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Correlation of Different Biochemical Parameters in Blood Sera of Healthy and Sick Cows

I. Klimienė[1]*, V. Špakauskas[1] and A. Matusevičius[2]
[1]*Lithuanian Veterinary Academy Veterinary Institute, Instituto g. 2, LT-4230 Kaišiadorys;* [2]*Lithuanian Veterinary Academy, Kaunas, Lithuania*
**Correspondence: klimienei@yahoo.com*

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ABSTRACT

Metabolic diseases of cows represent the leading internal pathology in Lithuania in terms of incidence and economic impact. This paper summarizes the mineral metabolic state of milk cows, and details the influence of feeding on serum levels of calcium, nonorganic phosphorus, magnesium, potassium, sodium, glucose, total protein, and urea quantity, and correlationd among them, in healthy dry or post-calving cows as well as in cows with osteomalacia and milk fever after calving. There was less pronounced hypocalcaemia and there were only minor changes in phosphorus, magnesium, potassium and sodium in the serum of healthy dry and post-calving cows that had silage and mineral-vitamin supplements, compared to cows that did not have supplements and silage. There was a fall in calcium and phosphorus (to 1.89 ± 0.12 mmol/L and 0.71 ± 0.06 mmol/L, respectively) in the blood of cows sick with milk fever after calving, while levels of magnesium and potassium were increased. The correlation between calcium and phosphorus was $r = 0.6993$, $p < 0.001$ in the serum of sick cows. There was a fall in calcium, phosphorus and magnesium (to 1.86 ± 0.46 mmol/L, 0.75 ± 0.37 mmol/L, and 0.60 ± 0.19 mmol/L, respectively) and an increase in sodium level (to 158.90 ± 19.30 mmol/L) in the blood of cows with osteomalacia in comparison with healthy cows.

Keywords: cows, macroelements, milk fever, osteomalacia

Abbreviations: NEL, net energy for lactation

INTRODUCTION

Calcium, phosphorus and magnesium ions play very important role in the metabolism of animal body tissues. Animals are often affected by disorders of mineral metabolism when the balance between intake and excretion of macronutrients is disturbed. The requirements for normal levels of mineral elements for agricultural animals differ in different countries. These requirements depend not only on the breed of animal, climatic conditions, stocking intensity and mode of digestion, but also on unknown factors that might ameliorate and/or inhibit incorporation of these elements (Correa *et al.*, 1993; Ebbesvik, 1993). The incidence and economic impact of metabolic diseases are a leading factor in internal pathology of cows in Lithuania. Approximately 19 000 cattle per year are affected by metabolic diseases in Lithuania.

Some authors report that up to 20% of herds of cows were identified having hypocalcaemia and hypomagnesaemia, which may manifest before calving, during calving or up to 48 h after calving (Coe, 1993; Fenwick, 1994; Kocabagli *et al.*, 1995; Riond *et al.*, 1995). Japanese researchers (Yamagishi *et al.*, 1995) have reported that among cows that did not recover after calving, hypocalcaemia was observed during the dry period in the majority cases. Other authors (Ellison, 1994) have linked this non-recovering syndrome with hypomagnesaemia, which may also cause milk fever after calving. On the other hand, some authors (Chandler, 1997; Goff and Horst, 1998) claim that rather than calcium, phosphorus or magnesium, it is potassium and sodium levels in the ration that play the key role in the aetiology of milk fever According to their data, a higher level of sodium in feedstuff increases the risk of milk fever, whereas the level of potassium in feedstuff is inversely proportional to the calcium level in serum. It has been experimentally established (Marten, 1995) that the levels of minerals in feedstuffs influence the levels of calcium, phosphorus, magnesium, sodium and potassium in serum. High levels of potassium and sodium in feedstuffs causes metabolic alkalosis and affects calcium homeostasis in dry cows, whereas calcium from feedstuffs cannot prevent milk fever. There is an opinion (Ewy and Lutz, 1997; Golf and Horst, 1997) that milk fever mostly affects cows whose diet includes 3.1% potassium and 0.5% calcium. It is seen that opinions differ significantly regarding the influence of the serum levels of macronutrients on the incidence of metabolic diseases of mineral nature in cows.

The aim of this work was to evaluate the levels of calcium, phosphorus, magnesium, potassium, sodium, glucose, urea and general proteins, and correlation between these parameters, in the serum of healthy dry cows, cows after calving, and cows suffering from osteomalacia and milk fever, in relation to different types of feedstuffs received.

