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Variation of 25-hydroxyvitamin D in sera of healthy and sick cows

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An interaction between the blood level of 25-OH Vit D, PTH, CT and the level of calcium, phosphorus and magnesium was examined in 182 cows which included healthy cows and cows with osteomalacia and paresis. The level of parathyroid hormone and calcitonin was determined using an IMMULITE analyser by means of immunometric assay. The level of vitamin D was measured using the enzyme linked immunosorbent assay (ELISA). The levels of calcium, phosphorus and magnesium were determined using an automated Eos-Bravo analyser with Hospitex reagents.

The blood serum level of 25-OH Vit D varied from 18.1 to 56.4 nmol/l. In healthy cows it changed depending on age, the level of vitamin being statistically significantly lower and the level of CT higher in cows 2–4 years old compared with cows aged 8 years and over. The level of vitamin D negatively correlated with the level of calcium. The blood serum level of 25-OH Vit D changed in healthy cows depending on the season of the year: a statistically significantly higher level of the vitamin was found during the winter period as compared with summer. Preparations of vitamin D (injected 3–5 times) administered in the last days before calving changed the level of calcium, phosphorus, PTH and 25-OH Vit D. The blood serum level of PTH and 25-OH Vit D in cows with parturient paresis was statistically higher than in healthy cows. There was no correlation between calcium and PTH in the blood serum of cows with parturient paresis, although a strong negative correlation relation was found between calcium and 25-OH Vit D. The level of 25-OH Vit D in cows with osteomalacia (compared with healthy cows) did not differ statistically significantly.

Key words: cows, 25-OH vitamin D, parturient paresis, osteomalacia
Abbreviations: 25-hydroxyvitamin D (25-OH Vit D), parathyroid hormone (PTH), calcitonin (CT), net energy for lactation (NEL)

INTRODUCTION

Calcium is strongly regulated in mammals because of the critical role of calcium ion concentrations in many physiological functions. The discovery of the vitamin D endocrine system has resulted in the realization that Ca regulation in mammals and birds involves a coordinated effort between the parathyroid hormone (PTH), calcitonin and the hormonally-active form of vitamin D_3. Failure of this system to maintain normal blood Ca concentrations at parturition is a common occurrence in ruminants, leading to clinical (parturient paresis, milk fever) and subclinical hypocalcaemia [1–5]. Vitamin D sterols have a significant role in efforts to avoid parturient hypocalcaemia, and our investigation highlights the advantages and disadvantages associated with their use [5–10]. Vitamin D is metabolized by sequential steps in the liver and kidney to its active form, a process that is strongly feedback-regulated. In old age, the activity of the enzyme 25-hydroxyvitamin D 1 hydroxylase which produces the vitamin D hormone is diminished [6, 7, 11–13].

Vitamin D metabolite not only stimulates the intestine to absorb calcium and phosphorus, the bones to mobilize calcium and phosphorus, and the kidney to cause increased renal reabsorption of calcium, but also directly suppresses the parathyroid hormone and is a developmental hormone necessary for the recruitment of cells for osteoclast formation, female reproduction, development of skin, and for the treatment of certain malignant conditions [5, 7, 14–16].

25-hydroxyvitamin D, also known as 25-hydroxycholecalciferol, calcidiol or abbreviated 25-OH Vit D, is the main vitamin D metabolite circulating in plasma

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[4, 17, 18]. Vitamin D deficiency can lead to abnormalities in calcium and bone metabolism such as rickets or osteomalacia. The measurement of the vitamin D hormone and its precursor may be of great value in the diagnosis of metabolic bone disease and, most importantly, the availability of new vitamin D compounds may play an important role in the treatment of these bone diseases [1, 7, 15, 19]. 25-OH Vit D has a longer half-life and its sera level shows less daily variations than calcitriol. Thus, its measurement is a better indicator of vitamin D status [11]. The measurement of 25-OH Vit D is becoming increasingly important in the management of cows with various calcium metabolism disorders [8]. Tissue 25-OH Vit D receptor concentrations decline with age, leaving the tissues less able to respond to 25-OH Vit D [4]. Also, tissue 25-OH Vit D receptor concentrations in-crease during pregnancy and lactation in cows.

