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METABOLIC PROFILE OF COWS BLOOD, SICK WITH POSTPARTUM HYPOCALCEMIA

Reviewer - Doctor of Veterinary Science O. I. Vischur

It was researched the content of 25-hydroxycholecalciferol (25-OH₂D₃), parathyroid hormone (PTH) and calcitonin (CT) and total calcium bounded with the protein and ultrafiltered inorganic phosphorus, magnesium, alkaline phosphatase, nonesterified fatty acids (NEFA), glucose and protein in the blood of cows suffering from postpartum hypocalcemia. It was found that in the blood of cows with clinical signs of postpartum hypocalcemia, 25-OH₂D₃ content was higher, but the content of PTH and CT - lower in comparison with healthy cows within 1-2 days after calving. However, in the blood of cows suffering from postpartum hypocalcemia was decreasing concentration of total, protein-linked and ultrafiltrates calcium, inorganic phosphorus, glucose, total protein and was increasing concentration of NEFA, magnesium and activity of alkaline phosphatase.

Keywords: *cows, postpartum hypocalcaemia, blood, 25-hydroxycholecalciferol, hormones, metabolism*

Setting the problem. Postpartum hypocalcemia (postpartum paresis, parturition paresis, milk fever) in cattle is characterized by a significant decreasing of calcium in blood and manifested by paresis, paralysis and coma [1, 8]. Postpartum hypocalcemia develops by sharp decrease of calcium in serum of blood due to its excessive loss of colostrum, which contains about 2.3 grams of calcium in 1 liter. Therefore one cow with production about 10 kg of milk loses about 23 grams of calcium, which is almost nine times greater than the amount of calcium contained in the extracellular fluid. In normal conditions, this loss is compensated by an increase of intestinal and bone resorption [1, 3, 6-9, 12, 13, 15].

Analysis of sources which discussing this issue. Most authors consider that the occurrence of postpartum hypocalcemia is associated with dysfunction of the thyroid, parathyroid and pancreas and vitamin D [3, 6, 9, 13] deficiency. However, recent data have shown that the disease is not always the result of lack of calcium regulating hormones (parathyroid hormone and calcitriol) and is caused by deficiency or dysfunction of receptors in the target cells of these hormones [12, 13]. Also it was found that hypocalcemia observed during parturition and accompanied by increasing of parathyroid hormone level in plasma [10, 12, 15] and 1,25-dihydroxyvitamin D (calcitriol), that is active hormonal form of vitamin D, which increases the concentration of calcium in blood [1, 3, 6-9, 12, 13, 15].

At the same time was found that the breast tissue contains receptors of 1, 25 - (OH) 2D₃ - and it reflects on the transport of calcium in response to the stimulating effect of 1, 25 - (OH) 2D₃ [3, 13].

These data are based on the idea that in the postnatal paresis cows was found higher level of circulating 1, 25 - (OH) 2D3, than in healthy cows during of parturition. Therefore, excessive formation of 1, 25 - (OH) 2D3 during parturition may contribute to the development of postpartum hypocalcemia [10, 15].

There are poorly researched questions of vitamin D metabolism and its interaction with calcium-regulating hormones in cows suffering from postpartum hypocalcemia. There is no information about relationship between changes of total, protein-linked and ultra-filtered calcium concentration, inorganic phosphorus, magnesium, glucose, total protein, on the one hand, and changes of the active metabolite of vitamin D - 25-hydroxycholecalciferol, parathyroid hormone and calcitonin content on the other.

The aim of this study was to investigate the state of mineral metabolism of vitamin D and calcium-regulating hormones in the body of cows suffering from postpartum hypocalcaemia in comparison with healthy cows during the first days after calving.

Research objectives:

- Set the level of PTH, CT and 25-OHD₃;
- To determine level of calcium, inorganic phosphorus, total protein and glucose, NEFA and activity of alkaline phosphatase.

Materials and research methods. Studies have been conducted on cows black-motley breed in the period from December to April. Clinical signs of postpartum hypocalcemia were confirmed in six cows during the first two days after calving.

In the control group were clinically healthy animals (n = 5). Cows were analogues for the period after calving with age and performance as sick cow. The researched group was consisted of cows with clinical signs of postnatal paresis (n = 6).

