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Levels of parathyroid hormone, calcium, phosphorus and magnesium in blood sera of healthy and sick cows

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Summary

The blood levels of parathyroid hormone (PTH), calcium, phosphorus and magnesium were examined in 122 cows, which included healthy cows as well as cows with osteomalacia and paresis. Levels of PTH were determined in vitro using IMMULITE analyzer, levels of macroelements - using the automated „Eos-Bravo" analyser with Hospitex reagents.

The blood level of PTH ranged from 1.7 to 7.5 pmol/l in healthy cows and from 3.95 to 20.19 pmol/l in sick cows. The highest and most significant increase in blood PTH level (up to 18.31 ± 1.88 pmol/l) was found in cows with parturient paresis. The blood serum levels of PTH in healthy cows changed depending on age and on the physiological status, being significantly lower in cows 2-4-years-old compared with cows 8-years-old and over. The levels of PTH were significantly higher in cows-in-calf and heifers-in-calf compared with cows of the lactation period. Higher levels of PTH were found during the winter than in the summer. The blood level of PTH correlated inversely with the level of calcium in cows with osteomalacia (r = -0.89) and with parturient paresis (r = - 0.49-0.61). The blood serum PTH levels were significantly increased in all groups of cows (p<0.05) on the day of parturition and one day after parturition compared with the measured PTH level 5 days before parturition. Vitamin D (injected 3-5 times) administered during the last days before calving effectively increased the levels of calcium, phosphorus and PTH.

Keywords: cows, parathormon, osteomalacia

Main regulatory roles of calcium metabolism belong to vitamin D, parathyroid hormone and calcitonin. The PTH and vitamin D endocrine system functions to maintain a normal Ca homeostasis (2, 9, 11, 27, 28). Parathyroid hormone (PTH) is secreted by the chief cells of the parathyroid gland, and plays an important role in calcium homeostasis. PTH secretion is altered by small physiological changes in extracellular ionized calcium concentrations, and there is an inverse sigmoidal relationship between serum calcium concentrations and PTH secretion (1, 8). Parathyroid hormone functions as a major mediator of bone remodelling and as an essential regulator of calcium homeostasis, producing several distinct and independent effects on the bone remodelling process, resulting in both bone formation (anabolic activity) and bone resorption (catabolic activity), e.g., continuous infusion of PTH decreases bone mass by stimulating osteoclast activity, while intermittent administration increases bone mass by stimulating osteoblast differentiation (11).

In diseases that involve one of the three calcitropic hormones, the serum concentrations of the other two will change to either amplify the effect of the primary abnormality or to defend against the calcium perturbation. Although these compensatory mechanisms act to restore serum calcium to normal, the homeostasis will not be complete until the primary abnormality has been corrected. In addition to these three calcitropic hormones, other hormones, cytokines, and growth factors play an important role in calcium metabolism (2, 13). As previously demonstrated (15, 20) the maximal response of PTH secretion to hypocalcaemia is dependent upon the rate of reducing the calcium levels. It is, therefore, a question whether in vivo studies the secretion of PTH, induced by hypocalcaemia, always is performed at the „maximal" rate and as such will result in a maximal response. The transient nature of PTH secretion during induction of hypocalcaemia adds difficulties to the interpretation of the maximum. During a severe and rapid decrease of Ca^{2+} an initial rapid increase of the PTH levels is seen, which soon declines to a lower level, despite a continuous fall in calcium. The PTH levels remain, however, considerably higher than before stimulation.

The change of PTH levels of healthy animals was researched using laboratory animals. Little articles are published about changes in PTH hormone levels of healthy and sick cows. It was found that PTH level started to increase 2 days before calving and the highest level of this hormone was found 12 hours after calving (9). Intestinal muco-serosal Ca transport and bone resorption increase (12) to meet fetal and neonatal Ca and P requirements as well as subsequent changes in vitamin D and parathyroid hormone metabolism and have been described in several studies (17-19).

The aim of the present was to determine variation particularities of the blood serum level of PTH, calcium, phosphorus and magnesium in healthy cows of different feeding, age and productivity, during different seasons of the year and in cows having parturient paresis and osteomalacia.

