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Changes of serum phosphatase activity in dairy cows supplemented whith calcium during early lactation

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The aim of this study was to examine the effects of calcium supplementation during the first trimester of lactation on the variations of serum phosphatases activity and mechanisms of calcium homeostasis. Twelve Holstein-Friesian cows with average daily production of 35 kg of milk were fed complete daily ration (hay, grass silage, maize silage and concentrates). This diet led to severe deficiency in calcium supply (-17.1±15.2 g of calcium day^{-1}) and mild excess of phosphorus (4.7±5.6 g of phosphorus day^{-1}). Two oral supplementation with 50 g of calcium-formiate were performed successively in 72 hour intervals. As the biochemical indicators of bone turnover the following parameters were measured before and 4 days after the calcium supplementation: serum calcium (Ca), inorganic phosphorus (iP), magnesium (Mg) concentrations, total alkaline phosphatase (ALP), bone alkaline phosphatase (bALP) and acide phosphatase (ACP) activity. A slight increase in Ca serum levels (2.09±0.11 mmol/L vs. 2.20±0.19 mmol/L; P=0.064) and decrease in iP (1.73±0.26 mmol/L vs. 1.66±0.24 mmol/L; P=0.373) and Mg (1.26±0.10 mmol/L vs. 1.16±0.11 mmol/L; P=0.058) was recorded. There was a rapid drop in mean bALP (-22.7%) but not in ALP (+1.4%) and ACP (+17.6%) activity, suggesting diminished osteoblastic mobilisation after the calcium supplementation. In contrast, osteoclastic resorption remained unchanged. The findings confirm the capacity of calcium supplementation on bone metabolism and the usefulness of bone ALP as biochemical indicator of bone turnover. Agricultura 1: 24-27 (2002)

Key words: dairy cows; early lactation; calcium supplementation; serum phosphatase activity

INTRODUCTION

The skeleton of an adult animal comprises at the most to their physique appearence. Beside skeletal frame, bone performs another role, that of the mineral pool in which the body deposit a minerals - primarily calcium - for withdrawal and replenishment as necessary. The hard, solid nature of the bone belies the fact that it is a dynamic, living tissue, a target of the endocrine system on the one hand and a self-regulating tissue on the other, constantly remodeling itself. Meticulously controlled formation and resorption cycles operate continuously and are essential for growth and maintenance of its mechanical strength and for calcium homeostasis.

The pathology of the skeleton in the bovine species was widely examinated during the past few years (Mosel et al. 1994, Philipov 1996, Bigras-Poulin and Tremblay 1998). The metabolic disturbance of particularly interest for nutritional management and large animal medicine are lactation-associated hypocalcemic syndrome - which include milk fever and episodes of transient hypocalcemia in dairy cows - osteomalacia and osteoporosis (Lappetelainen et al. 1993, Jonsson et al. 1999). It seems that parturient hypocalcemia results from diminished parathyroid gland activity at the initiation of lactation and temporarily insensitive osteoclast population (Mosel et al. 1994). Dhiman and Sasidharan (1999) and Hartigan (1999) reviewing efficacy and tolerance of calcium supplementation during the post-partum period. Collectively, there is overhelming agreement that treatment with different calcium salts could be beneficent to maintenance of calcium balance.

Studies mentioned above use different biochemical markers to asses bone turnover. Bone biopsy and histochemical techniques (Üstunel and Demir 1995) or cell hybridisation (Jemtland et al. 1998) are expensive and unpractical under the fields condition. Some of non-invasive techniques for monitoring bone metabolism are successfully applied in research and practice. Bone formation can be monitored by measuring total serum or bone-specific alkaline phosphatase (ALP, bALP) activity (Vojtic and Vengušt, 1994), procollagen propeptides and osteocalcin, which is non-collagenous osteoblastic protein (Philipov 1996, Scott et al. 1997, Collignon et al. 1996). On the other hand, bone resorption caused by osteoclasts can be detected by serum activity of acide phosphatase (ACP), L(+)-tartrate resistant

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acid phosphatase, urinary collagen cross-links (pyridinoline and deoxypyridinoline) and type I collagen telopeptides (reviewed by Eyre 1997).

