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CHANGES IN SELECTED BIOCHEMICAL BLOOD PARAMETERS FOLLOWING VARIOUS METHODS OF POSTPARTUM HYPOCALCAEMIA PROPHYLAXIS

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Abstract

The current methods of preventing calcium deficiency during the periparturient period are based primarily on a diet low in calcium and acidifying the rumen content before parturition, as well as on oral administration of liquid preparations or boluses. The available literature does not provide information on whether these forms of prevention are effective only immediately after the parturition, or whether they also affect an organism throughout the initial lactation period. The aim of the study was to examine the effects of three methods for the prevention of postpartum hypocalcaemia on selected indicators of mineral and energy metabolism, as well as on functional parameters of the parenchymal organs during the two week period following parturition in dairy cows diagnosed with low calcium concentrations before parturition, induced by a lower dietary mineral content. The study was conducted in 60 HF cows, aged 3-6 years old, fed TMR. The animals were divided into three groups: the first group received only a mineral preparation contained in the feed, adjusted to the physiological stage and milk production level; the second group received three additional doses (directly before the parturition, then 24 hours and 48 hours after the delivery) of an oral fluid preparation containing 62.5 g of Ca; while the third group received an intraruminal bolus containing 43 g of pure calcium (immediately after parturition). This research showed that physiological hypocalcaemia occurred at the beginning of lactation in the groups which had received additional calcium preparations (groups II and III), whereas in group I blood collection one week and two weeks after the parturition demonstrated subclinical hypocalcaemia. A statistically significant increase in magnesium and a decrease in phosphorus concentrations were also observed in the latter group, as well as an excessive GGTP activity and high bilirubin concentrations. Using only one complex mineral supplement in high yielding milk cows after parturition does not ensure the expected prophylactic effects, especially in the long term.

Keywords: prophylaxis, hypocalcaemia, milk cows, macroelements.

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INTRODUCTION

The periparturial period in dairy cows is characterised by considerable changes in an organism adjusting its metabolism for parturition and increased milk production. This results in various postpartum mineral disorders, which significantly increase the risk of metabolic disorders in a clinical or subclinical form (Horst et al. 1997, Mulligan et al. 2006, Goff 2008). Mineral disorders, in particular hypocalcaemia, also contribute to decreased milk production, development of secondary metabolic disorders, and changes in the reproductive system, which cause economic loss and elimination of cows from farms (Mulligan et al. 2006, Goff 2008, Reinhardt et al. 2011). The disorders are usually associated with macroelements, especially calcium (Ca), excreted in milk during lactation, which may result in a significant decrease of its concentration in the blood (Goff, Horst 1997, Kronqvist et al. 2011). Calcium in the organism affects the contractility of smooth muscles (Murray et al. 2008), therefore its deficiency reduces the motor function of the forestomach and increases the risk of abomasum displacement (Goff, Horst 1997, Chapinal et al. 2011), as well as causing teat sphincter inadequacy, which may lead to inflammation of the mammary gland and metritis (Goff 2008, Reinhardt et al. 2011) due to abnormal involution (Martinez et al. 2012). Reduced Ca concentration in the organism results in a loss of appetite, contributing to a negative energy balance, increased fat mobilisation and ketosis (Goff, Horst 1997, Mulligan et al. 2006, Jawor et al. 2012).

Calcium deficiency (hypocalcaemia) postpartum may occur in two forms: clinical and subclinical. The risk of hypocalcaemia in dairy cows increases with age and number of lactations, from 25% in the first lactation to 54% in the fifth lactation (Reinhardt et al. 2011). Clinical hypocalcaemia develops in approximately 5% of postpartum cows (Mulligan et al. 2006, Goff 2008, Mulligan, Doherty 2008, DeGaris, Lean 2009), and manifests itself through characteristic symptoms of postpartum paresis in the form of excitatory pattern or coma (Horst et al. 1997, Thilsing-Hansen et al. 2002, Lean et al. 2006, Sobiech et al. 2010, Reinhardt et al. 2011). Subclinical hypocalcaemia is presently the most common form of calcium deficiency in milk cows, and may affect as many as 25 to 50% of cows in the herd (Houe et al. 2001, Horst et al. 2003, Roche 2003, Oetzel 2004, Reinhardt et al. 2011). This disorder is described as a reduction in the blood serum Ca below 2.0 mmol L^{-1} (Mulligan et al. 2006, Goff 2008, DeGaris, Lean 2009, Reinhardt et al. 2011). It results in decreased productivity of dairy cow herds, causing larger economic losses than the clinical form. The subclinical form of calcium deficiency does not present specific symptoms; therefore, diagnosing it in a herd is much more difficult and takes more time. Biochemical blood tests facilitate the diagnosis. Apart from Ca blood concentration assays, blood tests should also include determinations of the content of other macroelements (Mg, Pn), because their metabolism is closely related. Testing hepatic enzymes and