Material and methods

The blood for analyses was taken from 122 cows of the Lithuanian Black-and-White breed in winter and in summer time. The groups of cows were formed using the principle of analogues and paying attention to the age, health status, time of parturition, productivity and type of received ration. Experimental cows and heifers were examined clinically before formation of groups. The cattle were divided into the following groups: I – clinically healthy heifers-in-calf during in-house period (n = 10), II – clinically healthy heifers-in-calf during pasturable period (n = 10), III – clinically healthy cows 2-4 years old during in-house period (n = 10), IV – clinically healthy cows 5-7 years old during in-house period (n = 10), V – clinically healthy cows 5-7 years old during pasturable period (n = 10), VI – clinically healthy cows 8 years old and above during in-house period (n = 10), VII – clinically healthy cows 8 years old and above during pasturable period (n = 10), VIII – cows with parturient paresis fed with mineral supplements (n = 10), IX – cows with parturient paresis fed without mineral supplements (n = 10), X – cows with osteomalacia (n = 12), XI – clinically healthy dry cows during the last decade of pregnancy: (Vit. D_3 used every day 50 mg 5 days before calving (n = 5), Vit. D_3 used thrice, 50 mg each time 5 days before calving (n = 5), Vit. D_3 used once, 50 mg 5 days before calving (n = 5), control group, for which the vitamin D_3 was not injected (n = 5).

The blood was collected from cattle of the groups I-X from jugular vein fourfold (during in-house and pasturable periods). The blood was taken 11 times from cows of the group XI: five times before calving every day, the day of calving and 5 days after calving.

Dry cows-in-calf, heifers-in-calf and lactating cows were fed with mineral supplements. The cows with parturient paresis were fed as with mineral supplements and without them as well. During wintering period, cows were fed with hay, straw, combined fodders, silo, root-stocks (concentration of nutrients per 1 kg of ration dry matter (DM): NEL – 5.7 MJ; green proteins – 13%, crude cellulose – 29%, crude fat – 3.0%), with mineral supplements; the animals grazed freely and received combined fodders and mineral supplements during pasturable period. Dry cows-in-calf and heifers-in-calf were fed with mineral supplements „Efekt Mineral Foder Lag." (Lactamin, Sweden), which contained the following: Ca – 9.8%, P – 12% (Ca : P = 0.8 : 1), Na – 7%, Mg – 9.2%, Cu – 400 mg/kg, Co – 30 mg/kg, I – 150 mg/kg, Mn – 300 mg/kg, Zn – 500 mg/kg, Se – 30 mg/kg, vit. A – 400 000 IU, vit. D_3 – 100 000 IU, vit.E – 100 mg/kg. Lactating cows were fed with mineral supplements „Efekt Mineral Foder Hog." (Lactamin, Sweden), which contained the following: Ca – 18.4%, P – 3.7% (Ca : P = 5 : 1), Na – 7%, Mg – 9.2%, Cu – 400 mg/kg, Co – 30 mg/kg, I – 150 mg/kg, Mn – 300 mg/kg, Zn – 500 mg/kg, Se – 30 mg/kg, vitamin A – 400 000 IU, vitamin D_3 – 100 000 IU, vitamin E – 100 mg/kg.

Blood from cows of the sample was taken in equalized conditions, i.e. at o'clock am, after overnight fast. Blood for analysis was collected by jugular venipuncture into single-use tubes Venoject (produced by Terumo Europe N. V., Belgium) without anticoagulant. Blood samples were delivered to the laboratory and centrifuged 5 minutes at the rate of rotation 3.000 times per minute. Separated blood serum was pumped out to Eppendorf tubes with lids (produced by Eppendorf AG, Germany, Hamburg) by means of dosimeter. Tubes filled with blood serum were frozen in a chamber of refrigerator at –20°C. All blood sera in tubes were brought to room temperature at once and investigated. The amounts of parathyroid hormone were determined using the Roche Elecsys 1010/2010 analyzer (Roche Diagnostics GmbH, USA). Amounts of macroelements (calcium, phosphorus, magnesium) were measured using the Eos-Bravo analyzer (Italy, Hospitex Diagnostics) and reagents of the company Hospitex.

