

## Effect of daily milk production levels on plasma calcium, phosphorus and magnesium concentrations in dairy cows

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### Research Article

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### ABSTRACT

The aim of this study was to determine the serum level of calcium (Ca), phosphorus (P) and magnesium (Mg) and hence determine the relationship and the effect of daily milk production on the mentioned essential macro elements. The metabolism and neurohumoral regulation of Ca, P and Mg are closely related, and the metabolic disorder of one of these electrolytes inevitably affects the metabolism of the other two. The study was carried out in the winter period of animal keeping and nutrition, and it included 63 Holstein-Friesian breed cows in the northern region of Bosnia and Herzegovina, aged 2-9 years in different lactation stages (1-8). The largest number of cows, eighteen of them, were in the second, third and fourth lactation stage, while nine cows were in the first lactation stage. This study was conducted on three different groups of cows corresponding to the amount of daily milk production. First was the group of lower daily milk production n=21, the second group of examined cows was the group of medium daily milk production n=23 and third was the group of higher daily milk production n=19. Ca, P and Mg were determined in blood plasma using the Beckmann spectrophotometer. By examining the obtained results and the dynamics of the tested mineral substances, we point out the different behaviour of the Ca-P relationship in correlation with the different level of daily milk produced. Although the average values of both minerals are lowered with an increase in daily milk production, the analysis of single linear regression shows that there is a negative correlation between P concentration in blood plasma and the amount of daily milk produced, while for Ca it has not been established. Although the concentration of Mg in the blood plasma increases as the daily milk production increases, the analysis of single linear regression does not show a significant interconnection of these two values. Lower average values of Ca and P concentrations in the blood of cows with higher daily milk production may be associated with increased total excretion of these minerals through milk, unlike the cows which daily produced lower amounts of milk.

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## Introduction

Maintenance of dairy cows in high lactation and good health condition are top priorities of modern breeding, whilst the control of nutritional metabolic status is of great importance. The metabolic overload of highly-productive cows is most evident in late gravidity and puerperium. Considering the needs of cows in the last two months of gravidity and in early lactation, especially

during the winter period, meals are often not well and rationally balanced, both in terms of nutrient selection and in terms of energy content, such as: proteins, dry matter and cellulose. During the production cycle, the organism of each cow has great demands such as labor and lactogenesis. Both processes require the change and increased metabolism of Ca.

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After the calving, Ca is directed to the milk gland with previous bone mobilization and enhanced absorption from the digestive system (Bigras-Poulin and Tremblay, 1998) aiming to maintain the physiological level in blood and other tissues (Hays and Swenson, 1993). Concentration of Ca in cow serum during lactation is associated with changes in the concentration of P in serum (Bigras Poulin and Tremblay, 1998). Mg is being excreted in cow milk during the lactation, while the homeostasis depends on the resorption of Mg from the digestive system and the excretion from the kidneys. Therefore, any deviation in the serum concentration of Mg can be quickly detected (Herdt, 2000, Kaneko et al., 2008). These specificities can at the same time disturb the normal flow of physiological processes and influence highly-productive cows to become particularly prone to metabolic disorders.

Metabolism and neurohumoral regulation of P, Ca, and Mg traffic are closely related, and the metabolic disorder of one of these electrolytes inevitably affects the metabolism of the other two. Therefore, the pathophysiological changes associated with these three electrolytes are studied together (Jovanovic, 2012; Zivancevic - Simonovic, 2006; Katica, 2007, Mukaca, 2015).

Puerperal paresis as a serious metabolic disorder implies visible or moderate hypocalcaemia with hypophosphatemia, but the concentration of Mg may be normal, reduced or most frequently increased (Jovanovic, 2012; Hadzimusic and Krnic, 2012). Milk fever is caused by insufficient Ca absorption in the initial part of lactation. The most common cause of this disease is the imbalance in the ratio of P, Mg and Ca. Hypocalcaemia may be the major cause of hypomagnesaemia (Aladrovic et al., 2018; Mukaca, 2015; Katica, 2007). Although there is no strong correlation between clinical signs and Mg in the serum, cows which have a concentration of serum Mg lower than 0.4 mmol / L, also have a higher risk of developing pasture tetanus.