renal function indicators (creatinine, GFR and, if necessary, urea) is also useful and important for for the diagnostic process because parenchymal organs are responsible for many changes necessary in the normal functioning of hormones (PTH, calcitriol) and proteins regulating the metabolism of calcium and phosphorus, as well as for producing proteins required for the transport of elements from the gastrointestinal tract (Horst et al. 1997). Parathormone (PTH) is responsible for increasing blood Ca concentration as a result of its resorption from bones, and inhibition of renal excretion. This hormone, along with Mg as a co-factor, affects transformations of vitamin D in the liver and kidneys, and the production of calcitriol (1.25-dihydroxycholecalciferol), necessary for normal Ca absorption from the gastrointestinal tract in case it is deficient (Horst et al. 1997).

Hypocalcaemia may be associated with elevated levels of non-esterified fatty acids (NEFA) in the blood, due to increased lipolysis. This condition leads to the increased accumulation of fat in hepatic cells and, as a result, in their damage (Van den Top et al. 1996). An elevated NEFA concentration is a direct cause of the increased activity of gamma-glutamyl transpeptidase (GGTP) and aspartate transaminase (AST) (Cebra et al. 1997, Chamberlin et al. 2013). When the activity of the enzymes tested is increased and simultaneously there is a decrease in urea or albumins concentrations, liver failure may be suspected.

Postpartum disorders in blood Ca concentrations are usually caused by an incorrectly balanced diet in dry cows or the use of a feed ration inadequate for milk productivity/production (Moore et al. 2000, Mulligan et al. 2006, Zelený et al. 2007, Sobiech et al. 2010, Kronqvist et al. 2011). Calcium deficiency prophylaxis in the periparturient period is based on nutritional methods or pharmacological preparations expected to increase Ca bioavailability and resorption from the gastrointestinal tract. It is common to reduce dietary Ca content in the feed, and to use a diet acidifying the rumen for two to three weeks before parturition (Beede et al. 1992, Charbonneau et al. 2006, Ramos-Nieves et al. 2009). This facilitates the absorption of the element, and stimulates increased PTH secretion (reduced Ca content in the organism), in addition to which it intensifies calcitriol synthesis (Goff 2008). Additionally, oral supplementation with calcium preparations in the form of calcium salts may be introduced in the periparturial period. It is usually applied three times: directly before, and then 12 and 24 hours after the parturition. It consists of the administration of liquid preparations with calcium chloride, a source of easily absorbable Ca ions which demonstrates a stronger acidifying effect on the rumen than other calcium salts (Goff, Horst 1993, 1994, Goff et al. 2004, Gelfert et al. 2010). However, this type of supplementation may cause burns in the oral mucosa and increases the risk of metabolic acidosis (Goff, Horst 1993). Another form of supplementation is the administration of calcium preparation in a bolus containing calcium chloride and calcium sulphate. In this case, the preparation is administered in a single dose, usu-

ally a few hours after parturition, and is believed to be equally effective (Pehrson, Jonsson 1991, Sampson et al. 2009).

Another method of supplementation is postpartum intraruminal drenching – 25-45 L of water with a high content of various ingredients (including minerals) to prevent abomasum displacement, negative energy balance and mineral deficiency.

Analysis of the available literature demonstrates that the authors focus only on the diagnostics of mineral deficiencies in the 2-3 day period after parturition. There are no data on the effects of periparturial prophylaxis and its various methods on concentrations of different elements in the bodily fluids and on the function of parenchymal organs in dairy cows in a longer time period after parturition.

The aim of the study was to determine the effects of different methods for postpartum hypocalcaemia prophylaxis on changes in the indicators of mineral and energy metabolism, as well as on the function of parenchymal organs during the period of up to two weeks following parturition in dairy cows with low Ca concentrations before parturition, induced by a lower Ca content in the feed.

