LITHUANIAN VETERINARY ACADEMY
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VARIATION OF BLOOD SERUM VITAMIN D, CALCITONIN, PARATHYROID HORMONE LEVELS IN COWS AND THEIR IMPORTANCE FOR PROCESSES OF MINERAL METABOLISM

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LIETUVOS VETERINARIJOS AKADEMIJOS VETERINARIJOS INSTITUTAS

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VITAMINO D, KALCITONINO IR PARATHORMONO KIEKIŲ KAITA KARVIŲ KRAUJO SERUME BEI REIKŠMĖ MINERALINIŲ MEDŽIAGŲ APYKAITOS PROCESAMS

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INTRODUCTION

The direct role of *vitamin D* and *parathyroid hormone* (PTH), hormone of parathyroid gland, and that of calcitonin (CT), hormone of thyroid gland, includes the regulation of the blood level of calcium (Capen, Martin, 1982, Enemark et al., 2003). Ionised calcium of the blood serum maintains normal functions of nerves and muscles. Calcium is indispensable for normal activity of the heart, besides it is one of the main ions, which regulate the acid-alcali balance. Calcium diminishes permeability of membranes and binding features of tissue colloids, activates such enzymes as ATP-ase, lecithinase, succinatedehydrogenase, it stabilises trypsin in the pancreas and inhibits enolase and dipeptidase. When the body experience a lack of calcium, the amounts of proteins, ATP, erythrocytes and haemoglobin become reduced and membrane permeability for ions increases (Urazaev, 1990). In addition, calcium, together with phosphorus and magnesium, forms mineral basis for skeleton. The blood level of ionised calcium is very stable. Main regulatory roles of calcium metabolism belong to *vitamin D, parathyroid hormone* and *calcitonin*. *Vitamin D* stimulates absorption of calcium and phosphorus from guts and creates opportunity for appropriate bone mineralization by maintaining uniform level of electrolytes in guts at the same time (Omdall, DeLuca, 1973, Horst et al., 2003). *Vitamin D* guarantees bone mineralization by securing sufficient level of mineral ions, meanwhile *parathyroid hormone* maintains a necessary calcium-phosphorus ratio in intercellular fluids. Besides, small levels of *vitamin D* are necessary for stimulation of *parathyroid hormone* to mobilize calcium from skeletal reserves to intercellular fluids ("facultative effect") (Haussler, McCain, 1977). The most important roles of *parathyroid hormone* are the following: to increase blood concentration of calcium and to diminish that of phosphorus, to stimulate excretion of phosphorus with urine, to increase reabsorption of calcium in canaliculi when the urine level of calcium is decreased, to increase osteolysis and the number of osteoclasts on the surface of bones, to stimulate excretion of hydroxiprolin with urine, to activate adenilate cyclase in certain cells (Rasmussen, 1974). *Calcitonin* stimulates accumulation of calcium in bones by inducing the osteoblast activity. In addition, it acts on phosphorus metabolism in the body. Because it decreases the blood level of calcium, *calcitonin* is antagonist of *parathyroid hormone*, which increases the level of this element in the blood.

Today's farming conditions require from animal owners to implement intensive rearing technologies, because it is necessary to avoid economic losses related with metabolic disorders of various character. At present, insufficient attention is paid to animal non-infectious diseases in Lithuania, though metabolic diseases take one of the first places in the pathology of internal diseases by their spread and economic losses. In conditions of insufficient means, not all cattle receive vitamins and also they do not receive mineral supplements everywhere and always. Biochemical tests of animal blood are not carried-out constantly and all-round, a computerized system for health evaluation of every animal, such as systems in the countries of developed animal husbandry, does not exist. It is difficult to define metabolic status of cows of agricultural companies and farmers, because biochemical control of

cows-in-calf is not carried out and metabolic processes are not investigated 15 to20 days before calving. It is not clear what is the situation in specific districts and during specific seasons of the year. In this study, it was therefore planned to investigate metabolic status of *vitamin D*, *calcitonin, parathyroid hormone* and macroelements – calcium, phosphorus and magnesium in herds of cows of our Republic.

Aim of the study:

To determine variation particularities of the blood serum level of *vitamin D, calcitonin* and *parathyroid hormone* in healthy cows of different feeding, age and productivity and during different seasons of the year and similar levels in cows having parturient paresis, osteomalacia and mastitis.

Pursuing the general aim of the study, the following goals were posed:

1. To investigate variation particularities of the blood serum levels of *vitamin D*, *calcitonin, parathyroid hormone* in cows of different age, productivity, physiological status, feeding type during different seasons and their relation with levels of calcium, phosphorus and magnesium.
2. To determine change particularities of the levels of *vitamin D, calcitonin* and *parathyroid hormone* and their relation with levels of calcium, phosphorus and magnesium in cows with parturient paresis, osteomalacia and mastitis.
3. To investigate effects of vitamin D preparations on mineral and hormone metabolism in healthy cows-in-calf.
4. To test suitability of methods developed for humans (electrochemiluminescence analysis, chemiluminescence immunometric analysis and immunoferment analysis) for measurement of the blood serum levels of *PTH, CT and 25-OH vitamin D* in cattle.

Novelty of the scientific study:

Change particularities of the blood serum levels of *vitamin D, calcitonin* and *parathyroid hormone* were analysed in healthy cows of different feeding, age and productivity and in sick cows with metabolic diseases. It was investigated how biochemical indicators vary in cows with metabolic diseases (parturient paresis, osteomalacia and mastitis) and the most informative indicators for diagnostics of these diseases (for levels of calcium, phosphorus and magnesium) were determined. Obtained findings were processed statistically.

The method of electrochemiluminescence analysis used for analysis of the blood serum levels of PTH in humans was applied. Compared with radioimmunic method, this method is not expensive and quite fast; in addition, this method is sensitive and reliable, because it permits to detect low levels of PTH in the blood serum (up to 0.127 pmol/l). The method of chemiluminescence immunometric analysis used to find the level of *CT* in humans was tested. Also the method of immunoferment analysis (ELISA) was tested to measure the blood serum level of 25-OH vitamin D in cattle using human antibodies.

MATERIALS AND METHODS

The study was performed in the Laboratory of Animal Health and Epidemiology, Microbiology and Food Safety Department, Lithuanian Veterinary Academy Veterinary Institute (LVA VI), in 2002–2005; in the Virology Laboratory, LVA VI; in the Biochemistry Laboratory of Kaunas University of Medicine Hospital; and in cow-houses of individual farms of Kaunas, Kaišiadoriai and Jurbarkas districts.

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 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 status of the animal was examined, the pulse rate counted, temperature measured, contractions of the rumen counted, rumination of the animal, diuresis and defecation were observed. The cows, which above mentioned indicators were in the range of normal, were considered healthy. The cattle were divided into the following groups:

1. Clinically healthy heifers in-calf (n = 20) (during in-house period (n = 10) and during pasturable period (n = 10));
2. Clinically healthy cows 2–4 years old (n = 10) (during in-house period and during pasturable period);
3. Clinically healthy cows 5–7 years old (n = 10) (during in-house period and during pasturable period);
4. Clinically healthy cows 8 years old and above (during in-house period and during pasturable period);
5. Clinically healthy cows of high and low productivity fed with mineral supplements and without them (during in-house period) (n = 40);
6. 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));
7. Clinically healthy dry cows-in-calf during in-house period (n =10);
8. Having parturient paresis (n = 20) (fed with mineral supplements (n =10), fed without mineral supplements (n = 10));
9. Having osteomalacia (n = 12);
10. Having mastitis (n = 10).

The blood was collected from cattle of the groups 1, 5, 7, 8, 9 and 10 from jugular vein once. The blood was taken from cows of the groups 2–4 twice (during in-house and pasturable periods). The blood was taken 11 times from cows of the group 6: five times before calving every day, the day of calving and 5 days after calving.

The cows and heifers of the groups 1–4, 6, 7, 9 and 10 were fed with mineral supplements. The cows of the groups 5 and 8 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.

METHODOLOGY OF COW BLOOD ANALYSIS

Blood from cows of the sample was taken in equalized conditions, i.e. at 7 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 *in vitro* using the Roche Elecsys 1010/2010 analyzer (*Roche Diagnostics GmbH,* USA). The amounts of the calcitonin were determined *in vitro* using the IMMULITE (USA, *Diagnostic Products Corporation*) analyzer, by the immunometric method. Amounts of 25-hidroxicholecalciferol were determined using ELISA method. Amounts of macronutrients (calcium, phosphorus, magnesium) were measured using the Eos-Bravo analyzer (Italy, Hospitex Diagnostics) and reagents of the company *HOSPITEX*.

Statistical data analysis.

Findings and statistical data were computed using a program Epi Info (1996; Centers for Disease Control & Prevention (CDC), U.S.A., Version 6.04). Arithmetical means of findings (M), absolute error (m) and level of significance (p) were calculated. The Student's multiple comparison method was used for identification of group significance criterion (p). Difference was considered significant when $p<0.05$.

Findings and discussion

Performing analysis of the blood serum levels of macroelements Ca, P and Mg and levels of calcium-regulating hormones PTH, CT and 25-OH vitamin D in heifers-in-calf, we determined that average levels of macronutrients Ca, P, Mg during in-house period (2.62±0.37, 1.51±0.22, 1.08±0.13, respectively) and

pasturable period (2.85±0.21, 2.01±0.10, 1.21±0.12, respectively) were in the range of normal. We found statistically significantly higher levels of phosphorus ($p<0.05$) and magnesium ($p<0.05$) during pasturable period compared with in-house period, however higher calcium levels found during pasturable period did not differ statistically significantly from those found during in-house period ($p>0.05$). Higher levels of calcium, phosphorus and magnesium during pasturable period were found by Horst (1986), Reinhardt et al. (1988) and Klimienė (2001). 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 statistically significantly from the groups of cows 2–4 years old ($p<0.05$), however it did not differ statistically 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 not strongly 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 statistically 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 ($r = – 0.779$). This corresponded with the findings of Potts et al. (1995) saying that PTH concentration correlates with calcium concentration inversely and the level of PTH increases with decrease of calcium concentrations and vice versa. When comparing heifers-in-calf with dried-off cows, we noticed that significantly higher levels of blood serum calcium ($p<0.05$) and magnesium ($p<0.05$) were found in heifers-in-calf, however the levels of phosphorus and calcitonin did not differed statistically significantly ($p>0.05$), though found higher. Measured lower levels of vitamin D differed statistically significantly ($p<0.05$) from these ones of dry cows-in-calf, however the levels of parathyroid hormone did not differ statistiscally significantly ($p>0.05$), though found lower. Also Klimienė (2001) reported that levels of calcium and posphorus diminished with age however our study did not corroborate that the level of magnesium increased in the course of life. The average level of calcitonin was 2.14±0.56 pmol/l in summer and 1.75±0.38 pmol/l in winter and they did not differ statistically significantly ($p>0.05$). The level of calcitonin found in winter time was highest in all investigated groups of clinically healthy cows during in-house period, however it differed statistically significantly only from the group of cows 8 years old and above ($p<0.05$) and from the groups of clinically healthy cows of low productivity fed as with mineral supplements, as without them ($p<0.05$). Levels of calcitonin correlated strongly with level of parathyroid hormone in summer and in winter as well ($r = 0.916$ and $r = 0.689$, respectively). Higher calcitonin levels in cows-in-calf were reported by Barlet (1972). In his opinion, the level of calcitonin increases in cows-in-calf and heifers-in-calf for protection of mother's skeleton from too high losses of calcium and phosphorus. According to the data of Deftos (1997), calcitonin is secreted constantly in presence of normal calcium concentration, however its secretion increases significantly by elevation of blood calcium level. That corresponded to our data, because we found higher level of calcitonin in summer compared with winter period, though there was no significantly higher difference ($p>0.05$). Cooper (1972) described that it is possible

to determine the elevation of calcitonin secretion when animal eats fodder containing much calcium still before the increase in plasma Ca. That corresponded also with our data, because we found higher level of calcitonin in summer time compared with winter period. Average level of vitamin D was 26.76±6.67 nmol/l during winter period and that was the lowest and statistically significant vitamin D level during in-house period found for all investigated groups of clinically healthy cows ($p<0.05$). In summer, the average level of vitamin D was 25.4±2.94 nmol/l and it was very close to level observed during winter time (26.76±6.67) ($p>0.05$); also it was lower than that of the groups of clinically healthy cows in summer time, but it differed statistically significantly from the group of cows 8 years old and above ($p<0.05$). The determined low level of vitamin D may be associated with age. That corresponded to the data of Scharla (1998) that the level of 25-OH vitamin D increased in the course of life.