Cutuk (2010) described hematological profile of cows in postpartum phase and shortly after delivery, while Khan et. al. (2002) as well as McNamara et.al. (2003) reported on influence of quality and composition of dairy cow's food on the blood metabolite concentration. Hadzimusic and Krnic (2012) analyzed values of Ca, P and Mg in blood plasma during the

summer period of breeding cows in lactation depending on reproductive cycle. However, there is not enough research on the effect of daily milk production on essential plasma minerals profile, especially during winter period.

It is obvious that the relationship between production characteristics of high dairy producing cows (daily milk production) and metabolic capacity of their organism is still very poorly researched; therefore the reason for this kind of research is highly justified. In this regard, the aim of this study was determining the levels of Ca, P i Mg in plasma, and as well determining the link between the daily milk production effect on the mentioned essential macro elements.

## Material and Methods

The research was carried out at the farm of Holstein-Friesian breed cows in the northern region of Bosnia and Herzegovina placed in modern and very good zoo-technical conditions. The Radio Frequency ID (RFID) technology was used during the breeding and production in this farm, which implies that each cow owns a chip through which the animal's activities related to reproduction, lactation and history of the diseases is being monitored and recorded.

The study was carried out during the winter period of, and it included 63 cows aged 2-9 years (the largest number of cows was between 3 and 5 years old) in different lactation stages (1-8). The largest number of cows, eighteen of them, were in the second, third and fourth lactation stage, while nine cows were in the first lactation stage. Three groups of cows were formed according to the amount of daily milk produced, based on the control of the amount of daily milk production which was performed during the last fifteen days, prior to the blood sampling:

- Group 1 = Low-level of daily productivity of cows: 20 - 28 L/day of milk (n = 21).
- Group 2 = Medium-level of daily productivity of cows: 29 - 34 L/day of milk (n = 23).
- Group 3 = High level of daily productivity of cows: 35 - 52 L/day of milk (n = 19).

Examined cows (n=63) were unselectively grouped into three groups (group: 1., 2., and 3.), regardless of age or lactation stage. In the winter period, the animals were fed depending on the physiological status and reproductive-production cycle: high milk producing cows were fed with 30 kg of silage, 15 kg of haylage,10

kg of concentrated mixture containing 18-20% of proteins. Cows in dry cow period are fed with 20 kg of silage, 10 kg of haylage and 3-4 kg of concentrate with 18-20% protein. Postpartum cows had meal consisted of silage ad libitum, 2 kg of concentrate mixture, 3 kg of haylage and 0.05 kg of soybean meal. All categories were provided with cattle salt and chalk in the amount of 0.05 kg.

Blood samples were taken in the morning, after cow's morning meal and after milking, by puncturing the coccygeal vein into 5 ml heparinized vacutainers. Blood from each animal was taken into two vacutainers. The blood was then transported to the laboratory while being placed in a handy refrigerator at a temperature of + 4 ° C. Immediately upon arrival at the laboratory, the blood was centrifuged (LC 320, 3000 rpm / 10 min) in order to extract the plasma. Ca (mmol / L), P (mmol / L) and Mg (mmol / L) were determined in blood plasma using the "Beckmann DU-64 UV / VIS" spectrophotometer. For the purpose of analysis were used the commercial kits of the manufacturer "Human", Germany.

#### Statistical analysis

The results were statistically processed using descriptive statistics. Differences in arithmetic mean were determined by using the ANOVA test. Testing differences in mean values of parameters between individual groups of cows was performed at the significance level of  $p < 0.05$ . After determining the

existence of statistically significant differences in the individual parameters regression analysis was used among the groups of cows (single linear regression). Based on this it is determined which blood parameters are dependent on the amount of milk produced.

Statistical analysis of the results obtained by the research was carried out using the software program SPSS 16.