Decreased 25-OH Vit D concentration is as an indicator of vitamin D deficiency and is associated with hypocalcaemia, hypophosphatemia, and elevated alkaline phosphatase [7, 17, 20].

The aim of the present study was to determine particularities of the blood sera level of 25-OH Vit D in healthy cows of different feeding, age and productivity, its seasonal variations and differences in cows with parturient paresis and osteomalacia.

MATERIALS AND METHODS

The blood for analyses was taken from cows of the Lithuanian Black-and-White breed in winter and in summer time. The groups of cows were formed on the principle of analogues and with regard to age, health status, time of parturition, productivity and the type of ration. Experimental cows and heifers were examined clinically before the formation of groups (Table 1).

Blood was taken from cows of groups 1–4, 5, 7 and 8 twice (during in-house and pasturable periods). From cows of group 6, blood was taken 11 times five times before calving every day, on the day of calving and five days following calving.

Cows and heifers of groups 1–4, 5, 6 and 8 were fed with mineral supplements. Cows of group 7 were fed both with mineral supplements and without them. During the wintering period, cows were fed 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 the pasturable period. Dry cows-in-calf and heifers-in-calf were fed with Efekt Mineral Foder. Lag. mineral supplements (Lactamin, Sweden) which contained 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 Efekt Mineral Foder. Hog. mineral supplements (Lactamin, Sweden) which contained 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.

Table 1. **Groups of cattle**

Groups No	Animals	During in-house period	During pasturable period
Clinically healthy cattle			
1.	Heifers in-calf (n = 20)	n = 10	n = 10
2.	Cows 2–4 years old (n = 20)	n = 10	n = 10
3.	Cows 5–7 years old (n = 20)	n = 10	n = 10
4.	Cows 8 years old and above (n = 20)	n = 10	n = 10
5	Dry cows-in-calf	n = 10	
6.	Dry cows during the last decade of pregnancy (n = 20)	6a) Vit. D_3 used every day 50 mg 5 days before calving (n = 5), 6b) Vit. D_3 used thrice, 50 mg each time 5 days before calving (n = 5), 6c) Vit. D_3 used once, 50 mg 5 days before calving (n = 5), 6d) control group, vitamin D_3 was not injected (n = 5)	
Sick cattle			
7.	Cows with parturient paresis (n = 20)	7a) fed with mineral supplements (n =10), 7b) fed without mineral supplements (n = 10)	
8.	Cows with osteomalacia	n = 12	

Blood from cows of the sample was taken in equalized conditions, i.e. at 7 o'clock a. m., after overnight fast. Blood for analysis was collected by jugular venipuncture into Venoject single-use tubes without anticoagulant. Blood samples were delivered to the laboratory and centrifuged for 5 min at the rate of rotation 3.000 times per minute. Separated blood serum was pumped out to Eppendorf tubes with lids using a 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 *in vitro* using the Roche Elecsys 1010/2010 analyzer (Roche Diagnostics GmbH, USA). The amounts of calcitonin were determined *in vitro* using the IMMULITE (USA, Diagnostic Products Corporation) analyzer, by the immunometric method. 25-OH Vit D content was determined using the ELISA method. The content of macronutrients (calcium, phosphorus, magnesium) was measured using the Eos-Bravo analyzer (Italy, Hospitex Diagnostics) and reagents of the HOSPITEX company.

Findings and statistical data were computed using Epi Info program (1996; Centers for Disease Control & Prevention (CDC), USA, Version 6.04). The arithmetical means of findings (M), standard error (SD) and the level of significance (p) were calculated. The Student's multiple comparison method was used for identification of the group significance criterion (p). The difference was considered significant at $p < 0.05$. Correlations were determined using Pearson's correlation coefficients (r).