MATERIAL AND METHODS

The study was conducted in 60 Holstein-Friesian (HF) cows from a herd which for several years had been under routine clinical control (full veterinary examination four times a year). The cows, aged 3-6 years, were in good condition (BCS 3-3.5) before calving, and during the previous lactation yielded, on average, approximately 9.000 kg of milk. Only clinically healthy animals were included in the study. Cows whose first blood tests revealed significant changes in the parameters describing parenchymal organ functions were excluded from the study.

Postpartum feeding of these animals was based on the total mix ration (TMR) system, including: corn silage (46.8%), haylage (25.0%), hay (1.5%), straw (3.5%), cereal mix (8.0%), brewer's spent grains (13.0%), protein supplement (2.0%) and mineral supplementation (0.2%). The animals were divided into three groups, 20 cows in each, according to the applied oral method of calcium deficiency prophylaxis. Directly after parturition, all groups began receiving a mineral and vitamin supplement for lactating dairy cows, containing 19.5% calcium, 3.0% phosphorus, 6.0% magnesium and 1.0% sulphur, depending on milk production level. In group I, adding this standard preparation to a feed ration was the only form of protection against macroelement deficiency associated with the onset of intensive milk production. In group II, directly before parturition, 24 hours after and 48 hours after parturition, an oral preparation containing 62.5 g of ionized calcium per animal (calcium

chloride) was additionally administered. In group III, instead of three administrations of a calcium preparation, a single dose as an intraruminal bolus was given after parturition, containing 43 g of calcium (70% calcium chloride and 30% calcium sulphate).

Blood samples were collected in test tubes from the external jugular vein using a clot activator in a closed system, always at the same time in the morning, after milking, for the first time 2-3 days before parturition, then on the 7th and 14th day after parturition. The animals were carefully examined clinically before each collection of the test material. The blood samples were centrifuged at 3.000 rpm for 10 minutes to obtain serum, and stored frozen at –80ºC. The serum content of total calcium (tCa), total magnesium (tMg) and nonorganic phosphorus (Pn), creatinine (Crea) and total bilirubin (tBil) concentrations, as well as aspartate transaminase (AST) and gamma-glutamyl transpeptidase (GGTP) activity were determined using a BS-130 Chemistry Analyzer by MINDRAY. The total Ca content was determined with an ACCENT-200 CALCIUM diagnostic kit, according to the o-cresolphthalein colorimetric method. The total Mg concentration was determined with the xylidyl blue colorimetric method, using an ACCENT-200 MG kit. The Pn concentration was determined with the ammonium molybdate colorimetric method, using an ACCENT-200 PHOSPHORUS kit. Aspartate aminotransferase (AST) activity was examined with an optimised, modified method based on the International Federation of Clinical Chemistry (IFCC) recommendations, without activation with pyridoxal phosphate, using an ACCENT-200 ASAT kit. Gamma-glutamyl transpeptidase (GGTP) activity was determined with the colorimetric method using γ-glutamyl-3-carboxy-4--nitroanilide reaction, with an ACCENT-200 GGT diagnostic kit. Blood serum total bilirubin (tBil) concentration was determined with a vanadate oxidation method, using an ACCENT-200 BIL TOTAL diagnostic kit. Creatinine (CREA) values were determined with the Jaffé method, without deproteinisation, using an ACCENT-200 CREATININE kit.

The test results were examined through mathematical and statistical analysis with a use of Statistica v.10.1. computer package, to calculate the mean (x), standard deviation (SD) and significance of differences for $p < 0.05$ and 0.01.

RESULTS

The total calcium (tCa) concentration in the study groups before parturition was within the norm for physiological hypocalcaemia (not a subclinical form). One week following parturition, the concentration of this element was reduced in all groups, but a statistically significant decrease was observed only in group I (Table 1). Moreover, subclinical hypocalcaemia occurred only in group I during that period, whereas this condition was found in