## Results

Concentration of Ca in blood plasma of all 63 examined cows at the farm ranged from 1.900 to 2.560 mmol / L with an average value of  $2.103 \pm 0.021$  mmol / L (Table 1). Comparison of these values with values of 2.2-3.0 mmol / L (Meyer et al., 2003; Jovanovic et al., 1997), 2.1-2.8 mmol / L (Merck Veterinary Manual, 2003), 2.43 - 3.1 mmol / L (Radostits et al., 2000; Kaneko, 2008), 2.0-3.0 mmol / L (Jazbec, 1990), show that these are slightly lower.

Our research confirmed that cows with higher daily milk production have lower concentration levels of Ca in the blood plasma than cows with the lowest milk producing characteristics (Figure 2). However, the intergroup differences are not statistically significant. However, our results show that there is no statistically significant effect of daily milk production on the Ca concentration in blood plasma of cows ( $R^2 = 0.000$ ,  $p = 0.976 > 0.05$ ; these indicators can be seen in Table 2 and Figure 2).

**Table 1.** Results of descriptive statistics for Ca, P and Mg from the blood of the entire sample.

Parameters	NVO	Mean	SE	Med.	SD	Var.	SDA	Min.	Max.
Calcium (mmol/L)	63	2.103	0.021	2.060	0.157	0.025	0.587	1.900	2.560
Magnesium (mmol/L)	63	1.197	0.031	1.185	0.246	0.061	0.315	0.720	1.720
Phosphorus (mmol/L)	63	1.850	0.052	1.795	0.402	0.161	1.018	1.230	3.080

**NVO** : Number of valid observations, **SE** : Standard error, **Med**: Median, **SD**: Standard deviation, **Var**: Variance, **SDA**: Skewness-distribution asymmetry, **Min**: Minimum, **Max** : Maximum.

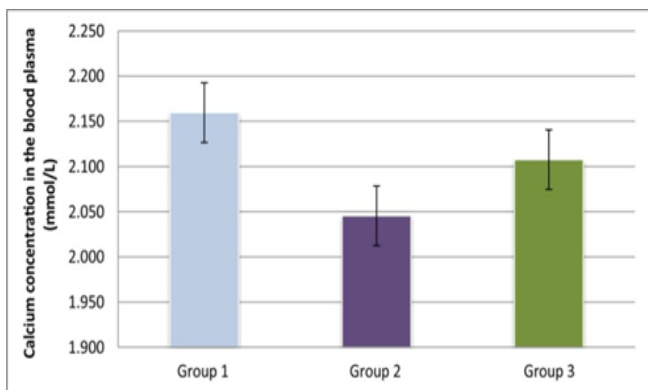
**Table 2.** Results of regression analysis for determination of Ca, P and Mg values depending on the amount of daily milk produced.

Regression parameters (sum)	Model		ANOVA		NSC (B)	
	R	R <sup>2</sup>	F	Sig.	Constant	Production (L/day)
Calcium (mmol/L)	0.004	0.000	0.001	0.976	2.106	-9.83E-02
Magnesium (mmol/L)	0.201	0.041	2.491	0.120	0.954	0.008
Phosphorus (mmol/L)*	0.388	0.150	10.780	0.002	2.586	-0.023

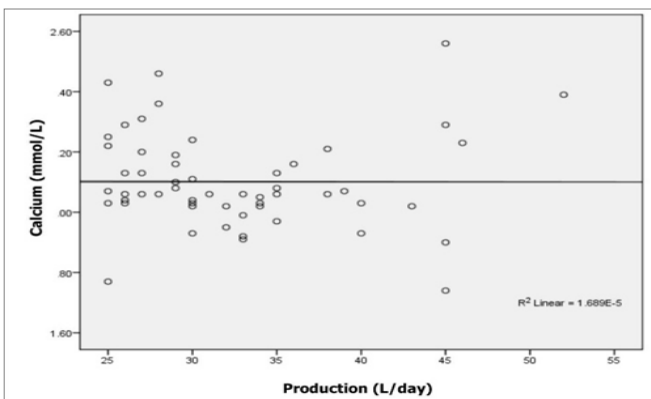
**NSC**: Non-standardized coefficients (B), \*Significantly on the level from 5% ( $p < 0.05$ )

According to the data of most authors, the concentration of inorganic P in the cattle blood amounts between 1.6 - 2.3 mmol / L), 1.81-1.1 mmol / L (Radostits et al., 2000; Whitaker, 2000; Kaneko, 2008), respectively 1.4-2.5 mmol / L (Merck Veterinary Manual, 2003).