RESULTS

Analysis of the blood serum level of calcium-regulating hormones PTH, CT and 25-OH Vit D in heifers-in-calf in winter showed that the average level of vitamin D was 26.76 ± 6.67 nmol/l, and this was the lowest and statistically significant 25-OH Vit D level during the in-house period for all investigated groups of clinically healthy cows (Table 2, Fig. 1). In summer, the average level of 25-OH Vit D was 25.4 ± 2.94 nmol/l and was very close to the level observed during winter time ($p > 0.05$); it was also lower than that of the groups of clinically healthy cows in summer time, but differed statistically significantly from the group of cows aged 8 years and over ($p < 0.05$). The level of 25-OH Vit D correlated with the level of PTH very strongly during winter ($r = 0.936$) and summer ($r = 0.908$), and they correlated strongly negatively with the level of calcium during winter ($r = –0.651$) and summer ($r = –0.779$).

The average level of 25-OH Vit D of clinically healthy cows 2–4 years old at the end of the in-house period was 34.55 ± 9.16 nmol/l, and it was higher than this level in heifers-in-calf ($p < 0.05$); there was no significant difference among the other groups. The level of 25-OH Vit D during the winter period varied from 19.6

Table 2. **Blood serum indicators of clinically healthy and sick cows**

Cattle groups	Blood serum indicators				
	PTH, pmol/l	CT, pmol/l	Ca, mmol/l	P, mmol/l	Mg, mmol/l
Clinically healthy cattle	Heifers-in-calf during in-house period (n = 10)				
	4.37 ± 0.75	1.75 ± 0,38	2.62 ± 0.37	1.51 ± 0.22	1.08 ± 0.13
	Heifers-in-calf during pasturable period (n = 10)				
	2.74 ± 0.71	2.14 ± 0.56	2.85 ± 0.21	2.01 ± 0.10	1.21 ± 0.12
	Cows 2–4 years old during in-house period (n = 10)				
	3.46 ± 0.75	1.71 ± 0.34	2.28 ± 0.28	1.88 ± 0.17	0.96 ± 0.19
	Cows 2–4 years old during pasturable period (n = 10)				
	2.62 ± 0.58	2.03 ± 0.44	2.82 ± 0.42	1.89 ± 0.18	1.05 ± 0.14
	Cows 5–7 years old during in-house period (n = 10)				
	4.42 ± 0.57	1.54 ± 1.14	1.97 ± 0.24	1.46 ± 0.25	0.98 ± 0.22
	Cows 5–7 years old during pasturable period (n = 10)				
	3.58 ± 0.67	2.1 ± 0.54	2.62 ± 0.20	1.82 ± 0.18	0.98 ± 0.12
	Cows 8 years old and above during in-house period (n = 10)				
	4.37 ± 0.82	1.46 ± 0.00	1.88 ± 0.27	1.52 ± 0.12	0.85 ± 0.10
	Cows 8 years old and above during pasturable period (n = 10)				
	3.64 ± 0.59	1.51 ± 0.09	2.51 ± 0.04	1.73 ± 0.19	0.81 ± 0.13
	Dried-off cows-in-calf during in-house period (n = 10)				
	5.2 ± 1.26	1.61 ± 0.20	2.32 ± 0.18	1.47 ± 0.24	0.86 ± 0.16
Sick cattle	Cows with parturient paresis (fed mineral supplements, n = 10)				
	12.93 ± 2.14	1.74 ± 0.61	1.54 ± 0.61	0.71 ± 0.30	1.18 ± 0.50
	Cows without parturient paresis (fed no mineral supplements, n = 10)				
	18.31 ± 6.18	1.46 ± 0.42	1.38 ± 0.45	0.65 ± 0.23	1.22 ± 0.34
	Cows with osteomalacia (n = 12)				
	3.95 ± 0.60	1.55 ± 0.19	1.86 ± 0.18	0.88 ± 0.19	0.73 ± 0.11