Table 1

Blood chemistry in particular groups of cows

Parameter	Group	Collection of the test material		
		before birth	one week after	two weeks after
Ca (mmol L^{-1})	I	2.07±0.12	1.83*±0.19	1.95±0.14
	II	2.08±0.09	2.07^{a}±0.14	2.15^{a}±0.17
	III	2.11±0.16	2.05^{a}±0.12	2.10^{a}±0.11
Mg (mmol L^{-1})	I	1.01±0.14	1.27^{a}* ±0.22	1.24±0.16
	II	0.98±0.21	0.89^{b}±0.08	0.93±0.14
	III	1.12±0.19	1.07^{c} ±0.13	1.01±0.21
P mmol L^{-1})	I	1.52±0.34	1.54±0.39	1.64±0.21
	II	1.56±0.29	1.84±0.46	1.89*±0.49
	III	1.66±0.19	1.97*±0.28	1.94±0.31
AST (U L^{-1})	I	83.4^{a}±22.3	123.4^{a}±37.1	117.0^{a}±47.2
	II	65.6^{b}±32.1	92.1^{b}±27.9	87.2^{b}±34.1
	III	79.3^{a}±36.3	$99.1^{a,b}$±41.0	93.1^{b}±41.2
GGT (U L^{-1})	I	24.1±13.1	31.9±11.1	43.3^{*a}±16.8
	II	20.4±12.3	23.1±10.2	30.2^{b}±11.9
	III	27.7±14.3	31.1±12.6	32.9^{b}±14.2
Total bilirubin (µmol L^{-1})	I	3.94±1.54	6.67*±3.08	8.23^{a}*±2.76
	II	5.30±1.03	6.50±2.74	6.88^{b}*±2.05
	III	3.08±2.05	4.96±2.39	5.64^{c} ±1.71
Creatynine (µmol L^{-1})	I	86.63±18.56	131.72*±37.13	139.67*±25.64
	II	68.07±23.87	120.22*±34.48	118.46*±38.01
	III	72.49±24.75	129.67*±45.97	121.99*±34.48

The significance of differences between mean values in different groups $^{a, b, c}$ at $p < 0.05$.
The significance of differences between mean values in the groups * at $p < 0.05$.

approx. 50% of the cows in the other two groups. Two weeks after parturition, tCa concentration increased in all the groups compared to the values from the week before, but still remained below the physiological norm: 2.25 - 3.03 mmol L^{-1} (Winnicka 2011). In the group in which no additional mineral periparturial prophylaxis was used (group I), Ca concentrations were statistically significantly lower than in the other groups, both one week and two weeks after parturition (Table 1).

Mg concentrations in the study animals were within the physiological norm (0.75 - 1.32 mmol L^{-1}) throughout the study period. In group I, one week following parturition, a statistically significant increase in Mg concentration was observed, compared to the baseline value (Table 1). In groups II and III, the Mg concentration decreased. Two weeks after parturition, a sli-

ght decrease in magnesium concentration was found in groups I and III compared to the value observed one week following parturition and contrary to group II, where Mg concentration increased to a level similar to the baseline values. One week after parturition, the highest mean Mg concentration was found in group I, and the lowest occurred in group II, in which periparturial prophylaxis was used in the form of four oral administrations of a calcium preparation.

One week after parturition, Pn in all groups increased, but concentrations in group II and III were within the physiological norm: 1.81 - 2.10 mmol L^{-1} (Winnicka 2011). Compared to the baseline values, this increase was statistically significant in group III. Two weeks after parturition, the Pn concentration increased in groups I and II, and in group II the difference was statistically significant. In group III, a slight decrease in Pn concentration was observed, compared to the value one week after parturition. No statistically significant differences in Pn concentrations between the groups were observed.

AST activity in all the study groups increased in the first week after parturition, and in groups I and III it rose above the upper limit of the physiological norm. During the second week, the activity of this enzyme was still raised in group I, whereas in the groups where the oral periparturial prophylaxis was used it decreased; group III reached values within the physiological norm. Two weeks after parturition, the highest mean AST activity was observed in group I, and was statistically significantly higher than those values obtained in groups II and III.

GGTP activity in all the study groups increased one week after parturition. Two weeks following parturition, mean GGTP activity in the study groups was higher than one week after parturition. In group I, this increase was statistically significant compared to previous results as well as in comparison to the values observed in groups II and III in this test.

Mean total bilirubin (tBil) concentration in the study groups increased one week after parturition, and this increase was statistically significant in group I. Two weeks after parturition, a further increase in mean tBil values was observed. The highest statistically significant values were observed in group I. In group II, the concentrations were statistically significantly higher than in group III.

Mean creatinine (Cre) concentrations in the study groups one week and two weeks after parturition were statistically significantly higher than the values before parturition. During the second week a downward trend began in groups II and III.