Table 1. **Blood serum indicators of clinically healthy cows**

	Blood serum indicators					
	PTH, pmol/l	CT, pmol/l	Ca, mmol/l	P, mmol/l	Mg, mmol/l	Vit. D, nmol/l
Clinically healthy heifers-in-calf during in-house period						
M±m	4.37±0.75	1.75±0,38	2.62±0.37	1.51±0.22	1.08±0.13	26.76±6.67
Clinically healthy heifers-in-calf during pasturable period						
M±m	2.74±0.71	2.14±0.56	2.85±0.21	2.01±0.10	1.21±0.12	25.4±2.94
Clinically healthy cows 2–4 years old during in-house period						
M±m	3.46±0.75	1.71±0.34	2.28±0.28	1.88±0.17	0.96±0.19	34.55±9.16
Clinically healthy cows 2–4 years old during pasturable period						
M± m	2.62±0.58	2.03±0.44	2.82±0.42	1.89±0.18	1.05±0.14	28.69±6.28
Clinically healthy cows 5–7 years old during in-house period						
M± m	4.42±0.57	1.54±1.14	1.97±0.24	1.46±0.25	0.98±0.22	46.9±4.22
Clinically healthy cows 5–7 years old during pasturable period						
M± m	3.58±0.67	2.1±0.54	2.62±0.20	1.82±0.18	0.98±0.12	28.45±5.86
Clinically healthy cows 8 years old and above during in-house period						
M± m	4.37±0.82	1.46±0.00	1.88±0.27	1.52±0.12	0.85±0.10	37.2±7.78
Clinically healthy cows 8 years old and above during pasturable period						
M± m	3.64±0.59	1.51±0.09	2.51±0.04	1.73±0.19	0.81±0.13	35.67±5.49
Clinically healthy dried-off cows-in-calf during in-house period						
M±m	5.2±1.26	1.61±0.20	2.32±0.18	1.47±0.24	0.86±0.16	37.8±6.45

Levels of vitamin D correlated with levels of PTH very strongly during winter ($r = 0.936$) and summer ($r = 0{,}908$) periods and they correlated also strongly negatively with levels of calcium during winter ($r = -0.651$) and summer ($r = -0.779$). According to Capen and Rosol (1989), low levels of calcium stimulate secretion of parathyroid hormone and the latter induces synthesis of vitamin D. Because the blood calcium levels found for heifers-in-calf in summer and in winter time as well were in the range of normal, the level of vitamin D was not high too.

Mild hypocalcemia was found doing analysis of blood serum macronutrient levels of clinically healthy cows 2–4 years old at the end of the in-house period; the mean of the calcium level was 2.28±0.28 mmol/l. Average levels of phosphorus (1.88±0.17 mmol/l) and magnesium (0.96±0.19 mmol/l) were in the range of normal. PTH varied from 2.2 to 5 pmol/l (it was 3.46±0.75 pmol/l in average). This was the lowest and statistically significant ($p<0.05$) PTH level among all groups of clinically healthy cows investigated during in-house period, except the cows of high productivity (3.43±0.38, $p>0.05$) and cows of low productivity fed with mineral supplements (2.5±0.61, $p<0.05$) and without them (2.9±0.60, $p>0.05$). Parathyroid hormone correlated strongly negatively with level of calcium ($r = -0.770$). The measured low PTH level may be associated with age (Potts et al., 1995), because the calcium levels decrease and PTH activity increases in older age. The average calcitonin level was 1.71±0.34 pmol/l and it differed statistically significantly only from the level in the group of cows 8 years old and above during pasturable and during in-house periods ($p<0.05$) and from the groups of cows of low productivity ($p<0.05$). Vitamin D correlated with levels of PTH satisfactorily ($r = 0.598$), with calcium strongly negatively ($r = -0.664$) and with phosphorus weakly negatively ($r = -0.334$). Average level of vitamin D was 34.55±9,16 nmol/l and it was higher than level in heifers-in-calf (26.76±6.67 nmol/l, $p<0.05$), however lower than this parameter in all other clinically healthy cows investigated during in-house period, but significant difference was only in case of cows 5–7 years old ($p<0.05$) and cows of low productivity, which did not received mineral supplements ($p<0.05$); there was no significant difference between the other groups. That may be also associated with age (Scharla, 1998) and blood serum level of calcium (Bruder et al., 2001).

Levels of macronutrients found by investigating cows 2–4 years old during pasturable period corresponded with the physiological norm or were close to it. Average level of calcium was 2.82±0.42 mmol/l. The level of phosphorus was above physiological norm in 5 cows of ten investigated, it was in the range of normal in others (1.89±0.18 mmol/l in average). The level of magnesium varied in limits of normal and was 1.05±0.14 mmol/l in average. The level of calcium was significantly higher ($p<0.05$) during pasturable period (2.82±0.42 mmol/l) compared with in-house period (2.28±0.28 mmol/l), and the level of calcium almost did not differ ($p>0.05$) compared with heifers-in-calf during pasturable period (2.85±0.21 mmol/l). The levels of phosphorus almost did not differ during in-house and pasturable periods (1.88±0.17 mmol/l and 1.89±0.18 mmol/l

respectively, p>0.05). The levels of magnesium were also very similar during in-house and pasturable periods (0.96±0.19 mmol/l and 1.05±0.14 mmol/l respectively, p>0.05). The PTH level 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 statistically significantly only from the group of cows 8 years old and above (p<0.05). There was no significant difference between PTH levels found in winter and summer (p>0.05). PTH correlated with levels of CT (r = 0.770) and levels of vitamin D (r = 0.774) strongly and with levels of calcium strongly negatively (r = – 0.656). The average level of calcitonin was 2.03 ±0.44 pmol/l and it did not differ statistically significantly from the level observed during winter period (p>0.05) and from the other groups of cows investigated during summer period except the group of cows 8 years old and above (p<0.05). The level of vitamin D varied from 19.6 to 39.5 nmol/l (28.69±6.28 nmol/l) and did not differ statistically significantly from the level found during winter period (p>0.05) and from the other groups investigated during summer period except the group of cows aged 8 years and above (p<0.05). Vitamin D correlated with levels of calcium strongly negatively (r = – 0.661) and with levels of phosphorus weakly negatively (r = – 0.355).

Hypocalcemia was revealed by performing blood serum tests of cows 5–7 years old during in-house period (average level of Ca was 1.97±0.24 mmol/l); the level of calcium differed statistically significantly from the level of calcium of heifers-in-calf (p<0.05) and cows 2–4 years old (p<0.05), but it did not differ statistically significantly from the group of cows 8 years old and above (p>0.05). The average level of phosphorus (1.46±0.25 mmol/l) was in the range of the recommended norm. The level of phosphorus found during in-house period differed statistically significantly only from the group of cows 2–4 years old (1.89±0.18, p<0.05). The mean of the magnesium level was 0.98±0.22 mmol/l. The level of magnesium did not differ statistically significantly among investigated groups of clinically healthy cows neither during winter (p>0.05) nor during summer (p>0.05) periods. The average PTH level was 4.42±0.57 pmol/l. During in-house period, a higher statistically significantly 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). PTH correlated with levels of calcitonin strongly (r = 0.703) and vitamin D (r = 0.89) and also with levels of calcium satisfactorily negatively (r = – 0.585). The average level of calcitonin was 1.54±1.14 pmol/l, no statistically significant difference was between the other groups of cows investigated during in-house period (p>0.05). The average level of vitamin D was 46.9±4.22 nmol/l and that was the highest statistically significant level of vitamin D found during in-house period in the blood serum of clinically healthy cows (p<0.05), except the group of high productivity cows, which did not receive mineral supplements where the level of vitamin D was also lower, but the difference was not statistically significant (43.3±9.41, p>0.05). The level of vitamin D correlated with the level of calcium strongly inversely (r = – 0.678).

The levels of calcium measured during pasturable period by analysing blood

serum of the cows 5–7 years old corresponded to the physiological normal and they were statistically significantly higher compared with winter period (2.62±0.20 mmol/l, p<0.05). Statistically significantly higher levels of calcium were found only in the blood of heifers-in-calf (2.85±0.21, p<0.05) when comparing with other groups of cows investigated during summer. Averages of phosphorus (1.82±0.18 mmol/l) and magnesium (0.98±0.12 mmol/l) corresponded to the recommended physiological norm. The level of phosphorus was significantly higher (p<0.05) compared with winter period; and significantly higher amount was only in the blood of heifers-in-calf (2.01±0.10, p<0.05) when comparing with other groups of clinically healthy cows investigated in summer. There was no significant difference between levels of magnesium found in winter (0.98±0.22) and summer (0.98±0.12) (p>0.05); a significantly higher amount was only in the blood of heifers-in-calf (1.21±0.12, p<0.05) and significantly lower in the blood of cows 8 years old and above (0.81±0.13, p<0.05) when compared with other groups of cows investigated in summer. The level of parathyroid hormone 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). It was statistically significantly higher than the level 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. That may be associated with age (Scharla, 1998) and productivity, because cows 5–7 years old are most productive and large amounts of calcium are secreted into milk (Klimienė, 2001), therefore the synthesis of parathyroid hormone becomes more active (Malz and Meyer, 1993; Axelsson, 1991). PTH levels correlated with levels of CT (r = 0.857) and vitamin D (r = 0.908) strongly and with levels of calcium strongly negatively (r = – 0.690). Average level of calcitonin was 2.1±0.54 pmol/l and it was statistically significantly higher than corresponding level during winter period (1.54±1.14, p<0.05). That corresponds with data of Horst (1986), Reinhardt (1988) and other researchers, saying that cows absorb calcium depending on how much they need it, i.e. when cows eat fodder containing large amounts of calcium, synthesis of calcitonin becomes more active and the blood level of calcium normalises. When compared with other groups of clinically healthy cows investigated in summer, a statistically significant difference was found with the group of cows 8 years old and above (1.51±0.09, p<0.05). Average level of vitamin D was 28.45±5.862 nmol/l and it was statistically significantly lower than corresponding level in winter (46.9±4.22, p<0.05). There was no significant difference among other groups of cows investigated in summer except the group of cows 8 years old and above where the level of vitamin D was statistically significantly higher (35.67±5.49, p<0.05). The level of vitamin D correlated with the level of calcium strongly negatively (r = – 0.678)**.**

Hypocalcemia was revealed at the end of in-house period by analysing biochemical indicators of the blood serum of 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, however it differed statistically 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, $p<0.05$), and did not differ statistically significantly from the group of cows aged 5 to 7 years (1.97±0.24, $p>0.05$). The average levels of phosphorus (1.52±0.12 mmol/l) and magnesium (0.85±0.10 mmol/l) were in the range of the recommended norm, however the level of phosphorus did not reach the recommended norm in the case of two cows from 10 investigated cows and the level of magnesium also in the case of two cows. The level of phosphorus among the groups of clinically healthy cows differed statistically significantly only from the group of cows aged 2 to 4 years (1.88±0.17, $p<0.05$) during winter period. A significantly higher level of magnesium was found only in the blood of heifers-in-calf (1.08±0.131, $p<0.05$) during in-house period. The level of PTH varied from 3.4 to 6,1 pmol/l (4.37±0.82 pmol/l in average). Significantly lower level of PTH was determined only in the blood of cows aged 2 to 4 years (3.46±0.75, $p<0.05$). The level of PTH correlated wit level of vitamin D strongly ($r = 0.680$) and with the level of calcium strongly negatively ($r = – 0.610$). The amount of calcitonin was lower than 1.46 pmol/l and it differed statistically significantly from corresponding levels of cows-in-calf (1.61±0.20, $p<0.05$) and heifers-in-calf (1.75±0.38, $p<0.05$) and cows 2–4 years old (1.71±0.34, $p<0.05$). It did not differ statistically significantly (1.54±1.14, $p>0.05$) from the level in cows 5–7 years old. The average level of vitamin D was 37.2±7.78 pmol/l. A lower amount was found only in heifers-in-calf (26.76±6.67, $p<0.05$), and significantly higher amount was found in cows 5-7 years old (46.9±4.22, $p<0.05$). No statistically significant difference ($p>0.05$) was observed when comparing with other groups of clinically healthy cows. The amount of vitamin D correlated with levels of calcium satisfactorily negatively ($r = – 0.423$) and with levels level of phosphorus weakly negatively ($r = – 0.317$).

Performin analysis of the blood serum of cows 8 years old and above, which received mineral supplements with fodder, during pasturable period, we found that the average level of calcium was in the lower range of the recommended norm (2.51±0.043 mmol/l) and it differed statistically significantly from the level found during winter period (1.88±0.27, $p<0.05$). The level of calcium was a little lower the recommended norm in half of investigated cows. The level of calcium measured during summer period was significantly lower than that of heifers-in-calf (2.85±0.21, $p<0.05$) and cows 2-4 years old (2.82±0.42, $p<0.05$), and did not differ statistically significantly (2.62±0.20, $p>0.05$) from the group of cows aged 5 to 7 years. The average level of phosphorus (1.73±0.19 mmol/l) was in the range of normal and was statistically significantly higher than this one during winter period (1.52±0.12, $p<0.05$). Statistically significantly higher level of phosphorus was found only for heifers-in-calf (2.01±0.10, $p<0.05$) during summer period. The level of magnesium did not amount to the recommended norm in half of investigated cows, however the difference was mild. The average level of magnesium was 0.81±0.13 mmol/l and it did not differ statistically significantly from the level

measured during winter period (0.85±0.10, $p>0.05$) and was significantly lower than corresponding level of other groups of cows investigated during summer period ($p<0.05$). The average level of parathyroid hormone was 3.64±0.59 pmol/l and that was the highest blood serum PTH level in healthy cows found during pasturable period, however it differed statistically significantly from heifers-in-calf (2.74±0.71, $p<0.05$) and cows 2–4 years old (2.62±0.58, $p<0.05$), and did not differ statistically significantly (3.58±0.67, $p>0.05$) from cows 5–7 years old. The amount of PTH correlated with CT strongly ($r = 0.728$), with vitamin D satisfactorily ($r = 0.415$) and with calcium strongly inversely ($r = – 0.364$). The level of calcitonin was 1.51±0.09 pmol/l in average, however there was no significant difference when compared with in-house period (1.46±0.00, $p>0.05$). The average level of vitamin D was 35.67±5,49 and it was statistically significantly higher than this one of other groups of clinically healthy cows investigated in summer ($p<0.05$), however t did not differ statistically significantly (37.2±7.78, $p>0.05$) when compared with this one found during winter period. The level of vitamin D correlated strongly with calcium ($r = – 0.691$) and phosphorus ($r = – 0.620$).