To investigate blood were taken from the jugular vein, in which were determined the vitamin D3 - 25-hydroxycholecalciferol (25-OND3), parathyroid hormone (PTH) and calcitonin (CT) content of active metabolite by ELISA [4]. For the determination of calcium, inorganic phosphorus and magnesium was used set for biotesting by company Pliva Lachema (Czech Republic) [4, 5]. The activity of alkaline phosphatase (AP) was determined using as substrate of p-nitrofenolphosphate [5]. The content of total protein, glucose and NEFA - conventional methods [4]. Statistical analysis of the obtained digital data was carried out by a computer program. Results of mean values were considered statistically significant at: p <0,05 - *, p <0,01 - ** and p <0,001 - ***, in comparison to healthy cows (control group).

The results of research. During clinical studies of sick cows was establish a lower body temperature, anorexia, recumbency, absence of reflexes to external stimuli, a expressed S-shaped curve of the neck, coma.

Analysis of biochemical blood parameters of clinically healthy cows during the first days after calving showed that the level of calcium in the blood serum of the control group was at the low level and amounted to 2.08 mmol / L (Fig. 1). It shows that the extra calcium needed for muscle contractions at

parturition and used for the formation of colostrum resulted on low concentrations in the blood. The content of total calcium in serum of cows with clinical signs of postpartum hypocalcemia was lower by 43% ($p < 0.01$) in comparison with clinically healthy animals. The reduction of total calcium content was mainly due to its ultra-filtrated fraction, which also includes ionized calcium. In the blood of sick cows content of ultra-filtrated fraction of calcium was lower by 71% in comparison to its content in the blood of healthy ($p < 0,01$).

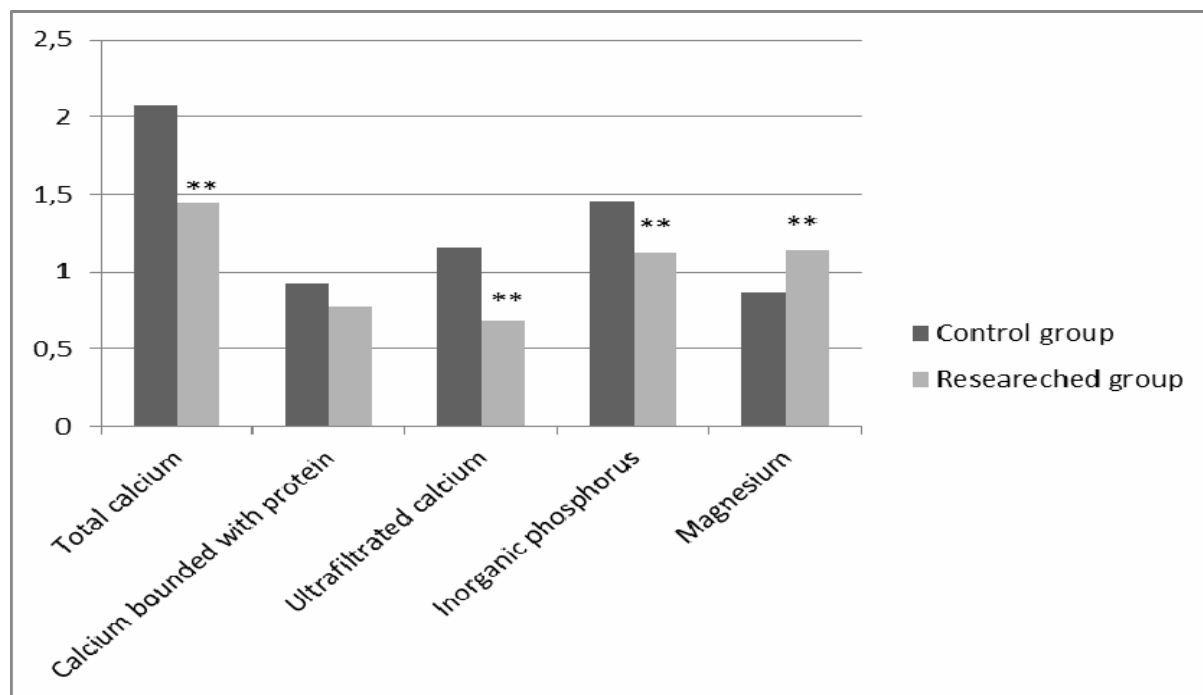


Figure 1. The content of macroelements in blood of healthy and postpartum hypocalcaemia sick cows ($M \pm m$; mmol / l; ** - $p < 0,01$, in comparison with the control group).