DISCUSSION

Changes during the periparturient period require use of mineral supplements and prophylactic methods to prevent deficiencies in dairy cows (Goff, Horst 1997, Goff 2008, DeGaris, Lean 2009). The animals are usually clinically examined only during the first three days after parturition. Further tests in the longer time period after parturition are not performed, as is it widely believed that the hormones regulating mineral metabolism reach normal levels in the period starting 72 hours after parturition,. However, in the first two-three weeks after parturition, milk production increases most rapidly and, simultaneously, the organism recovers after parturition, which is associated with a loss of mineral compounds (Goff 2006, Kronqvist et al. 2011).

This study was an attempt to evaluate the mineral status and functional condition of parenchymal organs in a two-week period following parturition. The study confirmed numerous reports implicating that reduced Ca supply in the preparturient period significantly prevented clinical hypocalcaemia, that is postparturient paresis (Goff 2006, Kronqvist et al. 2011). However, hypocalcaemia in the subclinical form persists for at least 2 weeks after parturition, despite administration of complex mineral supplements, as observed in group I. In the groups where oral prophylactic preparations were additionally used (groups II and III), blood serum total calcium concentrations were higher, although at the moment of test sample collection the preparations were no longer working. It may be assumed that this was a consequence of considerably higher calcium concentrations after parturition, resulting from the administration of oral liquid preparations or boluses. Such effects were observed by other authors, but usually only in the first days following parturition (Goff, Horst 1993, 1994, 1997, Sampson et al. 2009). In our own study on the long-term effect of administering calcium preparations, we observed considerably higher blood phosphorus concentrations in the study groups receiving additional Ca preparations. The results justify the conclusion that hypocalcaemia results in an increased concentration of parathormone, which – while having a beneficial effect on Ca transformation – does not cause phosphorus retention in the kidneys (Goff 2006). Another finding was that in the group receiving only the standard fodder supplement after parturition, relatively very high magnesium concentrations occurred, although they would be considered as normal by some authors (Goff 2008, Winnicka 2011). An increase in the magnesium concentration usually occurs in postparturient paresis and, despite the fact that the values are usually higher, it indicates the risk of metabolic disorders in the cows from group I (Goff 2006, 2008).

Numerous authors report increased AST and GGTP activity, as well as an elevated total bilirubin (tBIL) concentration in a course of hypocalcaemia (both in its clinical and subclinical forms) (Cebra et al. 1997, Chamberlin et

al. 2013). In our study, a particularly high and statistically significant GGTP activity was mostly observed in diseases associated with hepatic damage (Steen 2001, Sahinduran et al. 2010). Frequently, an increased activity of this enzyme is accompanied by a negative energy balance, which in our study could have been caused by a temporary loss of appetite or clinical/subclinical hypocalcaemia (Cebra et al. 1997, Goff 2008). Moreover, in the subsequent stages of lactation, it may affect the regulation of Ca absorption from the gastrointestinal tract, and result in further decreases of calcium concentration in the organism.

The present study also revealed a statistically significant increase of creatinine concentration in all the groups, which – while being within the physiological norm – could potentially indicate the beginning of reduced renal function. This organ is the site of changes in vitamin D_3 hydroxylation, important for the absorption of calcium from the gastrointestinal tract. The observed change may result in reduced calcium absorption and the development of subclinical hypocalcaemia. Regrettably, the available literature does not provide information on such a radical increase in this parameter after parturition, compared to the baseline values before parturition.

CONCLUSIONS

The results confirmed the benefits of an increased supply of calcium preparations in the initial lactation period (Pehrson, Jonsson 1991, Goff, Horst 1993, 1994, Sampson et al. 2009). In addition, it has been demonstrated that even a complex mineral supplement with a high calcium content does not ensure complete protection against subclinical hypocalcaemia in the postparturient period. According to our results, it is necessary to administer additional doses of calcium preparations after parturition for a rapid and effective reduction of Ca deficits. The findings indicate that the administration of a single intraruminal bolus shortly after parturition is a very quick and effective method. It should be noted that opinions on the significance of calcium concentration in the periparturient period concerning the risk of postparturient hypocalcaemia vary (Beede et al. 1992, Goff 2006, 2008, DeGaris, Lean 2009). However, this does not undermine the claim that maintaining the health of lactating cows is associated with an adequate calcium supply (particularly in forms which are easily absorbed from the gastrointestinal tract, such as oxides and chlorides) during parturition and at the beginning of lactation.

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