Table 2. **Blood serum indicators of high and low productivity clinically healthy cows fed with mineral supplements and without them**

	Blood serum indicators					
	PTH, pmol/l	CT, pmol/l	Ca, mmol/l	P, mmol/l	Mg, mmol/l	Vit. D, nmol/l
Clinically healthy cows of high productivity, which received mineral supplements						
M±m	3.43±0.38	1.6±0.28	1.98±0.30	1.63±0.26	1.04±0.13	39.5±6.74
Clinically healthy cows of high productivity, which did not receive mineral supplements						
M±m	5.85±0.97	1.5±0.14	1.79±0.16	1.47±0.12	0.98±0.15	43.3±9.41
Clinically healthy cows of low productivity, which received mineral supplements						
M±m	2.5±0.61	1.48±0.05	2.21±0.28	1.57±0.13	0.99±0.18	37.6±7.90
Clinically healthy cows of low productivity, which did not receive mineral supplements						
M±m	2.9±0.60	1.46±0.00	1.94±0.14	1.59±0.15	0.92±0.14	38.1±8.97

Hypocalcemia was revealed in all investigated cows during investigation of clinically healthy cows of high and low productivity during in-house period, fed with mineral supplements and without them. The highest hypocalcemia was found in most productive cows, which did not receive mineral supplements (1.79±0.16), the lowest found in cows of lowest productivity, which received mineral supplements (2.21±0.28). The level of calcium was dependent of productivity indicators of cows, because it correlated with amount of milk. That corresponds with findings of Klimienė (2001). The level of phosphorus was similar (in the rage of normal) in the blood serum of all investigated cows. A significant difference was

between high productivity cows fed with mineral supplements (1.63±0.26) and without them (1.47±0.12) ($p<0.05$). There was no statistically significant difference between other groups ($p>0.05$). The level of magnesium was in the range of normal in the blood serum of all investigated cows, however the highest amount of magnesium was found in the blood serum of productive cows, which received mineral supplements, but there was no significant difference ($p>0.05$). That did not correspond with data of Klimienė (2001) saying that a lower level of magnesium was found in cows of high productivity compared with cows of low productivity. The level of PTH among groups of cows varied from 2.5 to 5.85 pmol/l. The highest PTH amounts were found in most productive cows, which did not receive mineral supplements (5.85±0.97) and the lowest in low productivity cows, which received mineral supplements (2.5±0.61). We found that PTH levels in high productivity cows varied depending on feeding type (Table 2). Significantly higher PTH levels were found in the blood serum of cows, which did not receive mineral supplements (5.85±0.97, $p<0.05$) compared with those, which received them. Obtained findings correspond with reported data of Malz and Meyer (1993) and Axelsson (1991) 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. In case of high productivity healthy cows, which received mineral supplements, we found satisfactorily negative PTH correlation with calcium ($r = – 0.441$), strong correlation with vitamin D ($r = 0.666$) and very strong with CT ($r = 0.866$); and in case of cows, which did not receive mineral supplements, we found strong PTH correlation with calcium ($r = – 0.828$), satisfactory with vitamin D ($r = 0.559$) and calcitonin ($r = 0.599$). When doing analysis of PTH levels in the blood of low productivity cows (Table 2), we found that mineral supplements with fodder did not provide significant ($p>0.05$) difference in the analysed PTH indicators. That may be explained by the fact that low productivity cows use little amount of calcium for production of milk, the parathyroid gland functions in conditions of not increased physiological load and PTH activity increases thus very slightly in cows, which do not receive mineral supplements (Barnouin, Chassagne, 1994). We found strong PTH correlation with vitamin D ($r = 0.625$), satisfactory with calcitonin ($r = 0.572$) and strong negative with calcium ($r = – 0.774$) in the case of low productivity cows fed with mineral supplements. We determined strong PTH correlation with vitamin D ($r = 0.677$) and satisfactory negative with calcium ($r = – 0.539$) in the case of low productivity cows fed without mineral supplements. The level of calcitonin between the groups of cows varied from 1.46 to 1.6 pmol/l and did not differ statistically significantly ($p>0.05$). The average level of vitamin D between all four groups of cows varied from 37.6 to 43.3 nmol/l, however it did not differ statistically significantly ($p>0.05$). The highest amount of vitamin D was found in most productive cows, which did not receive mineral supplements (43.3±9.41), the lowest was found in low productivity cows, which received mineral supplements (37.6±7.90). In the case of productive cows, which did not receive mineral supplements, not only the highest level of vitamin D was found

(43.3±9.41), but also that of PTH (5.85±0.97), and the lowest calcium level too (1.79±0.16mmol/l). That corresponds with data of Bruder (2001) and other researchers that activity of vitamin D depends on blood calcium level, i.e. the lower is the amount of Ca, the higher is PTH level, which activates synthesis of vitamin D.

We found important changes of macronutrients and hormones by analyzing levels of macronutrients and calcitropic hormones in dry cows-in-calf, which were injected vitamin D (Romedat D_3 forte 1 ml (1 ml – 50 mg vit. D_3), Atarost, Germany) one week before calving. 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, PTH, CT and 25-OH vitamin D were observed. Measured values of macronutrients and hormones changed at similar intervals, independently of vitamin D injections. However after several days after calving the recorded levels of macronutrients and hormones differed (Figures 1–6).

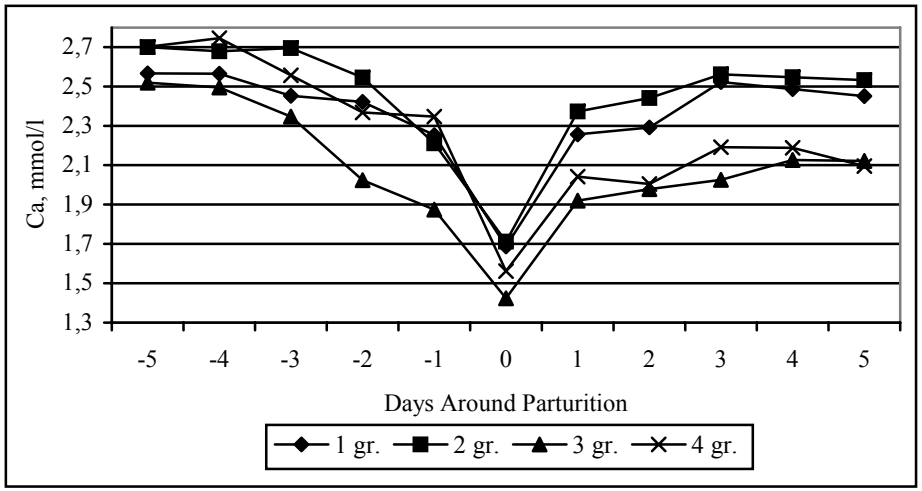

Figure 1. **Change of Calcium concentrations in the blood serum of cows**

The blood serum concentrations of phosphorus and calcium decreased significantly the day in all groups of cows ($p<0.05$), however the levels of calcium and phosphorus of the groups 1 and 2 found the fifth day after calving did not differ statistically significantly from valued observed 5 days before calving ($p>0.05$), and the levels of calcium and phosphorus found in the blood serum of cows of the groups 3 and 4 the 5th day after calving differed statistically significantly from levels present 5 days before calving ($p<0.05$).

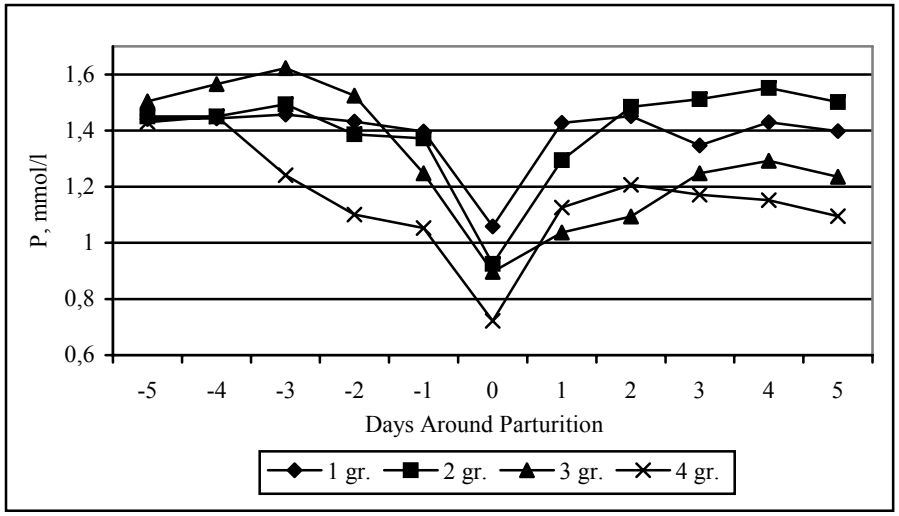

Figure 2. **Change of blood serum phosphorus concentration in cows**

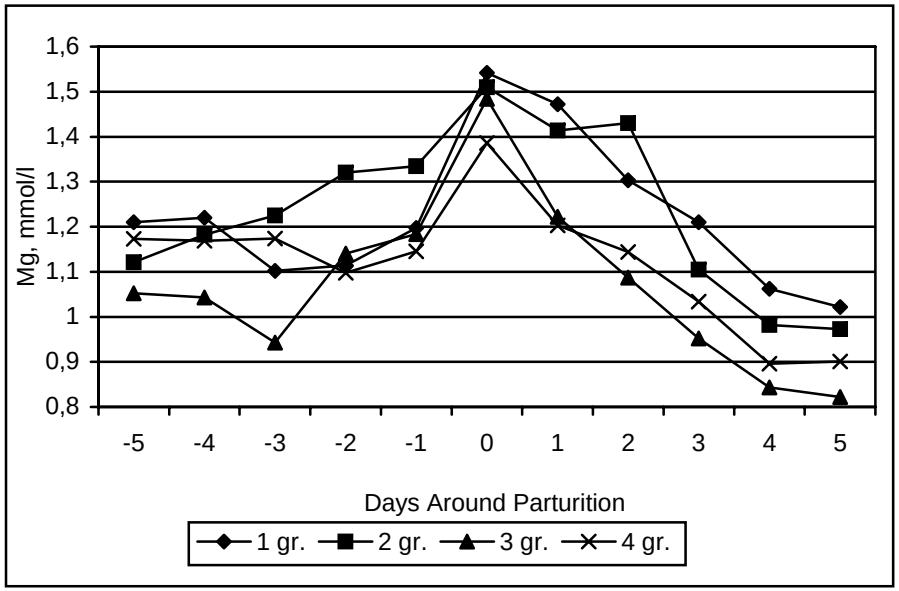

Figure 3. **Change of blood serum magnesium concentration in cows**

The decrease of blood calcium level before, during and after calving was reported also by other authors (Axelsson, 1991, Malz and Meyer, 1993, Goff and Horst, 1997). Also Riond et al. (1995) described that the levels of calcium and phosphorus decreased and the levels of magnesium increased after parturition.

