşıyan ortalamalar arası farklılık önemlidir ($P<0.05$). *, α, # = farklı işaret taşıyan gruplar arası farklılık önemlidir.

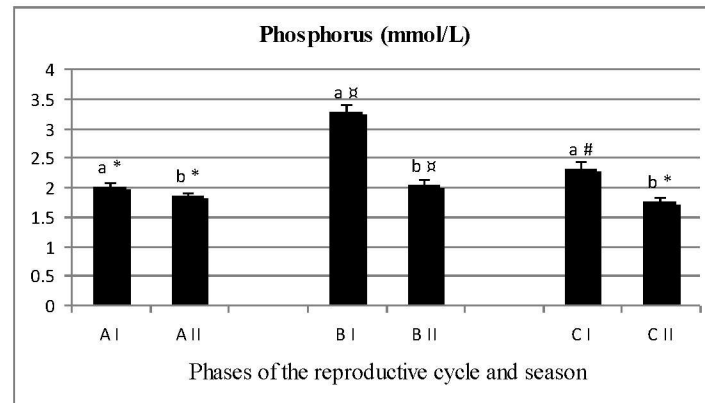


Figure 2. Mean value of phosphorus concentration (mmol/L) in blood plasma depending on the reproductive cycle of cows and sampling season. Values are mean±S.E. Marks I, II represent different seasons, where: I = summer period, II = winter period. A, B, C = different group of animals within different reproductive cycle, where A = lactating cows, B = dry period, C = cows up to 15 days from calving. a, b = values with different letters are statistically significant within the group ($P<0.05$). Marks *, α, # = values with different labels are statistically significant between groups.

Şekil 2. Reprodüktif dönem ve örnekleme mevsimine bağlı olarak kan plazması fosfor konsantrasyonu (mmol/L) ortalamaları (Ortalama değer ± standart hata). I= yaz dönemi, II = kış dönemi; A= laktasyondaki inekler, B= kuru dönem, C=buzağılamadan sonraki ilk 15 gün. a, b = grup içerisinde farklı harf taşıyan ortalamalar arası farklılık önemlidir ($P<0.05$). *, α, # = farklı işaret taşıyan gruplar arası farklılık önemlidir.

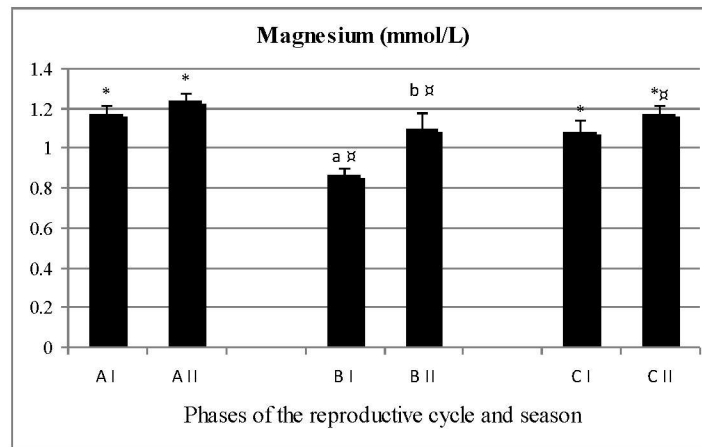


Figure 3. Mean value of magnesium concentration (mmol/L) in blood plasma depending on the reproductive cycle of cows and sampling season. Values are mean±S.E. Marks I, II represent different seasons, where: I = summer period, II = winter period. A, B, C = different group of animals within different reproductive cycle, where A = lactating cows, B = dry period, C = cows up to 15 days from calving. a, b = values with different letters are statistically significant within the group (P<0.05). Marks *, # = values with different labels are statistically significant between groups.

Şekil 3. Reprodüktif dönem ve örnekleme mevsimine bağlı olarak kan plazması magnezyum konsantrasyonu (mmol/L) ortalamaları (Ortalama değer ± standart hata). I= yaz dönemi, II = kış dönemi; A= laktasyondaki inekler, B= kuru dönem, C=buzağılamadan sonraki ilk 15 gün. a, b = grup içerisinde farklı harf taşıyan ortalamalar arası farklılık önemlidir (P<0.05). *, # = farklı işaret taşıyan gruplar arası farklılık önemlidir.

The range of reference values of calcium in cows is 2.2 – 3.0 mmol/L (Jovanović et al., 1997), 2.1 – 2.8 mmol/L (Merck, 2003), 2.43 – 3.1 mmol/L (Kaneko, 2008; Radostits et al., 2000).

Our research has proved the influence of sampling season on the value of calcium concentration in lactating cows and those in dry period (Figure 1). In cows up to fifteen days from calving, the influence of sampling season on the value of calcium has not been proved. The calcium level depends on the feed so that the varied botanical make-up of pastures, and grazing in general, may be a cause of significant differences established during various seasons of sample taking.

Certain values of calcium concentration have shown significant differences depending on the reproductive cycle of cows. The highest value of calcium concentration has been established in cows in the dry period with samples taken in summer, while lactating and postparturient cows have significantly lower values of calcium concentration in the same

period. The mean calcium level determined during summer in cows in dry period was found to be higher (3.1 ± 0.16 mmol/L) than those of the other published research findings (Kaneko, 2008; Merck, 2003). Hypercalcaemia is not a frequent phenomenon in ruminants. In cattle hypervitaminosis D, provoked by a generous addition of this vitamin in the animal feed, may simultaneously cause hypercalcaemia and hyperphosphatemia (Roberson et al., 2000). This fact could account for the high values of calcium in our research findings since during the above indicated period of sample taking in the group of cows in dry period the highest values of phosphorus concentration were determined (3.26 ± 0.14 mmol/L), while in other studies the reference values of phosphorus in the cows in this group are in the range of 1.81–2.1 mmol/L (Kaneko, 2008; Radostits et al., 2000; Whitaker, 2000) and 1.4–2.5 mmol/L (Merck, 2003).