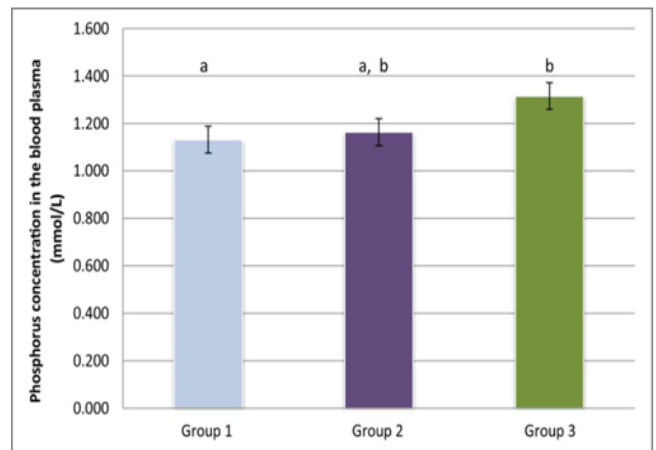
In our study, the determined P concentration in the blood plasma of 63 cows amounted  $1.850 \pm 0.052$  mmol / L within the range from 1.230 to 3.080 mmol / L (Table 1). These variations are slightly higher than the ranges indicated above. When it comes to the concentration of P in the blood plasma of cows with different amounts of daily milk production, our results show that the highest average concentration of P ( $2.091 \pm 0.114$  mmol / L) had cows with daily milk production of 20-28 L, and the lowest concentration of P have cows with the highest daily production of milk ( $1.715 \pm 0.066$ ; Figure 4). The P concentration of cows with the lowest daily milk production is significantly higher in comparison to cows with higher amounts of daily milk production ( $p < 0.05$ ) (Table 2). Daily milk production is higher and the concentration of P is lower ( $R^2 = 0.150$ ,  $p = 0.002 < 0.05$ ) (Table 2, Figure 3 and 4).



**Figure 1.** Ca concentration (mmol / L) in blood plasma of cows with different amounts of daily milk produced (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). All values are represented as  $\bar{x} \pm S\bar{x}$

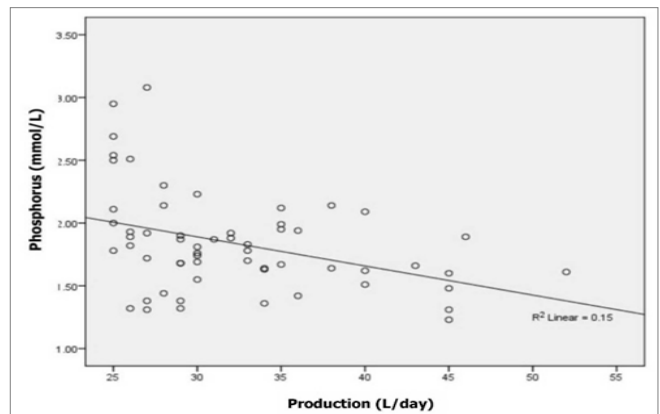


**Figure 2.** The dependence of Ca concentration (mmol/L) on the amount of daily milk produced.



**Figure 3.** P concentration (mmol/L) in the cow blood plasma with different levels of produced milk (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). a, b significantly differ  $p < 0.05$ .

The determined concentrations of Mg in the blood plasma of the examined cows ( $n = 63$ ) ranged from 0.720 to 1.720 mmol / L with an average value of  $1.197 \pm 0.031$  (Table 1). Values of Mg concentration in our study provide slightly higher contribution than those found in the available literature, whereas the following reference intervals are given: 0.8 - 1.3 mmol / L (Whitaker et al., 2000), 0.7-1, 2 mmol / L (Merck Veterinary Manual, 2003), 0.74-0.95 mmol / L (Radostits et al., 2000; Kaneko, 2008).