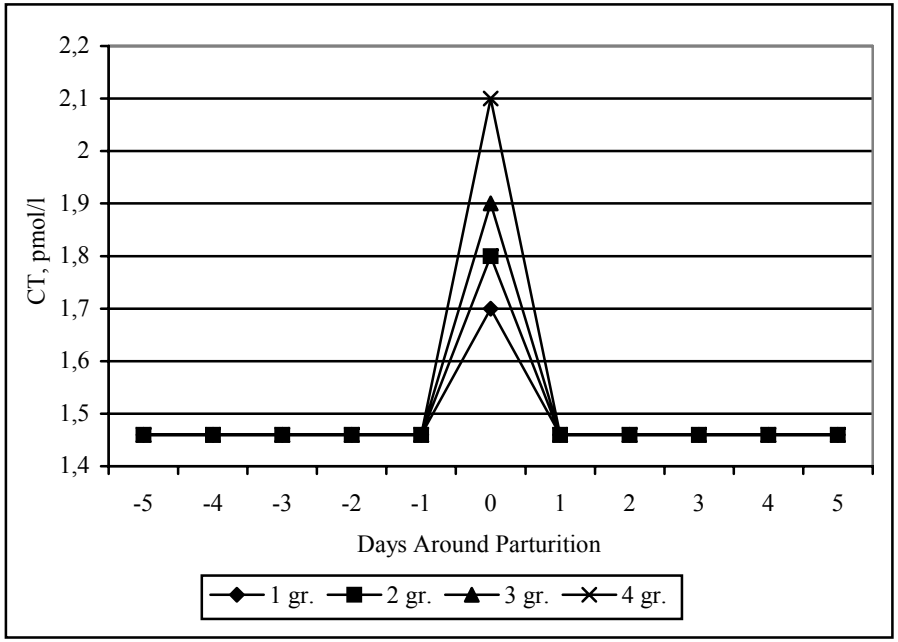

Figure 4. **Change of blood serum calcitonin concentration in cows**

Magnesium and calcium compete for fixation site in the cell. They are antagonists because ions of magnesium inhibit the passage of calcium ions into the cell. Depending on magnesium concentration, ions of magnesium replace ions of calcium on its membrane receptors and in the binding sites of enzymes (Niemack, 1985). Other authors (Schonewille, Klooster, 1994) found decreased blood level of calcium, phosphorus and magnesium only after parturition. According to Arney (1994), 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. When investigating effects of vitamin D on the levels of calcium and phosphorus, Zepperitz et al. (1994) 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. Besides, he indicates that different forms of this vitamin decrease the blood serum level of magnesium. According to reports of Beaudeau et al. (1994), Breves et al. (1995) 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 importantly 24 h after parturition and also increased little by little later depending on how often vitamin D was injected. According to Samanc et al. (1995), the level of hypocalcemia 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 did not restored 5 days after calving up to the level, which was 5 days before parturition.

We found increased level of calcitonin on the day of parturition, though the level of calcium was rather reduced. That contradicts the findings of Deftos (1997) that calcitonin is secreted constantly in conditions of normal concentration of calcium, however its secretion increases importantly by increase of the blood level of calcium. According to Barlet (1972) the level of calcitonin increases for protection of mother's skeleton from excessive loss of calcium and phosphorus.

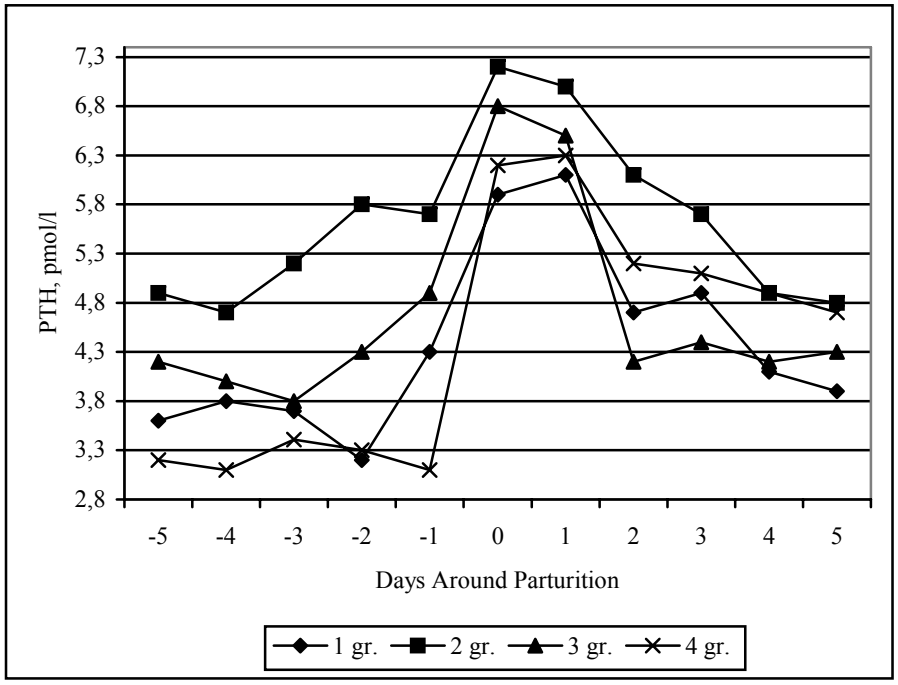

Figure 5. **Change of blood serum concentration of PTH**

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

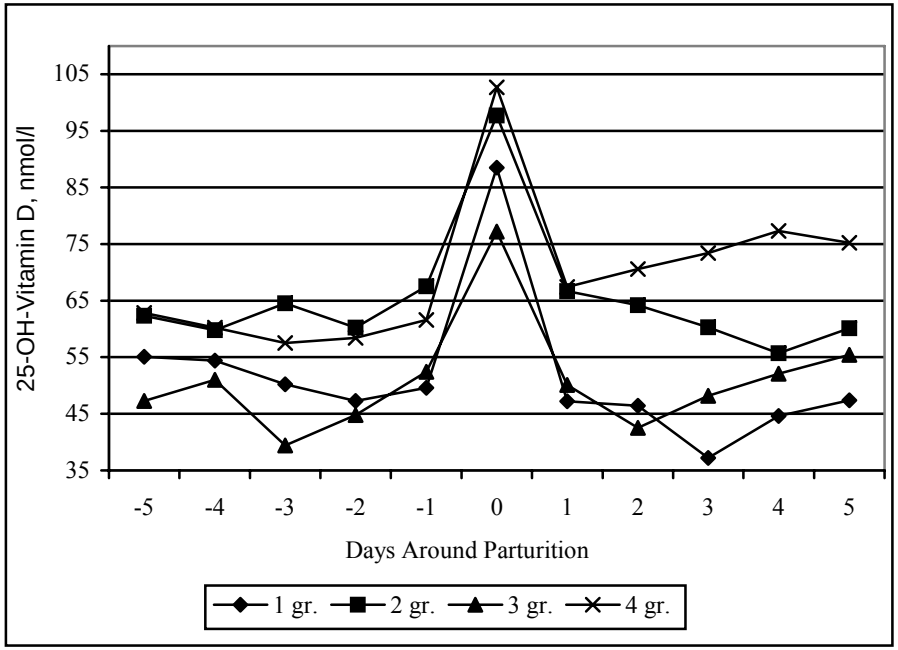

Figure 6. **Change of blood serum 25-OH vitamin D in cows**

The blood serum concentration of 25-OH vitamin D increased importantly ($p<0.05$) in all investigated cows on the day of parturition. It lowered much the first day after parturition until the former level observed before parturition. The blood serum concentration of vitamin D found the fifth day after parturition in groups 1, 2 and 3 did not differ statistically significantly from concentration found 5 days before parturition, however the blood serum concentration of vitamin D in the cow group 4 was statistically significantly higher ($p<0.05$). Measured blood serum levels of vitamin D of cows were dependent of vitamin D injections – we determined the highest amount of it in the blood of the control group of cows, which were not injected vitamin D. Injecting of vitamin D had impact on concentrations of calcium and phosphorus, because the levels of calcium and

phosphorus determined in the groups 1 and 2 the fifth day after parturition were close to those found 5 days before parturition; and concentrations of calcium and phosphorus did not differ statistically significantly from the control group in the case of cows whom vitamin D was injected only once. Based on our study it is possible to conclude that injecting of vitamin D has a big influence on blood serum levels of calcium and phosphorus and does not have greater impact on the level of magnesium. Vitamin D had impact on levels of PTH and CT as well because higher levels of PTH and CT were found in the control group whom vitamin D was not injected.

Hypocalcemia was found analysing blood levels of PTH, CT, vitamin D and macroelements Ca, P, Mg in clinically healthy cows-in-calf (Table 1), (2.32±0.18); and the levels of phosphorus (1.47±0.24) and magnesium (0.86±0.16) were in the range of normal. The level of calcium was statistically significantly lower than corresponding level in heifers-in-calf (2.62±0.37, $p<0.05$) and significantly higher than the level in cows 5-7 years old (1.97±0.24, $p<0.05$) and 8 years and above (1.88±0.27, $p<0.05$); and it did not differ statistically significantly (2.28±0.28, $p>0.05$) from cows 2-4 years old. Among the groups of clinically healthy cows, the level of phosphorus differed statistically significantly only from cows 2–4 years old (1.88±0.17, $p<0.05$). The blood level of magnesium was statistically significantly higher only in heifers-in-calf (1.08±0.13, $p<0.05$), it did not differ statistically significantly ($p>0.05$) from other groups of clinically healthy cows. PTH varied from 3.5 to 7.9 pmol/l and was 5.2±1.26 pmol/l in average. This was the highest PTH level found in the groups of clinically healthy cows, however it did not differ statistically significantly from heifers-in-calf (4.37±0.75, $p>0.05$); it was statistically significantly lower ($p<0.05$) in the blood serum of other groups of clinically healthy cows. It is supposed that requirement of calcium increase during growth of fetus and PTH activity increases also, therefore a higher amount of it is found (Malz, Mayer, 1993). When comparing dry cows-in-calf with heifers-in-calf, a lower blood PTH level was found in the latter (4.37±0.75 pmol/l), though there was no significant difference ($p>0.05$). Because cows aged 4–10 years were selected for the group of dry cows-in-calf, our data corresponded with statement of Rajala and Grohn (1998), that the levels of calcium decrease and PTH activity increases with age. The PTH level correlated with level of calcium strongly negatively ($r = – 0.726$) and with level of vitamin D very strongly ($r = – 0.917$). The level of calcitonin was 1.61±0.20 pmol/l in average and it did not differ statistically from other groups of clinically healthy cows except the group of cows 8 years old and above where it was statistically significantly lower (1.46±0.00, $p<0.05$). The level of vitamin D was similar to the level of other groups of clinically healthy cows, it was lower only in the case of heifers-in-calf, however no statistically significant difference was observed ($p>0.05$). Analogic findings were obtained also by S. H. Sharla in 1998. According to the statement of this researcher, findings of the study may be associated with age, because also the amount of 25-OH vitamin D increases in the course of life. Bruder and other researchers state that the activity of vitamin D depends on the blood level of

calcium (2001). According to our study this statement was supported, because levels of calcium and vitamin D found in the blood of dry cows-in-calf correlated importantly negatively one with another ($r = – 0.780$).

Found low values of macronutrients in the groups of cows with parturient paresis show that their blood serum levels depended on feeding type, but macroelements received with mineral supplement were not sufficient to maintain blood homeostasis in cows during critic time after parturition when large quantities of macroelements were excreted from blood to milk. Studies found that mineral supplements act on the status of mineral metabolism and have impact on levels of blood components (Baudet et al., 1996). However, it is not reported how they change the blood composition of cows with parturient paresis. According to Anot Horst et al. (1994), in the case of cows having parturient paresis, high levels of parathyroid hormone are found, as a rule. The literature indicates that the serum PTH level correlates with concentration of calcium most often inversely and, in presence of hyperthyroidism, the serum amount of calcium increases and this one of inorganic phosphorus decreases at the same time (Potts et al., 1995). According to our study, an important correlative dependence between PTH level and measured levels of calcium, phosphorus and magnesium was determined (correlation coefficient r varied respectively: – 0.023; 0.359; – 0.365). Low levels of calcium and phosphorus found during the study show incapacity of parathyroid gland to maintain homeostasis. These findings correspond to reports of other researchers that parathyroid gland in cows with parturient paresis, is incapable to maintain necessary levels of calcium and phosphorus though it secretes more PTH into the blood (Houe et al., 1999). Obtained findings correspond to reports of other researchers that low level of calcium stimulates secretion in parathyroid gland (Goff, Horst, 1997; Thilsing-Hansen et al., 2002). This is confirmed also by investigations of cows with parturient paresis, which did not receive mineral supplements. The blood PTH amount in these cows was higher ($p<0.05$) than that of cows having paresis, which received mineral supplement. Blood calcitonin levels in cows were decreasing with decreasing values of blood serum calcium. Also other researchers (Ciaramella et al., 2000) found low level of calcitonin when analysing parameters of mineral metabolism in the blood of buffalos. The blood level of vitamin D found in cows with paresis, which received mineral supplement, was 43.09±8.16 nmol/l, and that of cows, which did not receive mineral supplements, was 45.84±10.76 nmol/l. There was no statistically significant difference between these groups ($p>0.05$). However a significantly higher number was in the case of osteomalacia and mastitis ($p<0.05$) when comparing with other groups of cows during 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 significant difference ($p>0.05$) when compared with cows 5–7 years old and above. A higher blood level of vitamin D in the blood of sick cows may be explained by the fact that activity of vitamin 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 synthesis of vitamin D (Bruder et al., 2001).