The content of inorganic phosphorus in the blood of the investigated cows after calving was low and amounted 1,12-1,46 mmol / l (Fig. 1). In the blood serum of cows with clinical signs of postpartum hypocalcemia amount of inorganic phosphorus was 1.3 times lower ($p < 0,01$), in comparison with healthy. Our data consistent with other authors [1, 6, 7, 9, 12, 16]. However, some authors have noted that the level of inorganic phosphorus in cows by this disease did not differ from healthy cows [17]. At the same time, the concentration of magnesium was higher in patients cows compared to healthy ($p < 0.01$) and was 1.14 mmol / l.

As we can see in Figure 2 data the level of 25 hidroksyvitamin D in serum of healthy cows on the first day after calving was 19.2 nmol / l. This metabolite is the major circulating form of vitamin D and in normal conditions is converting to more polar metabolites, as a criterion for evaluating the D-vitamin status.

In cows suffering from postpartum hypocalcemia the content of 25-OH D3 was significantly higher, than in clinically healthy. Increasing of 25-CO-D3 level, as the main substrate probably occurs

simultaneously with increasing of 1, 25 - (OH) 2D3 levels and particularly 24,25 - (OH) 2D3 in the blood of cows with postpartum hypocalcemia, as evidenced by studies [4, 8, 9, 10]. Increasing of vitamin D active metabolite was during increasing of demand for calcium during lactation onset under effect of estrogen and prolactin, which induce the synthesis of 25-hydroxylase and increase the mobilization of calcium from bone and stimulates reabsorption of inorganic phosphate in the kidneys. Increase of calcium-diol may also be related to the fact that a group of sick cows were old cows and with age the content of 25-OH D₃ increases but decreases the number of receptors [16].

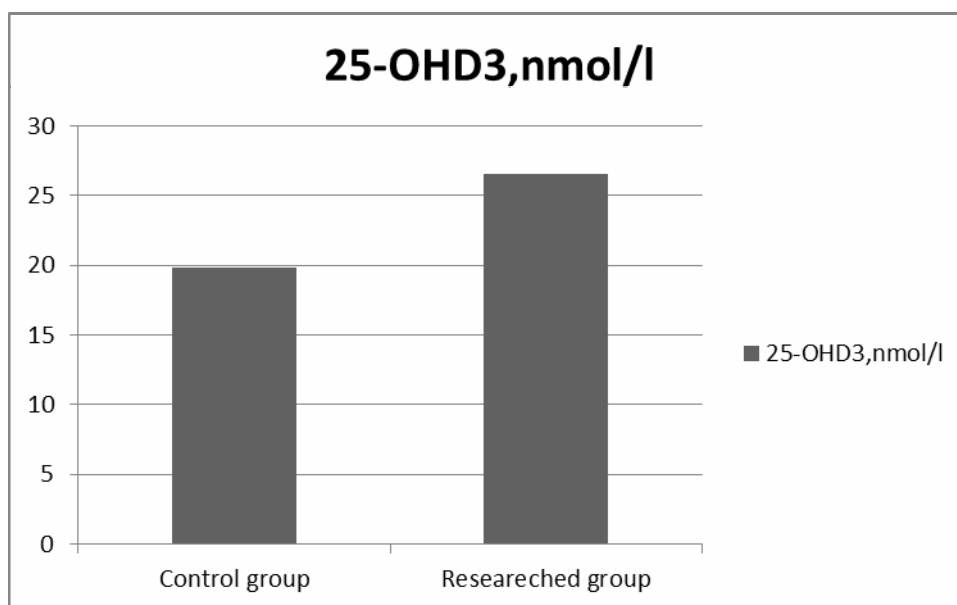


Figure. 2. The content of 25-OH D₃ in the blood of healthy and sick with postpartum hypocalcaemia cows ($M \pm m$, nmol / L; * - $p < 0,05$, in coparisant with the control group).

Reducing of the calcium content after calving resulted to the stimulation of PTH secretion - and its level in the range 14,63-18,74 pmol / L (Fig. 3). In addition, content of PTH in blood of the research group of cows was slightly lower in comparison with the control. One factor of inhibitory effect on the secretion of PTH is likely to increase in the content of active metabolites of vitamin D. Increased content of active metabolites of vitamin D in the blood of cows suffered from postpartum hypocalcaemia is confirmed by many authors [3,7, 12, 15, 17].