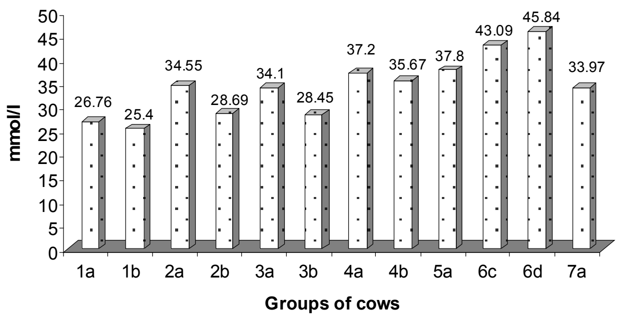

Fig. 1. Serum 25-OH Vit D of healthy and sick cows. Groups of cattle: 1 – heifers in-calf, 2 – cows 2–4 years old, 3 – cows 5–7 years old, 4 – cows 8 years old and above, 5 – dry cows-in-calf, 6 – cows with parturient paresis, 7 – cows with osteomaliacia, a – during in-house period, b – during pasturable period, c – fed mineral supplements, d – fed no mineral supplements.

to 39.5 nmol/l (28.69 ± 6.28 nmol/l) and did not differ statistically significantly from the level found during the winter period ($p > 0.05$) and from the other groups investigated during the summer period, except the group of cows aged 8 years and over ($p < 0.05$). 25-OH Vit D correlated with the level of calcium strongly negatively ($r = -0.661$) and with the level of phosphorus weakly negatively ($r = -0.355$).

The average level of 25-OH Vit D of cows 5–7 years old during the in-house period was 34.1 ± 3.07 nmol/l. The level of 25-OH Vit D strongly inversely correlated with the level of calcium ($r = -0.678$). The average level of 25-OH Vit D during the pasturable period was 28.45 ± 5.86 nmol/l and was statistically significantly lower than in winter ($p < 0.05$). There was no significant difference among the other groups of cows in summer, except the group of cows aged 8 years and over, in which the level of 25-OH Vit D was statistically significantly higher (35.67 ± 5.49, $p < 0.05$). The level of 25-OH Vit D strongly negatively correlated with the level of calcium ($r = -0.678$).

The average level of 25-OH Vit D of cows aged 8 years and over was 37.2 ± 7.78 pmol/l. A lower level was found only in heifers-in-calf (26.76 ± 6.67, $p < 0.05$). No statistically significant difference ($p > 0.05$) was observed when comparing with other groups of clinically healthy cows. The level of 25-OH Vit D correlated with the level of calcium satisfactorily negatively ($r = -0.423$) and with level of phosphorus weakly negatively ($r = -0.317$). According to analysis of the blood sera of cows aged 8 years and over, which received mineral supplements with fodder, during the pasturable period, we found that the average level of 25-OH Vit D was 35.67 ± 5.49 and was statistically significantly higher than in the other groups of clinically healthy cows investigated in summer ($p < 0.05$), however, the difference was not statistically significant (37.2 ± 7.78, $p > 0.05$) when compared with the winter period. The level of 25-OH Vit D strongly correlated with calcium ($r = -0.691$) and phosphorus ($r = -0.620$) levels.

We found important changes of hormones by analyzing the level of macronutrients and calcitropic hormones in dry cows-in-calf one week before calving, which were injected vitamin D (Romedat D_3 forte 1 ml – 50 mg vit. D_3). When performing analysis of the blood sera of cows immediately after parturition, an important fall in calcium and phosphorus concentrations and an increase in magnesium, PTH, CT and 25-OH Vit D concentrations were observed. The measured values of macronutrients and hormones changed at similar intervals, independently of vitamin D injections (Fig. 2). However, several days after calving the recorded level of macronutrients and hormones differed.