Table 3. **Blood serum indicators in cows with diseases of mineral metabolism**

	Blood serum indicators					
	PTH, pmol/l	CT, pmol/l	Ca, mmol/l	P, mmol/l	Mg, mmol/l	Vit. D, nmol/l
Cows with parturient paresis fed with mineral supplements						
M±m	12.93±2.14	1.74±0.61	1.54±0.61	0.71±0.30	1.18±0.50	43.09±8.16
Cows with parturient paresis fed without mineral supplements						
M±m	18.31±6.18	1.46±0.02	1.38±0.45	0.65±0.23	1.22±0.34	45.84±10.76
Cows with osteomalacia						
M±m	3.95±0.60	1.55±0.19	1.86±0.18	0.88±0.19	0.73±0.11	33.97±11.39
Cows with mastitis						
M±m	3.97±0.50	1.83±0.49	2.28±0.20	1.56±0.12	1.08±0.20	23.72±7.19

The blood serum levels of calcium, phosphorus and magnesium in cows with osteomalacia were statistically significantly lower (compared with healthy cows) ($p<0.05$). The average amount of parathyroid hormone was 3.95±0.60 pmol/l, however there was no significant difference when compared with healthy cows and cows with mastitis ($p>0.05$). However it was significantly lower than that in cows with paresis ($p<0.05$). The literature indicates that serum concentration of PTH correlates inversely with concentration of calcium, i.e. low level of calcium stimulates secretion of parathyroid glands (Goff, Horst, 1997, Potts, 1997). According to our study, the level of PTH in cows with osteomalacia correlated with level of calcium inversely ($r = – 0.497$). Possibly, in the case of acute hypocalcemia, the glands produce PTH in response to the decreased blood level of calcium and the parathyroid glands are not in position to compensate changes of blood calcium level in the blood of sick cows in the case of chronic hypocalcemia (having osteomalacia). The measured level of phosphorus depended little on the level of PTH, because the coefficient of correlation was small ($r = 0.389$). Average level of vitamin D was 33.97±11.39 nmol/l, however it did not differ statistically significantly from clinically healthy cows, except for cows 5–7 years old, where its amount was statistically significantly higher (46.9±4.22, $p<0.05$). Compared to sick cows, its level was statistically significantly lower than this one in cows with paresis ($p<0.05$) and statistically significantly higher than this one in cows with mastitis ($p<0.05$). The level of vitamin D, found in the blood of cows with osteomalacia, correlated with level of calcium inversely ($r = – 0.650$).

When analysing the levels of calcitropic hormones, vitamin D and macronutrients Ca, P, Mg in cows with mastitis, we found that the blood serum level of calcium varied without exceeding the lower physiological norm (2.28±0.20), and the levels of phosphorus (1.56±0.12) and magnesium (1.08±0.20)

were in the range of normal. Comparing the level of PTH in the blood of cows with osteomalacia, mastitis and paresis, the blood level of PTH in cows having osteomalacia and mastitis did not differ statistically significantly ($p>0.05$); it was increased importantly only in cows with parturient paresis ($p<0.05$). The level of calcitonin in cows with mastitis was very close to the level of calcitonin found in healthy cows ($p>0.05$). According to the data in literature, the level of calcitonin depends directly on the blood serum level of calcium (Deftos, Gagel, 2000; Austin, Heath, 1981). Because the blood serum level of calcium found in cows with mastitis was close to the lower limit of physiological norm, the level of calcitonin also was not high and it was close to the level of calcitonin found in healthy cows. The level of vitamin D, found in the blood serum of cows with mastitis, correlated inversely with level of calcium ($r = – 0.752$) and phosphorus ($r = – 0.901$). It is possible to see from the correlative relations found by us how long-lasting hypocalcemia influenced synthesis of vitamin D for intensification of resorption of calcium and phosphorus from the intestinal tract. The level of PTH in cows with mastitis and osteomalacia was very similar (3.97±0.50 and 3.95±0.60 pmol/l respectively, $p>0.05$), however the level of vitamin D differed (23.72±7,19 and 33.97±11.39 nmol/l respectively, $p<0.05$). The blood level of phosphorus in cows with mastitis corresponded to the physiological norm and the level of calcium varied without exceeding the lower physiological norm. The results of our study corresponded with findings of Haussler and other researchers (1998), which showed that the level of vitamin D depended on blood concentrations of calcium and phosphorus, however compared with PTH, this interaction was rather weaker. The PTH levels correlated with levels of calcium weakly inversely ($r = – 0.303$).

CONCLUSIONS:

1. The blood serum level of PTH in healthy cows varied from 1.8 to 7.9 pmol/l, this one of CT – from 1.46 to 2.9 pmol/l, vit. D – from 18.1 to 56.4 nmol/l. The levels of PTH correlated with levels of calcium inversely and with levels of vitamin D and calcitonin positively; and the levels of vitamin D correlated with levels of calcium negatively.
2. The blood serum levels of PTH, CT and vitamin D in healthy cows changed depending on age: the blood serum level of PTH and vitamin D were statistically significantly lower and the levels of CT were higher in cows 2-4 years old compared with cows 8 years old and above.
3. The blood serum levels of PTH and CT in healthy cows changed depending on the physiological status: the measured levels of PTH and CT were statistically significantly higher in cows-in-calf and heifers-in-calf compared with cows of lactation period.
4. The blood serum levels of PTH, CT and vitamin D changed in healthy cows depending on the season of the year – statistically significantly higher levels of PTH and vitamin D and lower levels of CT were found during winter period compared with summer period.
5. The highest levels of PTH were found in the blood serum of the most

productive cows fed without mineral supplements (5.85±0.97 pmol/l), the lowest – in the blood serum of low productivity cows fed with mineral supplements (2.5±0.61 pmol/l). The levels of CT and vitamin D depended little on cow productivity and feeding.

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

7. The blood serum levels of PTH and vitamin D in cows with parturient paresis were statistically significantly higher compared with levels in the blood serum of healthy cows. A statistically significantly higher blood serum PTH level (18.31±2.14 pmol/l; $p<0.05$), and statistically significantly lower level of calcitonin (1.46±0.02 pmol/l) were found in cows with parturient paresis fed without mineral supplements in comparison with cows fed with mineral supplements (12.93±2.14 pmol/l and 1.74±0.61 pmol/l respectively, $p<0.05$). There was no correlative relation between calcium and PTH in the blood serum of cows with parturient paresis, although a strong negative correlation relation was found between calcium and vitamin D.

8. A statistically significantly lower blood serum levels of calcium, phosphorus and magnesium were found in cows with osteomalacia (compared with healthy cows), however the levels of PTH, CT and vitamin D did not differ statistically significantly. The level of PTH correlated with level of calcium satisfactorily negatively and with level of vitamin D strongly negatively.

9. The methods developed for humans (electrochemiluminescence analysis, chemiluminescence immunometric analysis, immunofermentic analysis) are suitable for determination of levels of PTH, CT and vitamin D in the blood serum of cattle.

SUGGESTIONS:

1. To use the method of electrochemical luminescence analysis for determination of the blood serum level of PTH in cattle, which is sensitive (analytical sensitivity of 0.127 pmol/l), cheap and fast (duration of analysis of 18 min.).

2. To administer preparations of vitamin D parenterally at least thrice during the period of 10 days before supposed calving for prevention of parturient paresis of dry cows-in-calf.

LIST OF PUBLICATIONS ON THE DISSERTATION TOPIC:

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2. Bandzaitė V., Klimienė I., Špakauskas V., Snarskytė A. Parathormono ir kalcitonino kiekio kaita sveikų ir sergančių pareze apsiveršiavusių karvių kraujo serume. Veterinarija ir zootechnika. T. 29 (51). 2005. P. 17-21.

3. Bandzaitė V., Klimienė I., Špakauskas V., Šilkūnaitė J. Parathormono, kalcitonino ir vitamino D įtaka mineralinių medžiagų kiekiui sveikų ir sergančių karvių kraujo serume. Veterinarija ir zootechnika. T. 31 (53). 2005. P. 5-9.

4. Bandzaitė V., Klimienė I., Špakauskas V., Matusevičius A. Interaction between the levels of hormones and minerals in sera of healthy and sick cows. Polish Journal of Veterinary Sciences. 2005. (An article for publication).

5. Бандзайте В., Снарските А., Шпакаускас В., Климене И. Изменение калъция и его регулирующих гормонов в сыворотке крови болъных коров. Международная научно-практическая конференция “Актуалъные проблемы ветеринарной медицины в условиях современного животноводства”. 2005. С. 24-28.

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REZIUMĖ

ĮVADAS

Vitamino D, prieskydinės liaukos hormono *parathormono* (PTH), skydliaukės hormono *kalcitonino* (CT) tiesioginis vaidmuo yra reguliuoti kalcio kiekį kraujyje (Capen, Martin, 1982, Enemark ir kt., 2003). Kraujo serumo jonizuotas kalcis išlaiko normalias nervų ir raumenų sistemų funkcijas. Kalcis būtinas normaliai širdies veiklai, be to, jis yra vienas pagrindinių jonų, reguliuojančių šarmų ir rūgščių balansą. Kalcis mažina membranų pralaidumą, audinių koloidų surišimo savybes, aktyvina fermentus – ATF-azę, lecitilazę, sukcinatdehidrogenazę, kasoje stabilizuoja tripsiną, stabdo enolazę ir dipeptidazę. Trūkstant organizme kalcio, sumažėja baltymų, ATF, eritrocitų, hemoglobino, padidėja ląstelių membranų pralaidumas natrio jonams (Уразаев ir kt., 1990). Be to, kalcis kaip ir fosforas su magniu yra skeleto mineralinis pagrindas. Kraujo serume jonizuoto kalcio kiekis yra labai pastovus. Pagrindinį vaidmenį kalcio apykaitos reguliacijoje atlieka *vitaminas D, parathormonas* ir *kalcitonin*as. *Vitaminas D* skatina kalcio ir fosforo absorbciją iš žarnų tuo pačiu palaikydamas vienodą šių elektrolitų lygį tarpląsteliniuose skysčiuose, kad sudarytų galimybę atitinkamai kaulų mineralizacijai (Omdall, DeLuca, 1973, Horst ir kt., 2003). *Vitaminas D* išsaugo pakankamą mineralinių jonų kiekį, garantuodamas kaulų mineralizaciją, tuo tarpu *parathormonas* išlaiko reikiamą kalcio ir fosforo santykį tarpląsteliniuose skysčiuose. Be to, maži *vitamino D* kiekiai reikalingi, kad *parathormoną* skatintų mobilizuoti kalcį iš skeleto rezervų į tarpląstelinius skysčius (“fakultatyvus efektas”) (Haussler, McCain, 1977). Svarbiausias biologinis *parathormono* vaidmuo – didinti kalcio, mažinti fosforo koncentracijas kraujyje, skatinti fosforo ekskreciją su šlapimu, didinti kalcio reabsorbciją kanalėliuose sumažėjus kalcio

kiekiui šlapime, didinti osteolizę ir osteoklastų kiekį kaulų paviršiuje, skatinti hidroksiprolino ekskreciją su šlapimu, tam tikrose ląstelėse aktyvuoti adenilatciklazę (Rasmussen, 1974). *Kalcitoninas* skatina kalcio kaupimąsi kauluose – aktyvina osteoblastų veiklą. Be to, jis veikia fosforo apykaitą organizme. Mažindamas kalcio kiekį kraujyje, *kalcitoninas* yra *parathormono*, kuris didina šio elemento kiekį kraujyje, antagonistas.

Šiuolaikinės ūkininkavimo sąlygos iš gyvulių augintojų reikalauja ūkiuose įgyvendinti intensyvias auginimo technologijas, nes būtina išvengti ekonominių nuostolių dėl įvairaus pobūdžio medžiagų apykaitos sutrikimų. Dabartiniu metu Lietuvoje kreipiamas nepakankamas dėmesys gyvulių neužkrečiamosioms ligoms, nors pagal ligų paplitimą ir ekonominius nuostolius medžiagų apykaitos ligos užima vieną pirmųjų vietų vidaus ligų patologijoje. Neturint pakankamai lėšų, ne visi galvijai vitaminizuojami, ne visur ir visuomet jie pakankamai gauna mineralinių papildų. Pastoviai ir kompleksiškai neatliekami gyvulių kraujo biocheminiai tyrimai, nėra kiekvienam gyvuliui kompiuterizuotos sveikatos būklės nustatymo sistemos kaip išvystytos gyvulininkystės šalyse. Kokie yra karvių mineralinių medžiagų rodikliai kraujo serume, šiandien sunku pasakyti, nes nėra veršingų karvių kraujo biocheminės kontrolės, netiriami medžiagų apykaitos procesai 15–20 dienų prieš veršiavimąsi. Neaišku, kokia padėtis atskiruose rajonuose ir atskirais metų laikais. Todėl šio darbo metu numatoma ištirti *vitamino D*, *kalcitonino, parathormono* bei makroelementų – kalcio, fosforo, magnio apykaitos būklę mūsų respublikos karvių bandose.

Darbo tikslas: Nustatyti *vitamino D, kalcitonino* bei *parathormono* kiekių kaitos ypatumus sveikų skirtingo šėrimo, amžiaus, produktyvumo, metų laiko ir sergančių pareze po veršiavimosi, osteomaliacija ir mastitu karvių kraujo serume.