The ability to diagnose mineral balance during initiation of lactation in the dairy cow and to monitor this balance during the course of preventive treatment, is therefore a highly desirable goal. Thus, the objective of the current study was to examine (i) the influence of calcium on the calcium homeostasis in lactating cows during the first trimester of lactation and (ii) the usefulness of some biochemical markers, particularly serum phosphatases, for monitoring the changes in bone turnover before and after calcium supplementation.

MATERIAL AND METHODS

Twelve Holstein-Friesian cows, about 650 kg liveweight, with average (± s.d.) daily production of 35.3±4.6 kg of 4%FCM were used in this experiment. During the first trimester of lactation the basal diet consisted of a mixture of hay (2 kg), maize silage (19 kg) and grass silage (6 kg). To these were added 2.4 kg of soya-bean meal, 4.1 kg of corn-meal fodder, 0.16 kg of vitamin-mineral mix (which contains 140 g calcium and 70 g of phosphorus × kg^{-1} in pure chemical form, see Table 1) and 0.04 kg of limestone. Dietary ingridients were fed manually separated (except hay, which was offered only at morning) in two equal portions daily. The amounts of concentrate differed between 2 kg and 6 kg. Feed refusals were recorded for each cow and used to calculate daily matter intakes. The cows were tethered on the short stalls with rubber mats. Water bowls were placed in the stalls between each cow. The calcium and phosphorus balance was estimated by subtracting maintenance requirement (26 g for calcium and 26 g for phosphorus) and lactation requirement (3.2 g for calcium and 1.7 g for phosphorus; DLG 1991) from calcium and phosphorus intake (Table 1).

Table 1. Calcium and phosphorus content in daily ration according to the chemical analysis of particularly ingridients and calculated dry matter intake (Holstein-Friesian cows about 650 kg of liveweight, average daily production of 35.3 ± 4.6 kg of 4%FCM).

Ingridients	Weight (kg dry matter)	Calcium intake (g)	Phosphorus intake (g)
Hay (2nd cut)	1.78	12.6	5.4
Maize silage	7.98	17.5	18.3
Grass silage	3.42	18.3	13.7
Concentrate	3.70[a]	25.4	17.7
Soya-bean meal	2.10	5.2	13.2
Corn-meal fodder	3.60	2.2	12.3
Mineral mix[c]	0.15	21.0[b]	10.5[b]
Limestone	0.04	16.0	-
TOTAL intake	21.69	117	91
Energy concentration (MJ Nel/kg DM)	7.1	-	-
Crude protein (%)	15.9	-	-
Crude fiber (%)	16.4	-	-

[a] average consumption among 12 cows.

[b] sources of minerals were calcium carbonate and calcium phosphate (monobasic).

[c] 160 g of mixture provide 3.2 g Mg (as magnesium oxyde), 12.8 g Na, 368 mg Cu, 952 mg Zn, 320 mg Mn, 3.2 mg Co, 12.8 mg I, 112 mg S, 4.8 mg Se, 80.000 IU vitamin A, 16.000 vitamin D3, and 320 mg vitamin E.

Two oral supplementations with 350 mL gel drench which contain 50 g of calcium-formiate (Baymix-Calform®, Bayer, Leverkusen, FRG) were performed successively in 72 hour intervals. The blood samples were taken from a jugular vein immediately before the first supplementation and four days after the second supplementation of calcium-formiate gel. Total serum calcium (Ca), inorganic phosphate (iP) and magnesium (Mg) were measured using colorimetric methods (quantitative kinetic kits provided by bioMerieux, France) on Alysee (France) discrete analyser. Assays for total alkaline phosphatase (ALP) and acide phosphatase (ACP) activity where done in serum. Substrates for ALP and ACP were nitro-4-phenylphosphate with magnesium buffer (at 30°C, pH 10.5) and a-naphtylphosphate with citrate buffer (at 30°C, pH 5.1), respectively. Bone alkaline phosphatase (bALP) was distinguished from the rest of serum total ALP activity using inactivation procedure according to Moss and Whitby (1975).

Table 2. The effect of 2×50 g of calcium-formiate enteral supplementation on selected biochemical markers (values are expressed as mean ± s.d.) before and after the treatment in lactating dairy cows (n= 12, paired *t*-test).