Findings and statistical data were computed using a program Epi Info (1996; Centers for Disease Control&Prevention (CDC), USA., Version 6.04). Arithmetical means of findings ($\bar{x}$), standard deviation (SD) and Pearson correlation factor (r) were calculated. The Student multiple comparison test was applied to determine the significance criterion for difference between groups (p). The difference was considered statistically significant when $p < 0.05$.

Results and discussion

The blood serum levels of Ca, P and Mg in heifers-in-calf, during in-house period amounted 2.62 ± 0.37, 1.51 ± 0.22, 1.08 ± 0.13 respectively) and at pasturable period 2.85 ± 0.21, 2.01 ± 0.10, 1.21 ± 0.12, respectively (fig. 1) We found significantly higher levels of phosphorus ($p < 0.05$) and magnesium ($p < 0.05$) during pasturable period compared with in-house period and a tendency to higher calcium levels during pasturable period. Higher levels of calcium, phosphorus and magnesium during pasturable period were found by the other authors (13, 16, 22). Mild hypocalcemia was found in clinically healthy cows 2-4 years old at the end of the in-house period with the mean value of the calcium 2.28 ± 0.28 mmol/l. Average concentrations of phosphorus (1.88 ± 0.17 mmol/l) and magnesium (0.96 ± 0.19 mmol/l) were in the range of normal levels. The levels of calcium measured during pasturable period in the cows 5-7 years old corresponded to the physiological normal and were significantly higher compared with winter period (2.62 ± 0.20 mmol/l, $p < 0.05$). Significantly higher levels of calcium were found only in the blood of heifers-in-calf (2.85 ± 0.21, $p < 0.05$) when compared with other groups of cows investigated during summer. The level of phosphorus was significantly higher ($p < 0.05$) compared with winter period and significantly higher value was only in the blood of heifers-in-calf (2.01 ± 0.10, $p < 0.05$) when compared with other groups of clinically healthy cows investigated in summer. Hypocalcemia was revealed at the end of in-house period in cows 8 years old and above, which received mineral supplements (the average amount of calcium was 1.88 ± 0.27 mmol/l). That was the lowest level of calcium among the groups of clinically healthy cows determined during in-house period and it differed significantly from the level in heifers-in-calf (2.62 ± 0.37, $p < 0.05$) and cows 2-4 years old (2.28 ± 0.28, $p < 0.05$), but did not differ significantly from the group of cows aged 5 to 7 years (1.97 ± 0.24). The blood serum levels of calcium, phosphorus and magnesium in cows with osteomalacia were significantly lower in comparison with healthy cows ($p < 0.05$).

During winter period, the level of PTH of heifers-in-calf during in-house period varied from 3.5 to 5.9 pmol/l (4.37 ± 0.75 pmol/l) and it differed significantly from the groups of cows 2-4 years old ($p < 0.05$), however it did