Uždaviniai:

1. Skirtingais metų laikais ištirti įvairaus amžiaus, produktyvumo, fiziologinės būklės, šėrimo tipo karvių kraujo serume *vitamino D*, *kalcitonino, parathormono* kiekių kaitos ypatumus ir jų ryšį su kalcio, fosforo bei magnio kiekiais.
2. Nustatyti *vitamino D, kalcitonino* ir *parathormono* kiekių kaitos ypatumus ir jų ryšį su kalcio, fosforo bei magnio kiekiais pareze po veršiavimosi, osteomaliacija ir mastitu sergančių karvių kraujyje.
3. Ištirti vitamino D preparatų poveikį mineralinių medžiagų ir hormonų apykaitai sveikoms veršingoms karvėms.
4. Išbandyti žmonėms skirtų metodų (elektrocheminės liuminescencinės, chemiliuminescencinės imunometrinės ir imunofermentinės analizės) tinkamumą *PTH, CT ir 25-OH vitamino D* kiekiams nustatyti galvijų kraujo serume.

Darbo mokslinė ir praktinė reikšmė

Darbo metu tirti *vitamino D, kalcitonino* bei *parathormono* kiekių kaitos ypatumai sveikų skirtingo šėrimo, amžiaus, produktyvumo ir sergančių kai kuriomis medžiagų apykaitos ligomis karvių kraujo serume. Tirta, kaip kinta biocheminiai rodikliai karvėms sergant medžiagų apykaitos ligomis (pareze po veršiavimosi, osteomaliacija ir mastitu) ir nustatyti rodikliai (kalcio, fosforo, magnio kiekių), kurie informatyviausi šioms ligoms diagnozuoti. Gauti rezultatai

apdoroti statistiškai.

Buvo pritaikytas elektrocheminės liuminescencinės analizės metodas, naudojamas nustatant žmonėms PTH kiekį kraujo serume. Palyginus su radioimuniniu metodu, tai nebrangus ir gana greitas metodas, be to, šis metodas yra jautrus ir patikimas, nes galima aptikti žemus (iki 0,127 pmol/l) PTH kiekius kraujo serume. Buvo išbandytas chemiliuminescencinės imunometrinės analizės metodas, naudojamas nustatant *CT* kiekį žmonėms. Taip pat buvo išbandytas imunofermentinės analizės metodas (IFA) su žmogaus antikūnais matuoti 25-OH vitamino D kiekį galvijų kraujo serume.

TYRIMŲ MEDŽIAGA IR METODAI

Darbas atliktas 2002–2005 m. Lietuvos veterinarijos akademijos Veterinarijos instituto mikrobiologijos ir maisto saugos skyriaus gyvulių sveikatingumo ir epidemiologijos laboratorijoje, LVA VI virusologijos laboratorijoje, Kauno medicinos universiteto klinikų biochemijos laboratorijoje, Kauno, Kaišiadorių, Jurbarko rajonų individualių ūkių karvių fermose ir tvartuose.

Kraujas tyrimams imtas žiemos ir vasaros laikotarpiais iš Lietuvos juodmargių veislės karvių. Karvių grupės buvo suformuotos analogų principu, atsižvelgiant į amžių, sveikatos būklę, veršiavimosi laiką, produktyvumą, gaunamo raciono tipą. Prieš suformuojant grupes, bandomosios karvės ir telyčios buvo kliniškai ištirtos. Buvo stebima bendra gyvulio būklė, skaičiuojamas pulsas, matuojama temperatūra, skaičiuojami didžiojo prieskrandžio susitraukimai, stebimas gyvulio atrajojimas, diurezė, defekacija. Kliniškai sveikomis karvėmis laikytos tokios karvės, kurių aukščiau išvardinti rodikliai buvo normos ribose. Galvijai suskirstyti į grupes:

1. Veršingos kliniškai sveikos telyčios (n=20) (tvartiniu (n=10) ir ganykliniu laikotarpiais (n=10));
2. 2–4 metų amžiaus kliniškai sveikos karvės (n=10) (tvartiniu ir ganykliniu laikotarpiais);
3. 5–7 metų amžiaus kliniškai sveikos karvės (n=10) (tvartiniu ir ganykliniu laikotarpiais);
4. 8 metų amžiaus ir vyresnės kliniškai sveikos karvės (n=10) (tvartiniu ir ganykliniu laikotarpiais);
5. Didelio ir mažo produktyvumo kliniškai sveikos karvės, šeriamos su mineraliniais papildais ir be jų (n= 40);
6. Užtrūkusios kliniškai sveikos paskutinės veršingumo dekados karvės (n = 20) (Vit. D_3 naudojant kasdien po 1 ml likus 5 dienoms iki veršiavimosi (n =5), vit. D_3 naudojant 3 kartus po 1 ml likus 5 dienoms iki veršiavimosi (n = 5), vit. D_3 naudojant 1 kartą po 1 ml likus 5 dienoms iki veršiavimosi (n =5), kontrolinė grupė, kuriai vitaminas D_3 nešvirkštas (n = 5));
7. Užtrūkusios veršingos kliniškai sveikos karvės tvartiniu laikotarpiu (n=10);
8. Karvės,sergančios pareze po apsiveršiavimo (n=20) (šeriamos su mineraliniais papildais (n=10) ir be jų (n=10);
9. Karvės, sergančios osteomaliacija (n=12);
10. Karvės, sergančios mastitu (n=10).

1, 5, 7, 8, 9 ir 10 grupių galvijams kraujas iš jungo venos imtas 1 kartą. 2–4 grupių karvėms kraujas imtas du kartus (tvartiniu ir ganiavos laikotarpiais). 6 grupės karvėms kraujas imtas 11 kartų – 5 kartus iki veršiavimosi kiekvieną dieną, veršiavimosi dieną ir 5 kartus kasdien po veršiavimosi.

1–4, 6, 7, 9 ir 10 grupių karvės ir telyčios šertos su mineraliniais papildais. 5 ir 8 grupės karvės šertos ir su mineraliniais papildais, ir be jų. Žiemojimo periodu karvės šertos šienu, šiaudais, kombinuotais pašarais, silosu, šakniavaisiais (mitybos elementų koncentracija raciono 1 kg SM: NEL – 5,7 MJ; žaliųjų proteinų – 13%, ž. ląstelienos – 29%, ž. riebalų – 3,0%), mineraliniais papildais, ganiavos periodu gyvuliai buvo laisvai ganomi, gavo kombinuotuosius pašarus ir mineralinius papildus.

Karvių kraujas tyrimams imtas iš jungo venos į vienkartinius mėgintuvėlius "Venoject" (*Terumo Europe N. V.*, Belgija) be antikoagulianto. Pastarieji valandos bėgyje centrifūguoti 5 minutes 3 000 kartų per minutę apsisukimų greičiu. Atskirtas kraujo serumas dozatoriumi pipete nusiurbtas į Eppendorfo (*Eppendorf AG*, Vokietija) mėgintuvėlius su dangteliu. Mėgintuvėliai, užpildyti kraujo serumu, buvo šaldomi kameroje –20° C temperatūroje. Visus mėgintuvėlius su kraujo serumu vienu metu atšildžius, nustatyti 25-OH vitamino D, parathormono ir kalcitonino kiekiai, taip pat makroelementų Ca, P bei Mg kiekiai.

TYRIMŲ DUOMENYS IR JŲ APTARIMAS

Mūsų vykdomų bandymų metu tyrėme, kaip kinta vitaminoD, PTH, CT, Ca, P, Mg kiekiai sveikų ir sergančių karvių kraujo serume. Tvartiniu laikotarpiu ištyrus veršingų telyčių biocheminius kraujo serumo rodiklius, nustatyti atitinkantys fiziologinę normą arba jai artimi makroelementų kiekiai. Kalcio vidutiniškai buvo 2,62±0,37 mmol/l, fosforo – 1,51±0,22 mmol/l, magnio – 1,08±0,13 mmol/l. PTH kiekis svyravo nuo 3,5 iki 5,9 pmol/l (4,37±0,75 pmol/l) ir statistiškai patikimai skyrėsi nuo nustatyto 2–4 metų amžiaus karvių grupės kraujo serume ($p<0,05$), tačiau nuo nustatyto užtrūkusių veršingų karvių kraujo serume statistiškai patikimai nesiskyrė ($p>0,05$). Vidutinis kalcitonino kiekis buvo 1,75±0,38 pmol/l, tačiau statistiškai patikimai skyrėsi tik nuo 8 metų amžiaus ir vyresnių karvių grupės ($p<0,05$) ir nuo mažo produktyvumo sąlyginai sveikų karvių grupių, šeriamų tiek su mineraliniais papildais, tiek be jų ($p<0,05$). Vidutinis vitamino D kiekis buvo 26,76±6,67 nmol/l ir tai buvo mažiausias tvartiniu laikotarpiu nustatytas vitamino D kiekis ($p<0,05$) tarp visų tirtųjų kliniškai sveikų karvių grupių. Ganykliniu laikotarpiu ištyrus veršingų telyčių biocheminius kraujo serumo rodiklius, nustatyti atitinkantys fiziologinę normą kalcio kiekiai (2,85±0,21 mmol/l), padidėję ir fiziologinę normą atitinkantys fosforo (2,01±0,10 mmol/l) ir magnio (1,21±0,12 mmol/l) kiekiai. Parathormono kiekis kito nuo 2,0 iki 4,1 pmol/l (2,74±0,70 pmol/l) ir patikimai statistiškai skyrėsi nuo nustatyto tvartiniu laikotarpiu ($p<0,05$). Vidutinis kalcitonino kiekis buvo 2,14±0,56 pmol/l ir statistiškai patikimai nesiskyrė nuo nustatyto tvartiniu laikotarpiu ($p>0,05$). Vidutinis vitamino D kiekis buvo 25,4±2,94 nmol/l ir buvo labai artimas nustatytam žiemos laikotarpiu (26,76±6,67 nmol/l, $p>0,05$).

Tvartinio laikotarpio pabaigoje 2–4 metų amžiaus karvėms kraujo serume tirti kalcio, fosforo, magnio, PTH, CT, vitamino D kiekiai ir nustatyta, kad karvių kraujo serume vidutiniai fosforo (1,88±0,17 mmol/l) ir magnio (0,96±0,19 mmol/l) kiekiai buvo normos ribose. Kalcio kiekis rekomenduojamos normos ribose buvo nustatytas tik vienos karvės kraujo serume, kitų kraujo serume nustatyta nedidelė hipokalcemija, vidutiniškai kalcio buvo 2,28±0,28 mmol/l. PTH kito nuo 2,2 iki 5 pmol/l (3,46±0,75 pmol/l). Tai buvo mažiausias ir statistiškai patikimas ($p<0,05$) PTH kiekis iš visų kliniškai sveikų karvių grupių, tirtų tvartiniu laikotarpiu, išskyrus didelio produktyvumo karves, šeriamas su mineraliniais papildais(3,43±0,38 pmol/l, $p>0,05$) ir mažo produktyvumo karves, šeriamas tiek su mineraliniais papildais (2,5±0,61 pmol/l, $p>0,05$), tiek be jų (2,9±0,60 pmol/l, $p>0,05$). Vidutinis kalcitonino kiekis buvo 1,71±0,34 pmol/l ir statistiškai patikimai skyrėsi tik nuo 8 metų ir vyresnių karvių grupės tiek ganykliniu, tiek tvartiniu laikotarpiais ($p<0,05$) ir nuo mažo produktyvumo karvių grupių ($p<0,05$). Vidutinis vitamino D kiekis buvo 34,55±9,16 nmol/l ir buvo didesnis negu veršingų telyčių (26,76±6,67 nmol/l, $p<0,05$), tačiau mažesnis negu visų kitų tvartiniu laikotarpiu tirtų kliniškai sveikų karvių, bet patikimas skirtumas buvo tik su 5–7 metų karvėmis ($p<0,05$) ir su mažo produktyvumo karvėmis, negavusiomis mineralinių papildų ($p<0,05$), tarp kitų grupių patikimo skirtumo nebuvo ($p>0,05$). Ištyrus 2–4 metų amžiaus karves ganykliniu laikotarpiu, nustatyti atitinkantys fiziologinę normą arba jai artimi makroelementų kiekiai. Vidutinis kalcio kiekis buvo 2,82±0,42 mmol/l, fosforo – 1,89±0,18 mmol/l, magnio – 1,05±0,14 mmol/l. PTH kiekis kito nuo 1,9 iki 3,9 pmol/l ir vidutiniškai buvo 2,62±0,58 pmol/l, patikimai statistiškai skyrėsi nuo 8 metų ir vyresnių karvių grupės ($p<0,05$). Tarp žiemą ir vasarą nustatyto PTH kiekio patikimo skirtumo nebuvo ($p>0,05$). Vidutinis kalcitonino kiekis buvo 2,03±0,44 pmol/l ir statistiškai patikimai nesiskyrė nuo nustatyto žiemos laikotarpiu ($p>0,05$). Vitamino D kiekis kito nuo 19,6 iki 39,5 nmol/l (28,69±6,28 nmol/l) ir statistiškai patikimai nesiskyrė nuo nustatyto žiemos laikotarpiu (34,55±9,16 nmol/l, $p>0,05$) ir nuo kitų vasarą tirtų karvių grupių, išskyrus 8 metų ir vyresnes karves, kurių kraujo serume vitamino D kiekis buvo patikimai statistiškai didesnis (35,67±5,49 nmol/l, $p<0,05$).