MATERIALS AND METHODS

Experiments were carried out during the housing period using 5 to 12-year-old cows (dry and after calving) of Lithuanian black-and-white breed from holdings (group A) and farms (group B) with different feeding regimens. Cows in group A received a ration containing herbage 85.5%, mangel 4.7%, and barley flour 9.9%. Cows in group B received a ration containing herbage 70.8%, mangel 14.2%, and barley flour 15.0%. Based on analogue's principle and according to calving details, type of feeding and state of health, six trial groups of cows were formed (Table I). Diagnoses of milk fever and osteomalacia were made on the basis of results of clinical examination of animals and biochemical blood analysis.

Blood samples from the cows in experimental groups I and III were taken 15 days before expected calving; in groups II and IV a week after calving; and in groups V and VI before treatment. Samples were taken from the jugular vein and placed into containers (Terumo Europe N.V., Leuven, Belgium) without anticoagulant. Separated serum was centrifuged. Biochemical blood indices were analysed by semiautomatic biochemical analyser (Clin Check Plus Hospitex Diagnostics, Firenze, Italy) using Hospitex diagnostic reagents. Indices of blood serum analysed were the levels of calcium, phosphorus, magnesium, potassium, sodium, glucose, urea and total proteins.

TABLE I
Groups of cows according to calving date and feeding type

Group of cows	No. of animals	Type of group	Feeding type Nutritional element concentrations in 1 kg dry material
I[a]	10	Pregnant dry cows in group A[b]	NEL 5.7 MJ; green proteins 13%; crude fibre 29%; green fat 3.0%; calcium 0.74%; phosphorus 0.38%; magnesium 0.32%; Ca:P = 1.95:1; Ca:Mg = 2.35:1
II[a]	10	Cows after normal calving in group A	NEL 6.6 MJ; green proteins 15.5%; crude fibre 23.4%; green fat 2.7%; calcium 0.59%; phosphorus 0.39%; magnesium 0.26%; Ca:P = 1.5:1; Ca:Mg = 2.26:1
III	10	Pregnant dry cows in group B	NEL 5.9 MJ; green proteins 8.2%; crude fibre 25.04%; green fat 2.0%; calcium 0.44%; phosphorus 0.25%; magnesium 0.25%; Ca:P = 1.75:1; Ca:Mg = 1.75:1
IV	10	Cows after normal calving in group B	NEL 5.6 MJ; green proteins 7.8%; crude fibre 26.3%; green fat 2.0%; calcium 0.47%; phosphorus 0.25%; magnesium 0.26%; Ca:P = 1.88:1; Ca:Mg = 1.80:1
V	10	Cows suffering from osteomalacia	NEL 5.6 MJ; green proteins 7.8%; crude fibre 26.3%; green fat 2.0%; calcium 0.47%; phosphorus 0.25%; magnesium 0.26%; Ca:P = 1.88:1; Ca:Mg = 1.80:1
VI	14	Cows suffering from milk fever	NEL 5.6 MJ; green proteins 7.8%; crude fibre 26.3%; green fat 2.0%; calcium 0.47%; phosphorus 0.25%; magnesium 0.26%; Ca:P = 1.88:1; Ca:Mg = 1.80:1

[a]Cows (groups I and II) took 100–150 g/daily mineral and vitamin supplement containing 100 g: vitamin A 5000 UI, vitamin D_3 10 000 UI, vitamin E 100 mg, Ca 20.2 g, P 8.5 g, Na 5.8 g, Mg 3 g, Mn 0.08 g, Fe 0.09 g, Cu 0.025 g, Zn 0.075 g, I 6.83 mg, Co 2.1 mg, Se 2.26 mg

[b]Group A, cows from holdings; group B, cows from farms

The results were computed using Sigma Plot and Microsoft Excel'97 programs. Student's *t*-test was used for the estimation of the reliability criterion (p) on data differences among the groups. Differences were considered to be statistically reliable at $p < 0.05$. Correlation between related variables was estimated using Pearson's correlation matrices.

RESULTS

Calcium levels varied between 2.37 and 2.42 mmol/L (2.39 ± 0.025) in the serum of healthy (groups I–III) dry cows and cows after calving during housing period (Table II). Lower serum levels of calcium were found in cows fed without cereal grass silage after calving (group IV, 1.85 ± 0.38 mmol/L), cows suffering from osteomalacia (group V, 1.86 ± 0.46 mmol/L) and cows suffering from milk fever (group VI, 1.89 ± 0.46 mmol/L). The level of calcium varied from 0.55 to 2.35 mmol/L in the serum of individual cows suffering from milk fever. The decrease of serum calcium level is statistically significant in both cows suffering from osteomalacia (group V, $p < 0.05$) and cows suffering from milk fever (group VI, $p < 0.001$). The average serum level of phosphorus in cows from groups I and II, was higher compared to other groups ($p < 0.001$; Table II). The lowest levels of phosphorus were found in the serum of cows suffering from osteomalacia (0.75 ± 0.37 mmol/L) and milk fever (0.71 ± 0.24 mmol/L, $p < 0.001$) which represent 51.5–48.9% of the physiological norm (Marten, 1995).