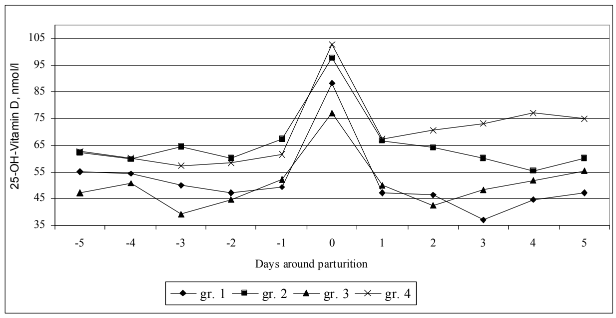

Fig. 2. Change of blood sera 25-OH Vit D in cows which were injected vitamin D.
Groups of cattle: 1 – Vit. D_3 used every day 50 mg 5 days before calving, 2 – Vit. D_3 used thrice, 50 mg each time 5 days before calving, 3 – Vit. D_3 used once, 50 mg 5 days before calving, 4 – control group with no vitamin D_3 injection.

The blood serum concentrations of calcium decreased significantly the day in all groups of cows ($p < 0.05$), however, the level of calcium in groups 1 and 2 on day 5 after calving did not differ from the values observed 5 days before calving ($p > 0.05$), and the level of calcium in the blood serum of cows of groups 3 and 4 on the 5th day after calving differed from the level fixed 5 days before calving ($p < 0.05$).

The blood serum concentration of 25-OH Vit D increased notably ($p < 0.05$) in all cows on the day of parturition. It lowered much the first day after parturition tothe former level. The blood sera concentration of 25-OH Vit D on the fifth day after parturition in groups 1, 2 and 3 did not differ from the concentration found five days before parturition, however, the blood serum concentration of 25-OH Vit D in group 4 was higher ($p < 0.05$). The blood serum level of 25-OH Vit D in cows was dependent on vitamin D injections – we determined its highest content in the blood of the control group of cows which were not injected vitamin D. Vitamin D injections had an impact on calcium and phosphorus concentrations, because their level in groups 1 and 2 on the fifth day after parturition was close to that found five days before parturition; the concentrations of calcium and phosphorus did not differ from those of the control group in the case of cows who had been vitamin D injected only once. Based on our study it is possible to conclude that vitamin D injection has a great influence on the blood serum level of calcium and phosphorus and has almost no impact on the level of magnesium. Vitamin D influenced the level of PTH and CT as well, because higher levels of PTH and CT were found in the control group to whom vitamin D has not been injected.

The blood level of 25-OH Vit D in paresis cows that received mineral supplement was 43.09 ± 8.16 nmol/l and in cows that did not receive mineral supplements 45.84 ± 10.76 nmol/l. There was no difference between these groups ($p > 0.05$). However, the numbers were significantly higher in the case of osteomalacia ($p < 0.05$) as compared with the other groups of cows during the winter period, also it was significantly higher in cows-in-calf and heifers-in-calf and cows aged 2–4 years ($p < 0.05$), however, there was no statistically significant difference ($p > 0.05$) as compared with cows aged 8 years and over (Table 2, Fig. 1).

The average level of 25-OH Vit D in cows with osteomalacia was 33.97 ± 11.39 nmol/l; however, it did not differ from that of clinically healthy cows (Table 2, Fig. 1). Compared to sick cows, its level was statistically lower than in cows with paresis ($p < 0.05$). The level of 25-OH Vit D in the blood of cows with osteomalacia inversely correlated with the level of calcium ($r = –0.650$).