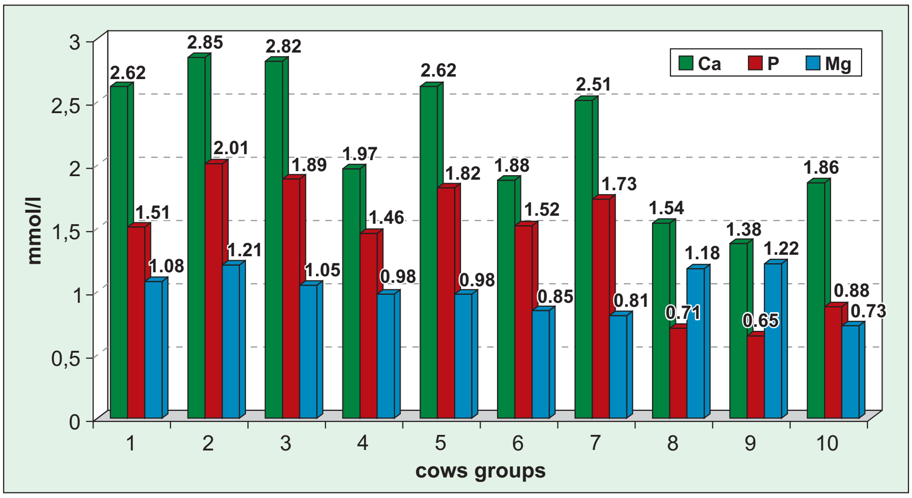

Fig. 1. The changes of blood calcium, phosphorus and magnesium levels in different cow groups (1 – clinically healthy heifers-in-calf during in-house period, 2 – clinically healthy heifers-in-calf during during in-house period pasturable period, 3 – clinically healthy cows 2-4 years old during in-house period, 4 – clinically healthy cows 5-7 years old during in-house period, 5 – clinically healthy cows 5-7 years old during pasturable period, 6 – clinically healthy cows 8 years old and above during in-house period, 7 – clinically healthy cows 8 years old and above during pasturable period, 8 – cows with parturient paresis fed with mineral supplements, 9 – cows with parturient paresis fed without mineral supplements, 10 – cows with osteomalacia)

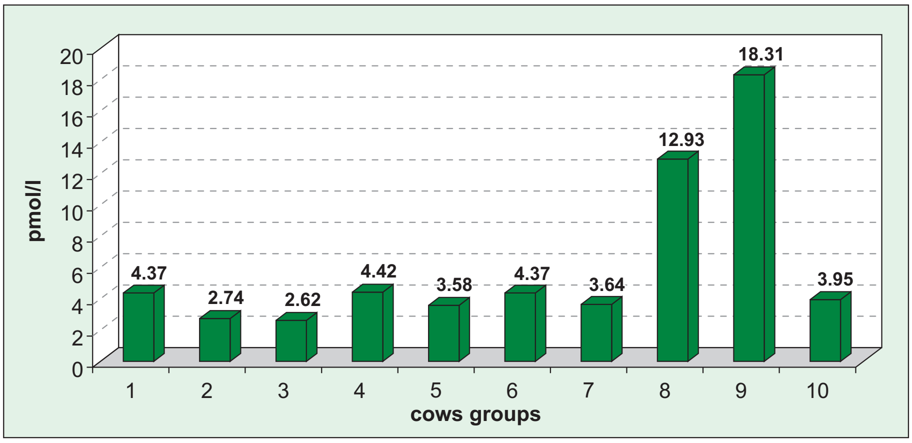

Fig. 2. The change of parathyroid hormone levels in different cows groups (1 – clinically healthy heifers-in-calf during in-house period, 2 – clinically healthy heifers-in-calf during pasturable period, 3 – clinically healthy cows 2-4 years old during in-house period, 4 – clinically healthy cows 5-7 years old during in-house period, 5 – clinically healthy cows 5-7 years old during pasturable period, 6 – clinically healthy cows 8 years old and above during in-house period, 7 – clinically healthy cows 8 years old and above during pasturable period, 8 – cows with parturient paresis fed with mineral supplements, 9 – cows with parturient paresis fed without mineral supplements, 10 – cows with osteomalacia)