Treatment	ALP (U/L)	BALP (U/L)	ACP (U/L)	Ca (mmol/L)	IP (mmol/L)	Mg (mmol/L)
Before	40.7±8.8	20.4±8.3	1.4±0.5	2.09±0.11	1.73±0.26	1.26±0.10
After	41.3±9.4	15.8±3.6	1.7±1.4	2.20±0.19	1.66±0.24	1.16±0.11
P=	0.497	0.050	0.586	0.064	0.373	0.058

Unless stated otherwise, all results are reported as mean ± standard deviation. The effect of a supplementation treatment on changes of selected biochemical markers was examined using paired *t*-test (Kenward 2000).

RESULTS

The effects of diet on estimated calcium and phosphorus balance are shown on Fig. 1 and Fig. 2. The above described diet lead to severe deficiency in calcium supply. The mean calcium intake was -17.1±15.2 g of calcium × day^{-1}, whereas on average a mild excess of phosphorus appears (4.7±5.6 g × day^{-1}). Slight increase in Ca serum levels (2.09±0.11 mmol/L vs. 2.20±0.19 mmol/L; P=0.064) and decrease in iP (1.73±0.26 mmol/L vs. 1.66±0.24 mmol/L; P=0.373) and Mg (1.26±0.10 mmol/L vs. 1.16±0.11 mmol/L; P=0.058) was recorded (Table 2).

The activity of ALP was 1.4% (40.7 U/L vs. 41.3 U/L, P=0.497) and the activity of ACP 17.6% increased (1.4 U/L vs. 1.7 U/L, P= 0.586). In contrast, the bALP activity was deminished for 22.7% (20.4 U/L vs. 15.8 U/L, P= 0.050; Table 2, Fig. 3).

DISCUSSION

The ALP (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) is a group of enzymes originated from bone, liver, gut, kidney, placenta and accessory sexual glands which catalyse the hydrolysis of phosphate esters at

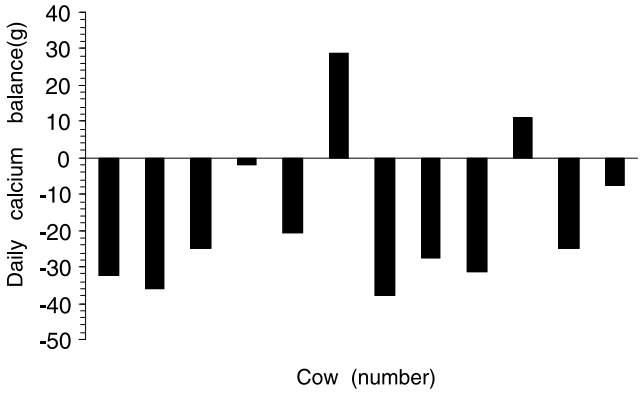

Fig. 1. Daily calcium balance (g × day^{-1}) for experimental cows (n=12). The basal diet consisted of a mixture of hay (2 kg), maize silage (19 kg) and grass silage (6 kg), 2.4 kg of soya-bean meal, 4.1 kg of corn-meal fodder, 0.16 kg of vitamin-mineral mix (with 140 g calcium and 70 g of phosphorus × kg^{-1}) and 0.04 kg of limestone. The range of concentrate intake was between 2 kg and 6 kg daily. Daily milk production ranged between 25.0 and 40.0 kg of 4%FCM.

an alkaline pH. Therefore, in various studies ALP was used as an indicator of the bone formation (Philipov 1992, Fürl et al. 1993, Wada et al. 1996) although ALP is not specific for bone tissue metabolism. According to these, no effects on ALP catalytical activity was detected after the calcium supplementation, suggested ALP activity is an insensitive measure of bone formation. The total ALP activity is controled only by 2 genes thus, only 2 true isoenzymes exist: intestinal ALP and tissue nonspecific ALP. Remaining isoforms differ as the result of posttranslational glycolysation, which is organ specific. One of these is bone specific ALP, which is present in osteoblast cell membranes simultaneously with parathyroid hormone receptors. The action of osteoblasts had two distinct consequences. The first is increased flow of Ca from bone to make fine adjustments in the blood calcemia and the second is to send some chemical mediators to enhance the bone resorption process through osteoclast cell population (Rosol and Capen 1990). The rise in bALP activity indicated that bone turnover is enhanced. Lower concentrations of bALP - as seen in our experiment - suggested reduced bone formation. On the basis of discrepancy between these two phasphatases it can be concluded that bALP, instead of total serum ALP, reflect the bone turnover.