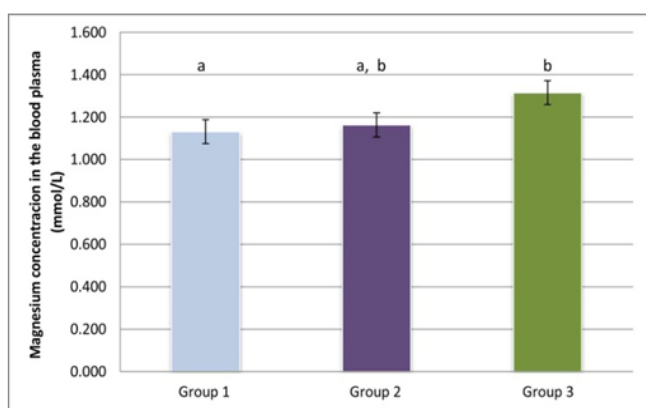


**Figure 4.** Dependence of P concentration (mmol / L) on the amount of daily milk produced

The lowest concentration value of Mg was found in cows with the lowest daily milk production ( $1.131 \pm 0.063$ ), and the highest in the blood plasma of cows with the highest daily milk production ( $1.315 \pm 0.055$ ), hence this difference was statistically significant ( $p < 0.05$ , Figure 5). The analysis of single linear regression does not show a significant interconnectedness of these two values ( $R^2 = 0.041$ ,  $p = 0.120 > 0.05$ , Table 2 and Figure 6).

## Discussion

In relation to the needs of cows in the last two months of gravidity and in early lactation, especially during the winter period, meals are often not well and rationally balanced in terms of nutrient selection and energy content- protein, dry matter and cellulose (Rajic et al., 1996; Jovanovic et al., 1997). Cow's supply with minerals depends exclusively on their intake through the digestive tract. Sufficient amount per meal does not assure the required amount of the organism. Mineral resorption depends on concentration of the minerals in the digestive system content, solubility, interaction between cations and anions, location of the resorption, loss through kidneys, fetus and milk, as well as the effects of a whole range of other factors, such as hormones and vitamins (Hadzimusic, 2010).

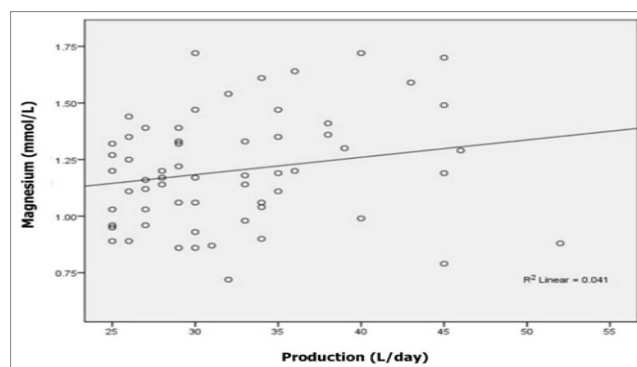


**Figure 5.** Mg concentration (mmol / L) in blood plasma of cows with different amounts of daily milk produced (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). a, b statistically significantly differ.  $P < 0.05$ .

Changes in the concentrations of some indicators of metabolic process show that the metabolic profile assessment cannot be strictly adhered to the physiological range from the existing literature, but should take into consideration all factors that could in any way affect the obtained results. Therefore for better assessment of the metabolic profile, it is necessary to consider all the anamnestic and clinical data concerning the pathology, nutrition, behaviour of animals, animal keeping conditions and production technology, as well as the knowledge of the physiological values of biochemical parameters of dairy cows (Bouraoui et al., 2002).

It is especially important to perform the determination of these elements in lactation. Namely, at the very beginning of milk secretion the needs of cows

for Ca and P are significantly increasing because the milk contains significant amount of these two elements.