DISCUSSION

The blood serum level of 25-OH Vit D varied from 18.1 to 56.4 nmol/l. In healthy cows it changed depending on age, being statistically significantly lower and the CT level higher in cows 2–4 years old compared with cows aged 8 years and over. Vitamin D is metabolized by sequential steps in the liver and kidney to its active form, a process that is strongly feedback-regulated. In old age, the activity of the enzyme, 25-hydroxyvitamin D 1 hydroxylase, which produces the vitamin D hormone, is diminished. Other authors [20] also obtained similar findings. Our study shows that the findings may be associated with age, because the blood level of 25-OH Vit D increases with age. The activity of 25-OH Vit D depends on calcium level in the blood [21]. The present findings confirm the data of [15] indicating that the level of 25-OH Vit D depends on the serum concentrations of calcium and phosphorus, but this interrelation is much weaker in comparison with its dependence on PTH. The highest blood level of 25-OH Vit D found in sick cows may be explained by the dependence of the 25-OH Vit D activity on the level of blood calcium, i.e. the lower is the level of calcium, the higher is the level of PTH activating the synthesis of vitamin D [15]. A higher blood level of 25-OH Vit D in the blood of sick cows may be explained by the fact that activity of 25-OH Vit D depends on the abundance of calcium in the blood, i.e. the lower is the level of Ca, the higher is the level of PTH which activates 25-OH Vit D synthesis [4]. The activity of 25-OH Vit D depends on the blood level of calcium [13]. In our study this statement was supported, because the level of calcium and 25-OH Vit D in the blood of dry cows-in-calf correlated strongly negatively ($r = –0.780$). The late stages of pregnancy and all stages of lactation are physiological conditions of Ca stress and therefore contribute to dramatic changes in Ca and P metabolism. Increased maternal mineral and bone metabolism is known to occur because of skeletal mineralization of the fetus during pregnancy and milk production during lactation in mammals [5, 22]. As cows aged 4–10 years were selected for the group of dry cows in-calf, the findings of our study correspond to the statements of [23] that the activity of calcium decreases and the activity of PTH increases with age.

The blood serum level of 25-OH Vit D changed in healthy cows depending on the season of the year: a statistically significantly higher level of PTH and 25-OH Vit D and a lower level of CT were found during the winter period as compared with summer. The level of 25-OH Vit D depended little on cow productivity and feeding. Low levels of calcium stimulate secretion of the parathyroid hormone, and the latter induces synthesis of 25-OH Vit D [24]. Because the blood calcium level in heifers-in-calf in summer and in winter time as well was in a normal range, the level of 25-OH Vit D was not high, either. In wintertime, serum 25-OH Vit D decreased by 15 nmol/l (from 77 ± 35 nmol/l to 62 ± 26 nmol/l) [17].

The seasonal decrease in 25-OH Vit D concentration in llamas and alpacas was significantly greater in neonates and yearlings than in adults. These results support the hypothesis that seasonal alterations in vitamin D_3 concentrations are a key factor in the development of hypophosphatemic rickets in llamas and alpacas [13].

Preparations of vitamin D (injected 3–5 times) administered during the last days before calving act effectively on the level of calcium, phosphorus, PTH and vitamin D. The level of calcium, phosphorus and 25-OH Vit D in cows injected vitamin D five times and thrice on the fifth day after parturition did not differ statistically significantly from the values found five days before calving.

The blood serum level of PTH and 25-OH Vit D in cows with parturient paresis was statistically significantly higher as compared with the level in healthy cows. There was no correlation between calcium and PTH in the blood serum of cows with parturient paresis, although a strong negative correlation was found between calcium and 25-OH Vit D levels.

When investigating the effects of 25-OH Vit D on the level of calcium and phosphorus, [25] has found that 25-OH Vit D acts increasing the blood level of calcium and phosphorus in cows and reduces the number of cases of parturient paresis after calving. Besides, the authors indicate that different forms of vitamin D decrease the blood serum level of magnesium. According to some reports [26, 27], the blood serum level of calcium and phosphorus in cows increased 36 h and 24 h after calving when vitamin D had been administered. According to the data of our study, the level of calcium and phosphorus decreased slightly five days before calving and notably increased 24 h after parturition and also increased little by little later depending on the frequency of vitamin D injections. According to some investigations [26], the level of hypocalcaemia is diminished by single doses of vitamin D administered for 3–10 days before parturition.Also, single doses of vitamin D did not have a marked impact on the level of calcium, because there was no significant difference in the level of calcium as compared with control cows, and the level of calcium did not restore five days after calving to the level which was found five days before parturition. Cows showed an increased bone resorption around parturition, and cows with a higher milk yield mobilize calcium more actively from bone than do cows with a lower milk yield [10, 28]. A daily administration of 650 nmol vitamin D3 for three days to pregnant rabbits caused a significant increase in calcium, phosphorus, 25-OH Vit D, and 24,25-(OH)2D3 in maternal plasma [29]. Continuous feeding of vitamin D reduced the incidence of milk fever in cows with previous milk fever from 60% in the controls to 26.1% in the group fed vitamin D. In the cows with no previous milk fever, feeding vitamin D did not reduce the incidence of milk fever (controls 23.7%, vitamin-D-fed 28.3%). The use of large doses of vitamin D metabolites and their analogues for milk fever prevention is controversial. Due to toxicity problems and an almost total lack of recent studies on the subject, this principle is not described in detail [30].