not differ significantly from dry cows-in-calf (fig. 2). Average parathyroid hormone levels correlated negatively with levels of calcium (r = –0.593) and positively with levels of magnesium (r = 0.439). This corresponds with the findings of Potts (21) who stated that PTH concentration correlates with calcium concentration inversely and the level of PTH increases with decrease of calcium concentrations and vice versa. The level of parathyroid hormone varied from 2.0 to 4.1 pmol/l (2.74 ± 0.71 pmol/l) during summer period and it differed significantly from the level found during in-house period ($p < 0.05$). During summer period, the level of PTH correlated negatively with level of calcium (r = –0.779). During pasturable period, the levels of PTH of cows 2-4 years old varied from 1.9 to 3.9 pmol/l and was 2.62 ± 0.58 pmol/l in average. That was the lowest PTH level among all groups of cows investigated during the summer period, however it differed significantly only from the group of cows 8 years old and above ($p < 0.05$). The measured low PTH level may be associated with age (21), because the calcium levels decrease and PTH increases in older age. There was no significant difference between PTH levels found in winter and summer ($p > 0.05$). PTH correlated with levels of calcium negatively (r = –0.656). During winter period, the level of PTH varied from 3.5 to 5.9 pmol/l (4.37 ± 0.75 pmol/l) and it differed significantly from the groups of cows 2-4 years old ($p < 0.05$), however it did not differ significantly from dry cows-in-calf ($p > 0.05$). Average parathyroid hormone levels correlated with levels of calcium negatively (r = –0.593) and with levels of magnesium positively (r = 0.439). The level of parathyroid hormone varied from 2.0 to 4.1 pmol/l (2.74 ± 0.71 pmol/l) during summer period and it differed significantly from the level found during in-house period ($p < 0.05$). During summer period, the level of PTH correlated with level of calcium strongly negatively (r = –0.779). The average PTH level of cows 5-7 years old during in-house was 4.42 ± 0.57 pmol/l. During in-house period, a higher PTH level among clinically healthy cows was found only for high productivity cows, which did not receive mineral supplements (5.85 ± 0.97, $p < 0.05$). The level of parathyroid hormone during summer period varied from 2.71 to 4.8 pmol/l (3.58 ± 0.67 pmol/l in average) and was significantly lower than that found during in-house period (4.42 ± 0.57 pmol/l, $p < 0.05$). That may be associated with age (24) and productivity, because cows 5-7 years old are most productive and large amounts of calcium are secreted into milk (16), therefore the synthesis of parathyroid hormone becomes more active (2, 7). The value was significantly higher than that in heifers-in-calf (2.74 ± 0.71, $p < 0.05$) and in cows 2-4 years old (2.62 ± 0.58, $p < 0.05$) when compared with other groups of healthy cows during pasturable period; there was no significant difference (3.64 ± 0.59, $p > 0.05$) when compared with cows 8 years old and above. The level of PTH of cows 8 years old and above in-house period varied from 3.4 to

6.1 pmol/l (4.37 ± 0.82 pmol/l in average). Significantly lower level of PTH was stated only in the blood of cows aged 2 to 4 years (3.46 ± 0.75, $p < 0.05$). The level of PTH correlated with the level of calcium negatively ($r = –0.610$). The average level of parathyroid hormone during pasturable period was 3.64 ± 0.59 pmol/l and that was the highest blood serum PTH level in healthy cows found during pasturable period, and it differed significantly from heifers-in-calf (2.74 ± 0.71, $p < 0.05$) and cows 2-4 years old (2.62 ± 0.58, $p < 0.05$), but did not differ significantly (3.58 ± 0.67, $p > 0.05$) from cows 5-7 years old. The average amount of parathyroid hormone in cows with osteomalacia was 3.95 ± 0.60 pmol/l, however there was no significant difference when compared with healthy cows ($p > 0.05$). A significantly higher blood PTH level was stated for the cows having the parturient paresis (12.93 ± 2.14 pmol/l, $p < 0.05$) and the appropriate level was even increased for the cows with the parturient paresis, which did not receive a mineral complement (18.31 ± 2.14 pmol/l, $p < 0.05$). The other authors (20) found that large amounts of calcium are used for production of milk and, when sufficient amount of calcium is not received with fodders, PTH secretion is activated for increase of blood calcium level by mobilizing it from bones. This supports the statements of other researchers that parathyroid glands of cows with parturient paresis, though secreting higher amounts of PTH to the blood, are not capable to maintain necessary levels of calcium and phosphorus (15). Our findings correspond with statements of other researchers that low calcium levels stimulate the secretion by the parathyroid gland (9, 10, 26). According to the findings of our study, the PTH level correlated inversely with the blood calcium level in cows with osteomalacia ($r = –0.89$) and in cows with parturient paresis ($r = –0.49$ and $r = –0.61$, respectively).