Žiemos periodu tiriant 5–7 metų amžiaus karvių, gaunančių mineralinius papildus su pašarais, kraujo serumo rodiklius, visų karvių kraujo serume buvo nustatyta hipokalcemija (vidutinis kalcio kiekis buvo 1,97±0,24 mmol/l), kalcio kiekis patikimai statistiškai skyrėsi nuo veršingų telyčių (2,62±0,37 mmol/l, $p<0,05$) ir 2–4 metų amžiaus karvių (2,28±0,28 mmol/l, $p<0,05$), o nuo 8 metų amžiaus ir vyresnių karvių grupės patikimai statistiškai nesiskyrė (1,88±0,27 mmol/l, $p>0,05$). Vidutinis fosforo kiekis (1,46±0,25 mmol/l) buvo rekomenduojamos normos ribose, mažesnis buvo nustatytas 4 iš 10 tirtųjų karvių. Tvartiniu laikotarpiu nustatytas fosforo kiekis patikimai statistiškai skyrėsi tik nuo 2–4 metų amžiaus karvių grupės (1,88±0,17 mmol/l, $p<0,05$). Magnio vidutiniškai buvo 0,98±0,22 mmol/l. Vidutinis PTH kiekis buvo 4,42±0,57 pmol/l. Didesnis statistiškai patikimas PTH kiekis tvartiniu laikotarpiu kliniškai sveikų karvių kraujo serume nustatytas tik didelio produktyvumo karvėms, negavusioms mineralinių

papildų (5,85±0,97 pmol/l, p<0,05). Vidutinis kalcitonino kiekis buvo 1,54±1,14 pmol/l, patikimo skirtumo tarp kitų tvartiniu laikotarpiu tirtų karvių grupių nebuvo (p>0,05). Vitamino D vidutiniškai buvo 46,9±4,22 nmol/l ir tai buvo didžiausias statistiškai patikimas vitamino D kiekis nustatytas tvartiniu laikotarpiu kliniškai sveikų karvių grupių kraujo serume (p<0,05), išskyrus didelio produktyvumo negavusių mineralinių papildų karvių grupę, kur vitamino D kiekis buvo mažesnis, bet skirtumas buvo statistiškai nepatikimas (43,3±9,4 nmol/l, p>0,05). Ištyrus 5–7 metų amžiaus karvių kraujo serumą ganykliniu laikotarpiu, nustatyti atitinkantys fiziologinę normą ir statistiškai patikimai didesni nei žiemos laikotarpiu kalcio (2,62±0,20 mmol/l, p<0,05) ir fosforo (1,82±0,18 mmol/l) kiekiai. Patikimo skirtumo tarp žiemą (0,98±0,22 mmol/l) ir vasarą (0,98±0,12 mmol/l) nustatyto magnio kiekio nebuvo (p>0,05). Parathormono kiekis kito nuo 2,71 iki 4,8 pmol/l (vidutiniškai 3,58±0,67 pmol/l) ir buvo patikimai mažesnis už nustatytą tvartiniu laikotarpiu (4,42±0,57 pmol/l, p<0,05). Lyginant su kitomis sveikų karvių grupėmis ganykliniu laikotarpiu, buvo patikimai statistiškai didesnis negu veršingų telyčių (2,74±0,71 pmol/l, p<0,05) ir 2–4 metų amžiaus karvių (2,62±0,58 pmol/l, p<0,05), lyginant su 8 metų ir vyresnėmis karvėmis, patikimo skirtumo nebuvo (3,64±0,59 pmol/l, p>0,05). Vidutinis kalcitonino kiekis buvo 2,1±0,54 pmol/l ir buvo statistiškai patikimai didesnis nei žiemos laikotarpiu (1,54±1,14 pmol/l, p<0,05). Vidutinis vitamino D kiekis buvo 28,45±5,86 nmol/l ir buvo statistiškai patikimai mažesnis nei žiemą (46,9±4,22 nmol/l, p<0,05).

8 metų ir vyresnėms karvėms, gaunančioms mineralinius papildus, tvartinio laikotarpio pabaigoje ištyrus kraujo serumo rodiklius, nustatyta hipokalcemija (vidutiniškai kalcio buvo 1,88±0,27 mmol/l). Vidutiniai fosforo (1,52±0,12 mmol/l) ir magnio (0,85±0,10 mmol/l) kiekiai buvo rekomenduojamos normos ribose. PTH kiekis kito nuo 3,4 iki 6,1 pmol/l (vidutiniškai 4,37±0,82 pmol/l). Patikimai mažesnis PTH kiekis nustatytas tik 2–4 metų amžiaus karvių grupės kraujo serume (3,46±0,75 pmol/l, p<0,05). Kalcitonino buvo mažiau nei 1,46 pmol/l ir patikimai statistiškai skyrėsi nuo veršingų karvių (1,61±0,20 pmol/l, p<0,05) ir telyčių (1,75±0,38 pmol/l, p<0,05) ir 2–4 metų amžiaus karvių (1,71±0,34 pmol/l, p<0,05). Nuo 5–7 metų amžiaus karvių patikimai statistiškai nesiskyrė (1,54±1,14 pmol/l, p>0,05). Vidutinis vitamino D kiekis buvo 37,2±7,78 nmol/l. Ganiavos periodo pabaigoje ištyrus 8 metų amžiaus ir vyresnių karvių, su pašaru gaunančių mineralinius papildus, kraujo serumą, nustatėme, kad vidutinis kalcio kiekis buvo rekomenduojamos fiziologinės normos žemutinėse ribose (2,51±0,04 mmol/l) ir patikimai statistiškai skyrėsi nuo nustatyto žiemos laikotarpiu (1,88±0,27 mmol/l, p<0,05). Vidutinis fosforo kiekis (1,73±0,19 mmol/l) buvo normos ribose ir buvo patikimai statistiškai didesnis nei žiemos laikotarpiu (1,52±0,12 mmol/l, p<0,05). Vidutinis magnio kiekis buvo 0,81±0,13 mmol/l ir patikimai statistiškai nesiskyrė nuo nustatyto žiemos laikotarpiu (0,85±0,10 mmol/l, p>0,05). Vidutinis parathormono kiekis buvo 3,64±0,59 pmol/l ir tai buvo didžiausias PTH kiekis sveikų karvių grupių kraujo serume, nustatytas ganykliniu laikotarpiu, tačiau patikimai statistiškai skyrėsi nuo veršingų telyčių (2,74±0,71 pmol/l, p<0,05) ir nuo 2–4 metų amžiaus karvių (2,62±0,58 pmol/l, p<0,05), o nuo 5–7 metų amžiaus

karvių patikimai statistiškai nesiskyrė (3,58±0,67 pmol/l, p>0,05). Kalcitonino vidutiniškai buvo 1,51±0,09 pmol/l, tačiau patikimo skirtumo palyginus su tvartiniu laikotarpiu nebuvo (1,46±0,00 pmol/l, p>0,05). Vidutinis vitamino D kiekis buvo 35,67±5,49 nmol/l ir palyginus su nustatytu žiemos laikotarpiu, patikimai statistiškai nesiskyrė (37,2±7,78 nmol/l, p>0,05), bet buvo patikimai statistiškai didesnis negu kitų kliniškai sveikų vasarą tirtų karvių (p<0,05).

Ištyrus didelio ir mažo produktyvumo kliniškai sveikų karvių, šeriamų su mineraliniais papildais ir be jų kraujo serumą, visoms tirtoms karvėms nustatyta hipokalcemija. Didžiausia hipokalcemija nustatyta produktyvioms karvėms, negavusioms mineralinių papildų (1,79±0,16 mmol/l), mažiausia – mažo produktyvumo karvėms, gavusioms mineralinius papildus (2,21±0,28 mmol/l). Fosforo kiekis visų tirtų karvių kraujo serume buvo normos ribose. Patikimas statistinis skirtumas buvo tarp didelio produktyvumo karvių, šeriamų su mineraliniais papildais (1,63±0,26 mmol/l) ir be mineralinių papildų (1,47±0,12 mmol/l), p<0,05. Tarp kitų grupių statistiškai patikimo skirtumo nebuvo (p>0,05). Magnio kiekis visų tirtų karvių kraujo serume buvo normos ribose, tačiau daugiausia magnio nustatyta produktyvių karvių kraujo serume, kurios gavo mineralinius papildus, tačiau patikimo skirtumo su kitomis grupėmis nebuvo (p>0,05). PTH kiekių vidurkis tarp karvių grupių svyravo nuo 2,5 iki 5,85 pmol/l. Daugiausiai PTH nustatyta produktyvioms karvėms, kurios negauna mineralinių papildų (5,85±0,97 pmol/l), mažiausiai – mažo produktyvumo karvėms, gaunančioms mineralinius papildus (2,5±0,61 pmol/l). Nustatėme, kad didelio produktyvumo karvių PTH kiekiai kinta priklausomai nuo šėrimo tipo. Negaunančių mineralinių papildų karvių kraujo serume nustatomi žymiai didesni PTH kiekiai (5,85±0,97 pmol/l, p<0,05), lyginant su gaunančiomis. Ištyrus mažo produktyvumo karvių kraujo serume PTH kiekius, nustatėme, kad mineralinis papildas su pašaru, patikimo (p>0,05) skirtumo tiriamuose PTH rodikliuose neduoda. Kalcitonino kiekis svyravo nuo 1,46 iki 1,6 pmol/l ir patikimai statistiškai nesiskyrė (p>0,05). Vidutinis vitamino D kiekis tarp visų keturių karvių grupių svyravo nuo 37,6 iki 43,3 nmol/l ir patikimai statistiškai nesiskyrė (p>0,05). Daugiausia vitamino D nustatyta produktyvioms karvėms, negavusioms mineralinių papildų (43,3±9,41 nmol/l), mažiausiai – mažo produktyvumo karvėms, gavusioms mineralinius papildus (37,6±7,90 nmol/l). Produktyvioms karvėms, negavusioms mineralinių papildų nustatytas didžiausias ne tik vitamino D kiekis (43,3±9,41 nmol/l), bet ir PTH (5,85±0,97 pmol/l), taip pat mažiausias kalcio kiekis (1,79±0,16 mmol/l).

Tvartinio laikotarpio pabaigoje, balandžio – gegužės mėnesiais buvo atliktas tyrimas. Buvo sudarytos 4 kliniškai sveikų užtrūkusių karvių grupės po 5 karves. Likus 5 dienoms iki numatomo veršiavimosi, 1–3 grupės karvėms tam tikrais intervalais švirkštas vitamino D preparatas “Romedat D_3 forte” po 1 ml (1 ml – 50 mg vit. D_3) (*Atarost*, Vokietija). 1 grupei leistas kiekvieną dieną (5 kartus), 2 grupei – tris kartus, 3 grupei – vieną kartą. Ketvirtoji grupė buvo kontrolinė. Kraujas tyrimams imtas 11 kartų – 5 kartus iki veršiavimosi kiekvieną dieną, veršiavimosi dieną ir po veršiavimosi 5 dienas iš eilės. Kraujas imtas prieš vitamino D

sušvirkštimą. Kraujo serume nustatėme makroelementų Ca, P, Mg, bei kalcitropinių hormonų – PTH, CT ir vitamino D kiekius. Veršiavimosi dieną kalcio koncentracija sumažėjo visų 4 tirtų karvių grupių kraujo serume ($p<0,05$). Penktą dieną po veršiavimosi 1 ir 2 grupių kraujo serume nustatytas kalcio kiekis patikimai statistiškai nesiskyrė nuo nustatyto 5 dienas prieš veršiavimąsi ($p>0,05$). 3 ir 4 grupės karvių kraujo serume nustatytas kalcio kiekis 5 dieną po veršiavimosi patikimai statistiškai skyrėsi nuo nustatyto 5 dienas iki veršiavimosi ($p<0,05$). Fosforo koncentracija veršiavimosi dieną sumažėjo visų karvių grupių kraujo serume ($p<0,05$). Penktą dieną po veršiavimosi 1 ir 2 grupių kraujo serume nustatytas fosforo kiekis patikimai statistiškai nesiskyrė nuo nustatyto 5 dienas prieš veršiavimąsi ($p>0,05$). 3 ir 4 grupės karvių kraujo serume nustatytas fosforo kiekis 5 dieną po veršiavimosi patikimai statistiškai skyrėsi nuo nustatyto 5 dienas iki veršiavimosi ($p<0,05$). Veršiavimosi dieną nustatytas magnio koncentracijos padidėjimas visų karvių grupių kraujo serume patikimai statistiškai skyrėsi nuo nustatytos magnio koncentracijos 5 dieną prieš veršiavimąsi ($p<0,05$). Po veršiavimosi tiriant magnio koncentraciją kraujyje, pastebėtas magnio koncentracijos mažėjimas. Penktą dieną po veršiavimosi nustatytas magnio kiekis visose karvių grupėse buvo patikimai statistiškai mažesnis už nustatytą 5 dienas prieš veršiavimąsi ($p<0,05$). Veršiavimosi dieną nustatytas PTH kiekis visų karvių grupių kraujo serume buvo patikimai statistiškai didesnis už nustatytą 5 dienas prieš veršiavimąsi ($p<0,05$). Pirmą dieną po veršiavimosi PTH kiekis išliko aukštas, tačiau patikimo skirtumo veršiavimosi dieną ir dieną po veršiavimosi nei vienoje grupėje nebuvo ($p>0,05$). Penktą dieną po veršiavimosi nustatytas PTH kiekis 1, 2 ir 3 grupių kraujo serume patikimai statistiškai nesiskyrė nuo nustatyto 5 dieną prieš veršiavimąsi ($p>0,05$), o 4 grupės kraujo serume buvo patikimai statistiškai didesnis ($p<0,05$). Kalcitonino kiekis tirtų karvių kraujo serume buvo mažas ir daugeliu atvejų aparatu nenustatomas, nes analizatoriaus IMMULITE techninėje charakteristikoje nurodoma, kad žemesnių verčių kaip 1,46 pmol/l jis nenustato. Tačiau iš gautų duomenų matome, kad veršiavimosi dieną kalcitonino koncentracija ženkliai padidėjo visose tirtose karvių grupėse ($p<0,05$). Lyginant 2 ir 3 grupę, patikimo skirtumo nebuvo ($p>0,05$), tačiau lyginant 1 grupę su 3 ir 4 grupėmis, pastebėtas statistiškai patikimas skirtumas ($p>0,05$). Veršiavimosi dieną 25-OH vitamino D koncentracija visų tirtų karvių kraujo serume ženkliai padidėjo ($p<0,05$). Pirmąją dieną po veršiavimosi ženkliai sumažėjo iki buvusio prieš veršiavimąsi lygio. Penktąją dieną po veršiavimosi 1, 2 ir 3 grupių kraujo serume vitamino D koncentracija statistiškai patikimai nesiskyrė nuo nustatytos 5 dienas prieš veršiavimąsi, tačiau 4 grupės karvių kraujo serume vitamino D koncentracija buvo patikimai statistiškai didesnė ($p<0,05$).