Besides the classical understanding of its action in calcium metabolism, it is clear that the hormonal form of vitamin D has more functions than those discovered as a result of following the appearance of its receptor. Furthermore, because of the vitamin D-based endocrine system, the use of vitamin D compounds in treating a variety of diseases has been expanded [7].

Statistically significantly lower blood serum levels of calcium, phosphorus and magnesium were found in cows with osteomalacia (compared with healthy cows); however, the level of PTH, CT and 25-OH Vit D did not differ statistically significantly.

The low values of macronutrients found in the groups of cows with parturient paresis show that their blood serum level depends on feeding type, but macroelements received with mineral supplements are not sufficient to maintain blood homeostasis in cows during the critical time after parturition when large quantities of macroelements are excreted from blood to milk. Studies have found that mineral supplements act on the status of mineral metabolism and have an impact on the level of blood components [31].

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25-OH VITAMINO D KAITA SVEIKŲ IR SERGANČIŲ KARVIŲ KRAUJO SERUME

Santrauka

Tirta 25-OH vitamino D kiekio kaita, jo koreliacija su kitais rodikliais skirtingai šeriamų, įvairaus amžiaus ir produktyvumo sveikų ir sergančių pareze po apsiveršiavimo bei osteomaliacija 182 karvių kraujo serume. Ca, P bei Mg kiekis nustatytas automatiniu „Eos-Bravo“ analizatoriumi su Hospitex reagentais, 25-OH vitamino D kiekis – imunofermentine analize, PTH kiekis – elektrochemine liuminescencine imunine analize Roche Elecsys 1010/2010 analizatoriumi, CT kiekis – IMMULITE analizatoriumi, chemiliuminescencine imunometrine analize.

Sveikų karvių kraujo serume 25-OH vitamino D kiekis kito nuo 18,1 iki 56,4 nmol/l. PTH kiekis neigiamai koreliavo su kalcio kiekiu ir teigiamai – su vitamino D ir kalcitonino kiekiu, 25-OH vitamino D kiekis neigiamai koreliavo su kalcio kiekiu. 25-OH vitamino D kiekis sveikų karvių kraujo serume kito priklausomai nuo amžiaus – 2–4 metų amžiaus karvių serume jo buvo statistiškai patikimai mažiau nei 8 metų. 25-OH vitamino D kiekis sveikų karvių kraujo serume kito priklausomai nuo metų laiko – žiemą nustatytas statistiškai patikimai didesnis vitamino kiekis nei vasarą. Vitamino D preparatai (švirkščiant 3–5 kartus), likus kelioms dienoms iki veršiavimosi, efektyviai veikia kalcio, fosforo, PTH ir vitamino D kiekį. Po veršiavimosi pareze sergančių karvių kraujo serume nustatytas statistiškai patikimai didesnis 25-OH vitamino D kiekis nei sveikų karvių. Šių karvių serume tarp kalcio ir vitamino D nustatyta stipri neigiama koreliacija. Osteomaliacija sergančių karvių kraujo serume vitamino D kiekis statistiškai patikimai nesiskyrė nuo sveikų karvių. PTH vidutiniškai neigiamai koreliavo su kalcio ir stipriai neigiamai – su vitamino D kiekiu.