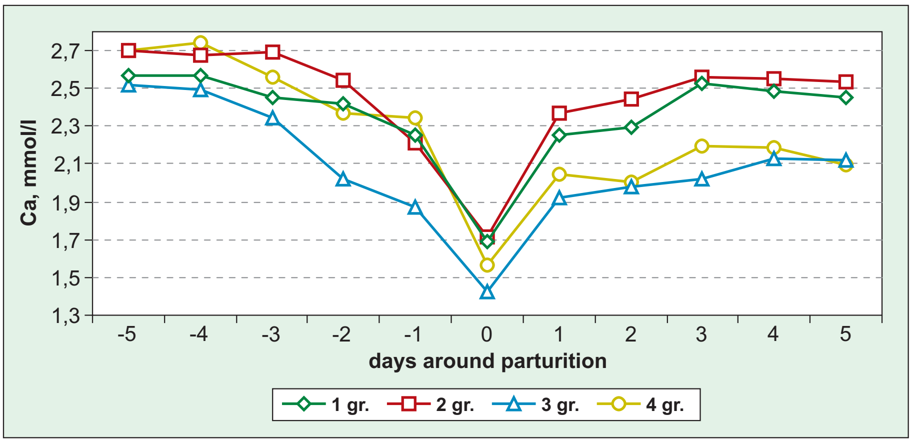

Fig. 3. Change of calcium concentrations in the blood serum of cows (groups of cows: 1 – Vit. D_3 used every day 50 mg 5 days before calving, 2 – Vit. D_3 used thrice, 50 mg each time 5 days before calving, 3 – Vit. D_3 used once, 50 mg 5 days before calving, 4 – control group, for which the vitamin D_3 was not injected)