During summer period, significantly lower levels of calcium were determined in cows immediately after calving. In interpreting the

calcium level we should not neglect the physiological hypocalcaemia which occurs after calving, especially in dairy cows. If we make a comparison with the established reference values we can notice hypocalcaemia in many periparturient cows. However, these values are appropriate for the postpartal physiological condition (Oltner and Bergland, 1983). Our research has also shown that the lowest values of calcium concentration have been determined in cows up to fifteen days from calving, while in winter period the calcium level amounted to 2 mmol/l. The calcium value below 2 mmol/L is considered to be critical (Underwood, 1969). During the same sampling season somewhat higher values, but not significantly higher, were determined in cows in the dry period. Significantly higher values of calcium were determined during the same sampling period in lactating cows (Figure 1). The studies conducted on dairy cows indicate that milder forms of hypocalcaemia during periparturient period may cause disease. In other words, the connection was established between hypocalcaemia in dairy cows in periparturient period and abomasum displacements as well as the uterus prolapse.

The research about calcium level in dependence on the physiological condition and seasonal variations did not show the influence of the above indicated factors on calcium values in cows (Yokus et al., 2006). However, Krnić et al. (2003) reported not only considerably lower calcium values in cows 1-5 days from calving but also hypocalcaemia in lactating cows and in cows 5-10 days before calving. Lower bottom values of calcium are also reported by Olayemi et al. (2001). They found that 39% of cows had the calcium concentration lower than 2 mmol/L.

According to literature data, the reference values of phosphorus in cows are in the range of 1.81–2.1 mmol/L (Kaneko, 2008; Radostits et al., 2000; Whitaker, 2000) and 1.4–2.5 mmol/L (Merck, 2003). The findings in our research indicate that phosphorus concentration was greatly influenced by the sampling season as well as the reproductive cycle of cows (Figure 2). Phosphorus values are primarily a reflection of phosphorus intake through feed

(Whitaker, 2000). The influence of phosphorus concentration on phosphorus values in blood plasma was reported by Huffman et al. (1933). Palmer and Eckles (1927) reported low values of phosphorus in the blood of animals fed in phosphorus-deficient regions. Our research findings indicate a greater need of cows for phosphorus than that provided by plants contained in the animal diet, and this is a direct consequence of low values of this mineral in the soil composition. The age of animals also influence the value of phosphorus concentration. In other words, the phosphorus level increases with the age of animals (Roussel et al., 1982). As early as 1919, Meigs et al. (1919) observed phosphorus variations in the blood plasma of cows commensurate with their age, gravidity and lactation. Health disorders which are a direct outcome of hypophosphatemia and hypocalcaemia occur most commonly in the period around calving. This is the most vulnerable phase in respect of homeostasis of calcium and phosphorus since the organism must provide in a short time interval big quantities of these macro elements indispensable for lactic glands at the beginning of lactation. The imbalance of calcium and inorganic phosphorus results in the disturbance of locomotors system slowed down involution of the uterus, irregular cycles, abortions and endometritis.

The values of magnesium concentration in literature varies widely such as 0.8–1.3 mmol/L (Whitaker, 2000), 0.7–1.2 mmol/L (Merck, 2003), 0.74–0.95 mmol/L (Kaneko, 2008; Radostits et al., 2000). In our research we have established the influence of the sampling season on the value of magnesium in cows in the dry period, while in other two groups of cows this influence was not detected (Figure 3). Since the value of magnesium in blood is a reflection of the nutritious value of animal feed, we assume that this factor affected the values obtained in our research. Mg resorption from the rumen may become decreased due to the excessive intake of azotes and calcium and the decreased intake of phosphorus and sodium. The soils rich in calcium or those intensively fed with mineral fertilizers based on calcium carbonate and azote

represent high-risk areas for the emergence of hypomagnesaemia in cattle.

Considering the fact that the level of magnesium in blood is primarily a reflection of its intake through feed rather than its reserves in the organism (Whitaker, 2000), we can assume that animals have obtained different quantities of this element through animal feed.

The supply level of an organism with minerals depends exclusively on their intake through the digestive system. In spite of this, a sufficient quantity of minerals in a ration does not at the same time imply that it is a sufficient quantity for the organism as such. Resorption of minerals depends on their loss through procreation and milk. In our research we have established the influence of the sampling season and the physiological condition, namely, the phase of the reproductive cycle, on the values of the examined minerals. Significantly lower values of calcium were determined during summer sampling in postparturient cows than those of lactating cows and those of cows in the dry period. Calcium is especially important in the diet of dairy cows because of the high calcium content in milk, but also because of postpartal paresis. The values of phosphorus indicate the influence of the sampling season, but also the physiological condition of cows. The biggest variations in phosphorus concentrations were determined in the period around calving with the increasing need of lactic glands at the beginning of lactation. The influence of sampling season on magnesium concentration was determined only in cows in the dry period, while the influence of the phase of the reproductive cycle was manifested in significantly lower values of magnesium concentration in cows in the dry period during summer sampling.

In conclusion, in order to clarify the conditions which bring about the above indicated changes we suggest to carry out our future research under the controlled conditions in respect of nutrition.

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