Our study found that the level of calcitonin in blood of healthy cows in the period after calving was high and amounted to 5.57 pmol / L (Fig. 3). In blood of cows with clinical signs of postpartum hypocalcemia we have marked decreasing of calcitonin by 70% compared with healthy ($p < 0,01$).

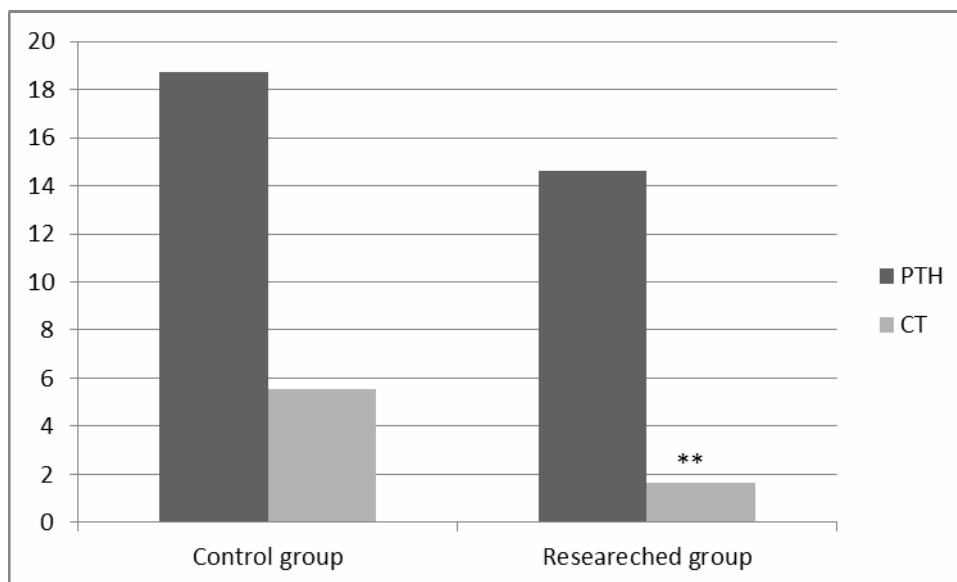


Figure 3. Content of PTH and CT in the blood of healthy and cows suffering postpartum hypocalcaemia ($M \pm m$; pmol / l; ** - $p < 0,01$, compared with the control group).

Reducing of the concentration of total calcium and inorganic phosphorus in the blood of cows after calving caused by enhanced release them from colostrum. These changes are also caused by tension of metabolism in cows, due to the importance of these macronutrients in certain key metabolic processes in their body. In particular, calcium plays a key role in the regulation of metabolic processes in the cell, and phosphorus - in energy metabolism [3, 14, 17].

At the same time, we have marked changes in energy metabolism of cows after calving, that accompanied by increased content of NEFA and decreased blood glucose levels, indicating increased lipolysis in adipose tissue (Table). Thus, concentration of NEFA in the plasma of sick cows was higher in 36% ($p < 0,01$), and concentration of glucose was lower in 24% ($p < 0,05$), in comparison with their levels in healthy (2.85 mmol / l). In this time decreased the content of total protein in the blood of cows suffered from postpartum hypocalcaemia ($p < 0,01$).

1. Content of NEFA, total protein, glucose and activity of alkaline phosphatase in the blood of healthy and sick cows suffered from postpartum hypocalcaemia ($M \pm m$)

Indicators	Groups of animals	
	control (n=5)	research (n=6)
NEFA (mkmol / l)	569±23,47	777±17,72***
Total protein (g/l)	70,06±2,08	59,84±2,12**
Glucose (mmol / l)	2,85±0,16	2,17±0,14*
Alkaline phosphatase, IU	49,22±3,25	56,02±4,75

The activity of alkaline phosphatase in the serum of sick cows was increasing. Probably the reason for the increasing of activity of AP was in negative influence of such state on the liver and thus increasing of activity was due to hepatic of isoenzyme.

Conclusions. Thus, assessing the hormonal regulation of the cows, which manifested clinical signs of postpartum hypocalcemia established that levels of PTH and CT were lower, and the content 25-OH D was higher, than in healthy cows. Diseases of cows by postpartum paresis accompanied with infringement of mineral, protein and energy metabolism, which manifested a decreasing of calcium concentration, inorganic phosphorus, total protein and glucose and increased activity of alkaline phosphatase and content of NEFA in their blood.