Ištyrus10 kliniškai sveikų užtrūkusių veršingų 4–10 m. amžiaus karvių kraujo serumą užtrūkimo pradžioje, nustatyta hipokalcemija (2,32±0,18 mmol/l), o fosforo (1,47±0,24 mmol/l) ir magnio (0,86±0,16 mmol/l) kiekiai buvo normos ribose. PTH kito nuo 3,5 iki 7,9 pmol/l ir vidutiniškai buvo 5,2±1,26 pmol/l. Tai buvo didžiausias PTH kiekis, nustatytas kliniškai sveikų karvių grupių kraujo serume, tačiau patikimai statistiškai nesiskyrė nuo veršingų telyčių (4,37±0,75 pmol/l,

$p>0,05$), kitų sąlyginai sveikų karvių grupių kraujo serume buvo patikimai statistiškai mažesnis ($p<0,05$). Kalcitonino kiekis vidutiniškai buvo 1,61±0,20 pmol/l ir statistiškai nesiskyrė nuo kitų kliniškai sveikų karvių grupių, išskyrus 8 metų ir vyresnių karvių grupę, kur buvo patikimai statistiškai mažesnis (1,46±0,00 pmol/l, $p<0,05$). Vitamino D kiekis buvo panašus į nustatytą kitų kliniškai sveikų karvių grupių kraujo serume, mažesnis buvo tik veršingų telyčių kraujyje, tačiau statistiškai patikimo skirtumo nebuvo ($p>0,05$).

Analizuojant sergančių pareze po veršiavimosi karvių kraujo serumo biocheminius rodiklius, pastebėti ryškūs tiriamų hormonų ir makroelementų kiekių pokyčiai. Mūsų tirtų pareze po veršiavimosi sirgusių karvių, šertų pašarais su mineraliniais papildais, kraujo serumo PTH buvo daug didesnis palyginti su sveikų ir vidutiniškai buvo 12,93±2,14 pmol/l ($p<0,05$). Vidutiniai kalcio (1,54±0,61 mmol/l) ir fosforo (0,71±0,29 mmol/l) kiekiai buvo patikimai statistiškai mažesni nei kliniškai sveikų karvių ($p<0,05$). Gavusių mineralinį papildą pareze sergančių karvių kraujo serume kalcio ir fosforo nustatėme daugiau nei pareze sergančių ir negavusių mineralinio papildo. Gavusių papildą pareze po veršiavimosi sergančių karvių kraujo serume kalcio buvo 10,4 %, fosforo 8,5 % daugiau nei negavusių, tačiau jų kiekis išliko mažas. PTH kiekis kito nuo 9,7 pmol/l iki 15,9 pmol/l (vidutiniškai 12,93±2,14 pmol/l). Vitamino D vidutiniškai buvo 43,09±8,16 nmol/l. Pareze po veršiavimosi sergančių užtrūkimo metu negavusių mineralinių papildų karvių kraujo serume nustatėme patikimai mažesnius kalcio ir fosforo kiekius nei kliniškai sveikų karvių, $p<0,05$. Kraujo serumo PTH buvo daug didesnis palyginti su sveikų ($p<0,05$) ir vidutiniškai buvo 18,31±6,18 pmol/l. PTH kiekis kito nuo 11,2 pmol/l iki 29,2 pmol/l. Kalcitonino kiekis šios grupės karvėms buvo mažas ir daugeliu atveju aparatu nenustatomas, nes analizatorius IMMULITE techninėje charakteristikoje nurodoma, kad žemesnių verčių kaip 1,46 pmol/l jis nenustato. Tačiau iš gautų duomenų galime spręsti, kad kalcitonino sergančių pareze ir negavusių mineralinio papildo karvių kraujyje buvo labai mažai. Tyrimo rezultatai rodo, kad mineralinis papildas su pašaru didina makroelementų kiekį tiek sveikoms, tiek sergančioms pareze karvėms ir skatina PTH išsiskyrimą. Papildas teigiamai veikia homeostazę, nors su preparatu gauto kalcio nepakako palaikyti reikiamo jo kiekio kraujo serume po veršiavimosi. Vitamino D vidutiniškai buvo 45,84±10,76 nmol/l.

Osteomaliacija sergančių karvių kraujo serume buvo statistiškai patikimai mažesni (lyginant su sveikomis karvėmis) kalcio, fosforo, magnio kiekiai ($p<0,05$). Parathormono vidutiniškai buvo 3,95±0,60 pmol/l, tačiau palyginus su sveikomis ir mastitu sergančiomis karvėmis, patikimo skirtumo nebuvo ($p>0,05$). Tačiau buvo patikimai mažiau negu pareze sergančių karvių ($p<0,05$). Kalcitonino vidutiniškai buvo 1,55±0,19 pmol/l, tačiau patikimo skirtumo su sveikomis karvių grupėmis nebuvo ($p>0,05$). Vidutinis vitamino D kiekis buvo 33,97±11,39 nmol/l, tačiau nuo kliniškai sveikų karvių patikimai statistiškai nesiskyrė, išskyrus 5–7 metų amžiaus karves, kur buvo patikimai statistiškai daugiau (46,9±4,22 nmol/l, $p<0,05$). Palyginus su sergančiomis karvėmis, buvo patikimai statistiškai mažiau negu sergančių pareze ($p<0,05$) ir patikimai statistiškai daugiau, negu sergančių mastitu

(p<0,05).

Tirdami kalcitropinių hormonų, vitamino D ir makroelementų Ca, P, Mg kiekį mastitu sergančių karvių kraujyje, nustatėme, kad kalcio kiekis kraujo serume kito neviršydamas žemutinės fiziologinės normos (2,28±0,20 mmol/l), o fosforo (1,56±0,12 mmol/l) bei magnio (1,08±0,20 mmol/l) kiekiai buvo normos ribose. PTH kiekis svyravo nuo 2,9 iki 4,62 pmol/l ir vidutiniškai buvo 3,97±0,50 pmol/l. Lyginant PTH kiekį osteomaliacija, mastitu ir pareze sergančių karvių kraujyje, sergančių osteomaliacija ir mastitu karvių kraujyje PTH kiekis patikimai statistiškai nesiskyrė (p>0,05), žymiai padidėjęs buvo tik pareze po veršiavimosi sergančių karvių kraujo serume (p<0,05). Mastitu sergančių karvių kraujo serume kalcitonino kiekis (1,83±0,49 pmol/l) buvo labai artimas sveikų karvių kraujyje nustatytam kalcitonino kiekiui (p>0,05). Vidutinis vitamino D kiekis buvo 23,72±7,19 nmol/l ir buvo patikimai statistiškai mažesnis negu sergančių osteomaliacija ir pareze po apsiveršiavimo (p<0,05). Lyginant su kliniškai sveikomis karvėmis tvartiniu laikotarpiu, buvo patikimai statistiškai mažesnis (p<0,05), išskyrus veršingas telyčias, nuo kurių patikimai statistiškai nesiskyrė (p>0,05).

IŠVADOS:

1. Sveikų karvių kraujo serume PTH kiekis kito nuo 1,8 iki 7,9 pmol/l, CT – nuo 1,46 iki 2,9 pmol/l, vit. D – nuo 18,1 iki 56,4 nmol/l. PTH kiekiai neigiamai koreliavo su kalcio kiekiais ir teigiamai – su vitamino D ir kalcitonino kiekiais, o vitamino D kiekiai neigiamai koreliavo su kalcio kiekiais.

2. PTH, CT ir vitamino D kiekiai sveikų karvių kraujo serume kito priklausomai nuo amžiaus – 2–4 metų amžiaus karvių kraujo serume nustatyti patikimai statistiškai mažesni PTH, vitamino D ir didesni CT kiekiai, lyginant su 8 metų ir vyresnėmis karvėmis.

3. PTH ir CT kiekiai sveikų karvių kraujo serume kito priklausomai nuo fiziologinės būklės – veršingų karvių ir telyčių kraujo serume nustatyti patikimai statistiškai didesni PTH ir CT kiekiai, lyginant su karvėmis laktacijos laikotarpiu.

4. PTH, CT ir vitamino D kiekiai sveikų karvių kraujo serume kito priklausomai nuo metų laiko – žiemos laikotarpiu nustatyti patikimai statistiškai didesni PTH, vitamino D ir mažesni CT kiekiai, lyginant su vasaros laikotarpiu.

5. Didžiausi PTH kiekiai nustatyti produktyvių be mineralinių papildų šeriamų karvių kraujo serume (5,85±0,97 pmol/l), mažiausi – mažo produktyvumo su mineraliniais papildais šeriamų karvių kraujo serume (2,5±0,61 pmol/l). CT ir vitamino D kiekiai mažai priklausė nuo karvių produktyvumo ir šėrimo.

6. Vitamino D preparatai (švirkščiant 3–5 kartus) likus kelioms dienoms iki veršiavimosi efektyviai veikia kalcio, fosforo, PTH ir vitamino D kiekius. Penktą dieną po veršiavimosi, karvėms, kurioms vitaminas D buvo švirkštas 5 ir 3 kartus, kalcio, fosforo, PTH ir vitamino D kiekiai patikimai statistiškai nesiskyrė nuo nustatytų 5 dienas prieš veršiavimąsi.

7. Pareze po veršiavimosi sergančių karvių kraujo serume nustatyti patikimai statistiškai didesni PTH ir vitamino D kiekiai nei sveikų karvių kraujo serume. Pareze po veršiavimosi sergančių karvių šertų pašarais be mineralinių papildų

kraujo serume nustatytas patikimai statistiškai didesnis PTH kiekis (18,31±2,14 pmol/l; p<0,05) ir patikimai statistiškai mažesnis kalcitonino kiekis (1,46±0,02 pmol/l), negu karvių, šertų su mineraliniais papildais (atitinkamai, 12,93±2,14 pmol/l, 1,74±0,61 pmol/l, p<0,05). Pareze sergančių karvių kraujo serume koreliacinės priklausomybės tarp kalcio ir PTH nėra, tačiau tarp kalcio ir vitamino D nustatytas stiprus neigiamas koreliacinis ryšys.

8. Osteomaliacija sergančių karvių kraujo serume buvo patikimai statistiškai mažesni (lyginant su sveikomis karvėmis) kalcio, fosforo ir magnio kiekiai, tačiau PTH, CT ir vitamino D kiekiai patikimai statistiškai nesiskyrė. PTH vidutiniškai neigiamai koreliavo su kalcio ir stipriai neigiamai – su vitamino D kiekiais.

9. Žmonėms skirti metodai (elektrocheminės liuminescencinės, chemiliuminescencinės imunometrinės, imunofermentinės analizės) tinka nustatyti PTH, CT ir vitamino D kiekius galvijų kraujo serume.

PASIŪLYMAI:

1. PTH kiekiui galvijų kraujo serume nustatyti naudoti elektrocheminės liuminescencinės analizės metodą, kuris yra jautrus (analizinis jautrumas 0,127 pmol/l), pigus ir greitas (analizės trukmė – 18 min.).

2. Užtrūkusių veršingų karvių parezės po apsiveršiavimo profilaktikai, likus 10 dienų iki numatomo veršiavimosi, vitamino D preparatus vartoti parenteraliai mažiausiai tris kartus.

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