Acid phosphatase is secreted into the circulation by osteoclasts which resorb bone. The bone degradation products, especially hydroxyapatit, are endocytosed to the cell, and vesicles containing ACP are released into the blood stream. Thus, the amount or activity of ACP in the blood

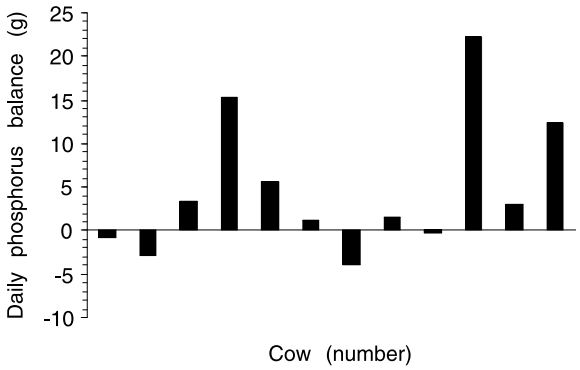

Fig. 2. Daily phosphorus balance (g × day^{-1}) for experimental cows (n=12). The basal diet consisted of a mixture of hay (2 kg), maize silage (19 kg) and grass silage (6 kg), 2.4 kg of soya-bean meal, 4.1 kg of corn-meal fodder, 0.16 kg of vitamin-mineral mix (with 140 g calcium and 70 g of phosphorus × kg^{-1}) and 0.04 kg of limestone. The amounts of concentrate differed between 2 kg and 6 kg daily. Daily milk production ranged between 25.0 and 40.0 kg of 4%FCM.

should be used as the measure of bone resorption. After the calcium supplementation the ACP activity was still at the same level as before, although the serum Ca concentrations were elevated up to the lower reference value. This suggests that negative calcium balance in the total daily ration could not be overcome using additional 100 g calcium by enteral supplementation.

The effect of treatment was clearly evident firstly, on the Ca level and secondly, on the iP level in the serum. Before supplementation the mean serum level of Ca was slightly under the Ca reference limit *i.e.* 2.2 mmol/L. After the treatmnet the Ca serum level increased up to the lower threshold value. This change was statistically insignificant but had a great clinical importance because it confirms the capacity of a calcium-formiate supplementation under the

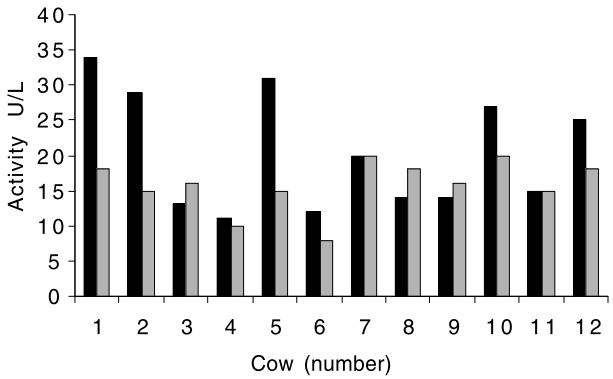

Fig. 3. Activity (U/L) of serum bone alkaline phosphatase (bALP) before (□ block) and after (■ block) the enteral supplementation with 2 × 50 g of calcium-formiate in lactating dairy cows (n= 12).

terms of negative calcium balance. In contrast to Ca, iP mean serum level was decreased. It seems that increased Ca concentration leads to reciprocal reduction in serum iP concentration because of the mass interactions that keep the Ca×iP ion product as constant. This is possible because of renal reabsorbtion of calcium is diminished and renal excretion of phosphorus is enhanced (Rosol and Capen 1990).

These results showed the potential of oral calcium supplementation on bone turnover during the first lactation trimester under terms of severe calcium deficiency. On the basis on changes in Ca serum levels is clear that calcium-formiate was highly effective supporting normal Ca serum concentrations. In addition, the bALP, instead total ALP, was real marker of bone metabolism.

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