The average level of magnesium was within physiological limits and did not differ in the serum of healthy dry pregnant cows (groups I and III) and cows after calving (group II), whereas it was lower (0.68 ± 0.29 mmol/L, $p < 0.05$) in the serum of cows from group IV and correlated with the level of sodium ($r = 0.7243$, $p < 0.001$). Even lower magnesium (0.60 ± 0.18 mmol/L, $p < 0.001$)) was found in the serum of cows suffering from osteomalacia (group V). Such a level of magnesium is 73.1% of the physiological norm and correlated with the serum level of phosphorus ($r = 0.8467$, $p < 0.001$) and potassium ($r = 0.6681$, $p < 0.001$). The level of magnesium exceeded the physiological norm by 17% in the serum of cows suffering from milk fever ($p < 0.05$).

The average level of potassium was close to physiological norm (Marten, 1995) in the serum of cows from groups I and II. Levels of potassium were statistically significantly increased in the serum of cows from group III (6.10 ± 0.24 mmol/L, $p < 0.05$) and group IV (5.33 ± 0.11 mmol/L, $p < 0.05$) compared to groups I and II. The serum level of potassium correlated with the level of magnesium ($r = 0.9385$, $p < 0.05$) in cows from group II. The level of potassium correlated with the level of calcium in cows from group IV ($r = 0.9607$, $p < 0.05$). Potassium level increased up to 5.17 ± 0.91 mmol/L, ($p < 0.05$) and correlated inversely with the level of magnesium ($r = 0.6681$) in the serum of cows suffering from osteomalacia compared to groups I and II. The average serum level of potassium was normal (4.15 ± 0.62 mmol/L) in cows suffering from milk fever, although in six cows it did not reach the physiological norm (Marten, 1995).

TABLE II
Biochemical indices in the serum of cows

Index and physiological norm[b]	Groups[a] I (n = 10)	II (n = 10)	III (n = 10)	IV (n = 10)	V (n = 10)	VI (n = 14)
Calcium (2.5–3.0 mmol/L)	2.42 ± 0.182	2.37 ± 0.418	2.40 ± 0.347	1.85 ± 0.388**	1.86 ± 0.464*	1.89 ± 0.468**
Phosphorus (1.4–2.0 mmol/L)	2.04 ± 0.417	2.09 ± 0.674	1.11 ± 0.470**	1.13 ± 0.660**	0.75 ± 0.370**	0.71 ± 0.240
Magnesium (0.7–1.0 mmol/L)	0.93 ± 0.133	0.99 ± 0.191	0.83 ± 0.184	0.68 ± 0.297	0.60 ± 0.185**	1.44 ± 0.609*
Potassium (3.5–4.5 mmol/L)	3.92 ± 0.102	4.06 ± 0.163	6.10 ± 0.244**	5.33 ± 0.109*	5.17 ± 0.917*	4.15 ± 0.625
Sodium (135–157 mmol/L)	139.0 ± 2.09	132.6 ± 2.49	143.0 ± 2.89	153.3 ± 6.75	158.9 ± 19.33*	168.5 ± 4.67**
Total proteins (60–89 g/L)	77.6 ± 7.39	75.6 ± 8.59	76.5 ± 9.03	68.8 ± 10.52	65.8 ± 8.28**	74.3 ± 8.77
Urea (3.6–8.2 g/L)	4.32 ± 0.97	4.68 ± 0.97	4.80 ± 0.89	4.60 ± 1.18	4.16 ± 0.99	4.33 ± 0.84
Glucose (2.4–4.4 g/L)	1.55 ± 0.32	1.05 ± 0.53*	1.48 ± 0.65	1.39 ± 0.52	2.01 ± 0.91*	2.43 ± 0.80

Group I, pregnant dry cows of group A; group II, cows after normal calving of group A; group III, pregnant dry cows of group B; group IV, cows after normal calving of group B; group V, cows suffering from osteomalacia; group VI, cows suffering from milk fever

[a]Group A, cows from holdings; group B, cows from farms

[b]According to Marten (1995)