REFERENCES

1. Кондрахин И.П. Послеродовая гипокальциемия коров / И.П. Кондрахин //Ветеринарна медицина України. — 2010. — №1. — С.17-19.
2. Кузнецов С.Г. Закономерности обмена кальция, фосфора и магния у телок, нетелей и коров / С.Г. Кузнецов, О.В. Харитонов, В.Н. Скурихин, И.Н. Валуев //Ветеринария. — 1992. — №1. — С.51-53.
3. Куртяк Б. М. Жиророзчинні вітаміни у ветеринарній медицині і тваринництві / Б.М. Куртяк, В.Г. Янович. — Львів: Тріада Плюс, 2004. — 426 с.
4. Лабораторні методи досліджень у біології, тваринництві та ветеринарній медицині : довідник /В.В.Влізла, Р.С.Федорук, І.Б.Ратич та ін. ; за ред. В.В Влізла. — Львів: СПОЛЮМ, 2012. — 764 с.
5. Лабораторные методы исследования в клинике: справочник. / [В.В. Меньшиков, Л.Н. Делекторская, Р.П. Золотницкая и др.; под ред. В.В. Меньшикова . — М.: Медицина, 1987. — 368 с.
6. Левченко В.І. Патогенез і профілактика післяродової гіпокальціємії корів / В.І. Левченко, О. С. Петренко // Біологія тварин. — 2008. — Т. 10, № 1–2. — С. 52-60.
7. Левченко В.І. Післяродова гіпокальціємія і гіпофосфатемія високопродуктивних корів / В.І.Левченко, І.П.Кондрахін, В.В. Сахнюк і ін.. //Ветеринарна медицина України. — 2011, №12 (190). — С.8-12.
8. Левченко В.І. Внутрішні хвороби тварин /В.І. Левченко, І.П. Кондрахін, В.В. Влізла та ін.; За ред. В.І. Левченка. — Біла Церква, 2001. —544с.
9. Яблонський В.А. Нові підходи до діагностики, лікування та профілактики післяродового парезу в корів / В.А. Яблонський // Ветеринарна медицина України. — 2009. — №5. — С.20-21.
10. Variation of 25-hydroxyvitamin D in sera of healthy and sick cows / V. Spakauskas, I. Klimiene, M. Ruzauskas, V. Bandzaite // Biologia. — 2006. — № 4. — P. 80–86.

11. *Goff J. P.* Effect of synthetic bovine parathyroid hormone in dairy cows: Prevention of hypocalcemic parturient paresis / J. P. Goff, E. T. Littledike, R. L. Horst // *J. Dairy Sci.* . — 1986b Vol.69 . — P.2278.
12. *Goff J. P.* Regulation of enzymes controlling vitamin D metabolism in normal and milk fever cows / J. P. Goff // *J Dairy Sci.* — 1990. — Vol. 73. — P.230.
13. *Goff J. P.* Bone resorption, renal function, and mineral status in cows treated with 1,25-dihydroxycholecalciferol and its 24-fluoro analogues / J. P.Goff, R. L. Horst, E. T. Littledike et al. // *J. Nutr.*— 1986a. — Vol.116. —P.1500.
14. *Horst R. L.* Calcium and vitamin D metabolism in the dairy cow / R. L. Horst, J. P. Goff, T. A. Reinhardt // *J. Dairy Sci.*. — 1994. — Vol. 77. — P. 1931–1951.
15. *Horst R. L.* Vitamin D metabolism in ruminants and its relevance to the periparturient cow / R. L. Horst, T. A Reinhardt // *J. Dairy Sci.* — 1983. — Vol. 66. — P. 661–678.
16. *Littledike E. T., Goff J.* Interactions of Calcium, Phosphorus, Magnesium and Vitamin D that Influence their Status in Domestic Meat Animals /E. T. Littledike, J. Goff // *J Anim Sci.* 1987. — Vol. — 65. — P. 1727-1743.
17. *Yamagishi N., Naito Yo.* Calcium metabolism in hypocalcemic cows with myocardial lesion / N. Yamagishi // *J. Vet. Med.Sci.* —1997. — Vol. 59 (1). — P.71-73.