**Figure 6.** Dependence of Mg concentration (mmol / L) on the amount of daily milk produced.

Underwood (1969) states, that Ca concentration below 2 mmol / L can be considered as a critical value and indicates to hypocalcaemia. In our study only eight cattle had insignificant hypocalcaemia - lower than 2 mmol / L, but none showed clinical manifestations of hypocalcaemia. Olayemi et al. (2001) reported that in 39% of the examined cows was noticed a Ca concentration of less than 2 mmol / L, and Hadzimusic (2010) states that out of total 442 cows, 30.77% of cows had Ca values below 2 mmol / L. However, as examined animals did not show symptoms of hypocalcaemia, a possible reason for slightly lower calcemia of the examined cows could be related to possible errors in blood sampling and laboratory procedures. It is also possible that the lower limit of the reference values of Ca concentration is slightly lower in our geographical area. According to Cvetkovic et al. (1978), the calcemia values of recently calved cows were 1.87 mmol / L. These authors consider that high-milk producing cows normally experience the decrease in the value of calcemia, whilst later, during lactation the values stabilize and range within the physiological limits for cattle, regardless of their production characteristics.

A cow that produces 9.000 kg of milk during a single lactation meanwhile excretes 63.6 kg of minerals from its organism (Jovanovic et al., 2001). Bearing in mind that 1 L of milk contains 1.2 g of Ca means that about 10.8 kg of Ca is excreted during milk lactation (Sjaastad et al., 2003). Consequently, the increase in milk production also increases the secretion of mineral elements from the organism, which is accompanied by a possible decrease in the level of Ca in the blood.



It's obvious 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 (Mukaca, 2015; Hadzimusic and Krnic, 2012; Cutuk, 2010). The analysis of single linear regression, which determined that the concentration of P in the blood plasma and the amount of daily milk produced is in the negative correlation. Daily milk production is higher and the concentration of P is lower (Table 2, Figure 3 and 4). This finding can be explained with the fact that during lactation with higher milk production consequently higher amounts of P are being "lost", therefore cows with higher amounts of daily milk production are spending more P. Our results obtained for P showed high accordance with study of Hadzimusic and Krnic, (2012). Because, according to their research; 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.

Considering the obtained results and the dynamics of the examined mineral matter, outlined is the different behavior of the relation between Ca and P in correlation with the different amount of daily milk produced. Although the average values of both minerals are decreasing with an increase in daily milk production, however, the analysis of single linear regression indicates a negative correlation between the concentration of P in blood plasma and the amount of daily milk produced, whilst for Ca it has not been identified. A possible explanation for this occurrence might be due to increase in the amount of excreted Ca through colostrum and milk, which consequently creates a tendency towards lowering the Ca, which in return stimulates the increased secretion of the parathyroid hormone. Parathormone leads to increased mobilization of Ca and P from the bones, hence encouraging the Ca reabsorption

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- from primary urine, and increases the elimination of P through urine.
- In many respects Mg metabolism is specific in relation to other microelements. Since it is considered that the level of Mg in the blood is more a reflection of its intake through food and not necessarily its body reserves (Whitaker et al., 2000), therefore it is assumed that the animals examined in our research have received different amounts of this element through food intake. However, the literature allegations treat as hypomagnesaemia conditions in which the level of Mg is less than 0.7 mmol / L, while the symptoms of hypomagnesaemia manifest when its value drops below 0.4 mmol / L (González, 2000).
- Although it is noticed that concentration of Mg in the blood plasma increases as the daily milk production increases, however, the analysis of single linear regression does not show a significant interconnectedness of these two values ( $R^2 = 0.041$ ,  $p = 0.120 > 0.05$ ). Our results obtained for Mg were slightly different than similar study of Cutuk (2010).

## Conclusion

The values of the Ca, P and Mg concentration in the blood of the examined cows varied within the physiological limits which is a result of satisfactory mineral ingestion through food and mineral-vitamin supplements, but also well-coordinated neurohumoral mechanisms which control the transport of these minerals into the organism. Lower average values of Ca and P concentrations in the blood of cows with higher daily milk production may be associated with increased total excretion of these minerals through milk, unlike the cows which daily produced lower amounts of milk. On the other side, concentration of Mg in the blood plasma increases as the daily milk production increases, may be associated. Therefore it is assumed that the animals examined in our research have received sufficient amounts of this element through food intake. 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, including a dry period, age of cows within the lactation stage.

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