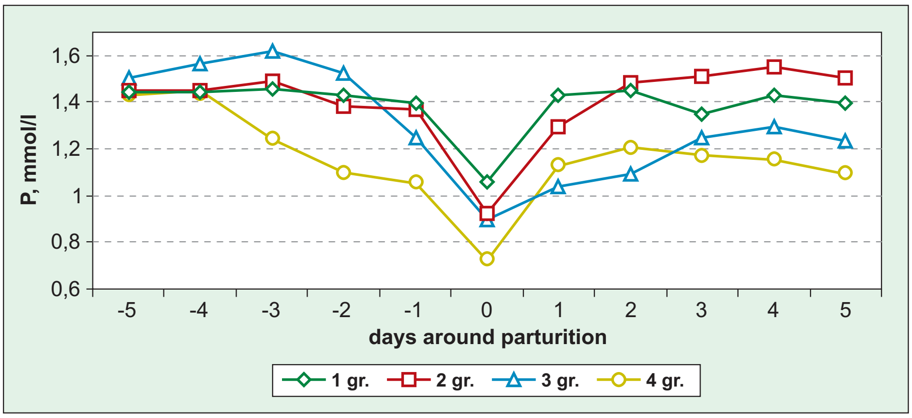

Fig. 4. Change of blood serum phosphorus concentration in cows

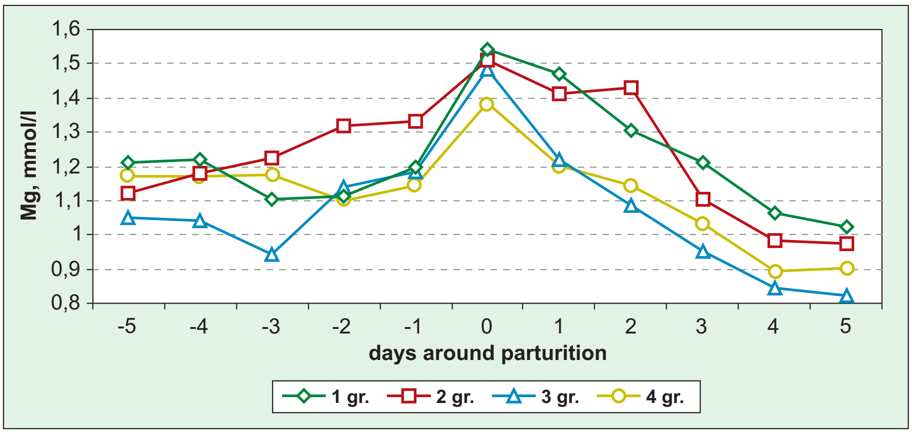

Fig. 5. Change of blood serum magnesium concentration in cows

There were founded important changes of macroelements and PTH hormone in dry cows-in-calf, which were injected vitamin D one week before calving (fig. 3-6). When performing analysis of the blood serum of cows at once after parturition, an important fall in concentrations of calcium and phosphorus and also an increase in concentrations of magnesium and PTH were observed. The decrease of blood calcium level before, during and after calving was reported also by other authors (9, 20). Also Riond et al. (23) described that the levels of calcium and phosphorus decreased and the levels of magnesium increased after parturition. The other authors (25) found decreased blood level of calcium, phosphorus and magnesium only after parturition. According to Arney (3), the decrease in calcium is determined by elevated loss of calcium with colostrum at the onset of lactation and insufficient resorption from the gastrointestinal tract together with decrease of PTH synthesis and lack of active forms of vitamin D. Measured values of macroelements and PTH changed at similar intervals, independently of vitamin D injections. However after several days after calving the recorded levels of macroelements and PTH differed (fig. 3-6). The blood serum concentrations of phosphorus and calcium decreased significantly these days in all groups of cows ($p < 0.05$), however the levels of calcium and phosphorus of the groups 1 and 2 at the fifth day after calving did not differ significantly from values observed 5 days before calving ($p > 0.05$). The levels of calcium and phosphorus found in the blood serum of cows of the groups 3 and 4 at the 5th day after calving differed significantly from levels at 5 days before calving ($p < 0.05$). When investigating effects of vitamin D on the levels of calcium and phosphorus, some of the authors (12, 29) found that vitamin D acts increasing the blood level of calcium and phosphorus in cows and reduce the number of cases of parturient paresis after calving. The other authors (5, 6) found that the blood serum levels of calcium and phosphorus in cows increase 36 h and 24 h after calving when vitamin D is administered. According to the data of our study, the levels of calcium and phosphorus decreased slightly 5 days before calving and increased significantly 24 h after parturition and also increased little by little later depending on how often vitamin D was injected. According to some authors (4, 14) the level of hypocalcaemia is diminished by single doses of vitamin D administered 3-10 days before parturition. According to our findings, single doses of vitamin D did not have greater impact on the level of calcium because there was no significant difference between levels of calcium compared with control cows and the level of calcium was not restored 5 days after calving up to the level, which was 5 days before parturition.

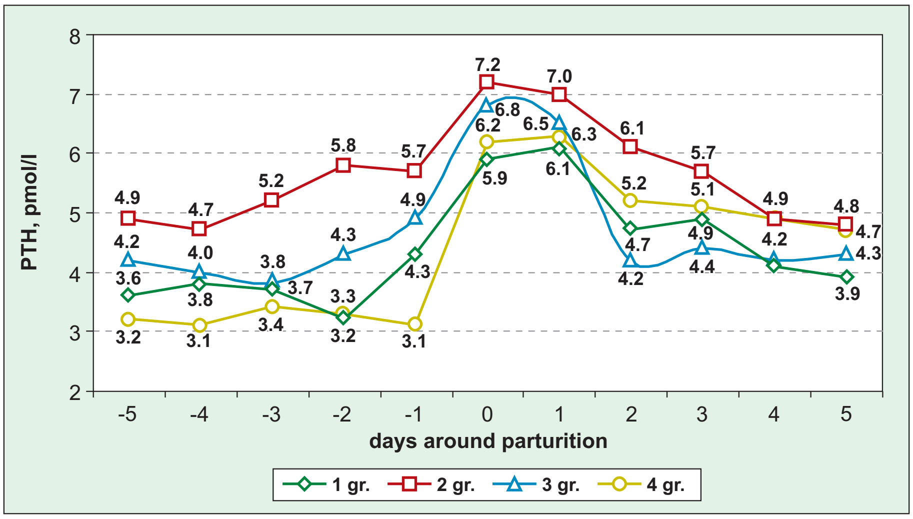

Fig. 6. Change of blood serum PTH in cows, which were injected vitamin D

The blood serum PTH levels were significantly increased in all groups of cows ($p < 0.05$) on the day of parturition and one day after parturition compared with PTH level 5 days before parturition. The blood serum PTH levels in groups 1, 2 and 3 did not differ significantly on the fifth day after parturition from the levels found 5 days before parturition ($p > 0.05$), and they were significantly higher in the blood serum of the group 4 ($p < 0.05$).

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