*$p < 0.05$; **$p < 0.01$ compared to healthy cows

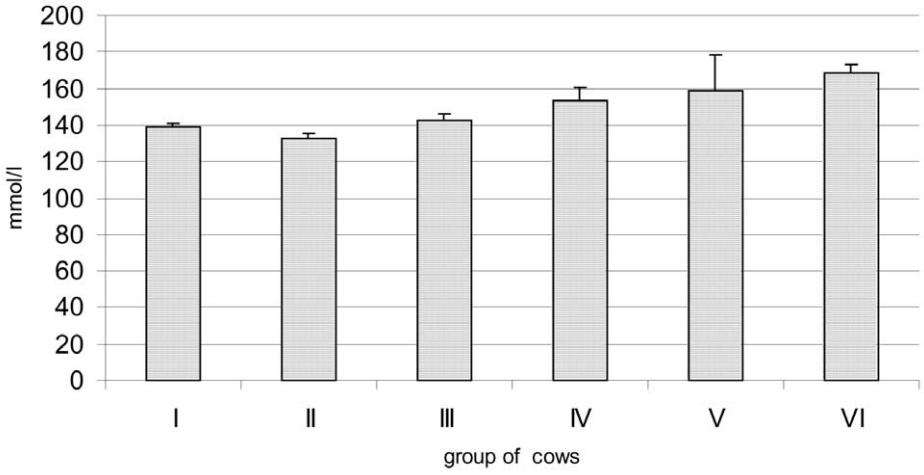

Figure 1. Changes of levels (+SD) of sodium in the serum of healthy and sick cows (group I, pregnant dry cows of group A; group II, cows after normal calving of group A; group III, pregnant dry cows of group B; group IV, cows after normal calving of group B; group V, cows suffering from osteomalacia; group VI, cows suffering from milk fever)

The serum level of sodium was within the physiological limits (Figure 1) in healthy cows (groups I–IV); it exceeded the physiological norm by 7.3–13.8%, ($p < 0.001$) in sick cows.

The level of general proteins was within physiological limits (Table II) and did not differ in the serum of cows from groups I–III; it was lower than the recommended norm (on average 68.88 ± 10.52 g/L) in the serum of cows from group IV and decreased to 65.80 ± 8.28 g/L ($p < 0.001$) in cows suffering from osteomalacia, which is 91.3% of the physiological norm ((Marten, 1995).

The level of urea, the final product of protein metabolism, was within normal physiological limits in the serum of cows in all groups. It was influenced mildly by the levels of calcium ($p < 0.05$), phosphorus ($r = 0.4644$, $p < 0.05$), magnesium ($r = 0.7834$, $p < 0.001$), potassium ($r = 0.5050$, $p < 0.05$) and sodium ($r = 0.7197$, $p < 0.05$) in the serum. The level of glucose decreased in the serum of healthy cows (groups I–IV). The lowest level of glucose (1.05 ± 0.53 mmol/L) was found in the serum of cows from group II ($p < 0.05$). Glucose level was within the physiological norm or 1.5–2.3 times higher in the serum of sick cows comparing with healthy ones ($p < 0.05$).

DISCUSSION

The levels of minerals in feedstuffs of cows influence the serum levels of calcium, phosphorus, magnesium, sodium and potassium, alteration or deficiency of which result in milk fever and osteomalacia. Milder hypocalcaemia, as well as milder alteration of the serum phosphorus, magnesium, sodium and potassium levels were detected in dry cows and cows after calving fed with corn silage and mineral additives. The results concur with the conclusions of others (Kocabagli *et al.*, 1995; Marten, 1995; Yamagishi *et al.*, 1995) that hypocalcaemia and hypomagnesaemia appear in pregnant cows before calving. However, contrary to the literature data, we did not find hypermagnesaemia in such cows. An increased level of magnesium was detected in the serum of 71.42% of cows suffering from milk fever. The ratio of calcium and phosphorus was increased (2.60–2.65:1) in the serum of all groups of cows; the ratio of calcium and magnesium decreased (2.6:1), which means that the feedstuffs used were not properly balanced regarding minerals. Large amounts of minerals are used for the growth of the fetus and later in the lactation period for the production of milk. Hypocalcaemia was less significant in dry cows or cows after calving that were fed with corn silage containing mineral additives, whereas the levels of calcium and phosphorus did not attain the physiological norms in cows fed with unbalanced feedtuffs.

The level of calcium, phosphorus and magnesium statistically decreased in the serum of cows suffering from osteomalacia, compared to healthy cows (1.86 ± 0.46 mmol/L, 0.75 ± 0.37 mmol/L, 0.60 ± 0.18 mmol/L, respectively); the level of sodium increased (to 158.9 ± 19.13 mmol/L). Increased levels of potassium (to 5.17 ± 0.91 mmol/L) correlated inversely with magnesium level in serum ($r = 0.6681$). According to the literature data (Fenwick, 1994; Kocabagli *et al.*, 1995; Riond *et al.*, 1995) calcium levels decreased to 1.9 mmol/L and nonorganic phosphorus levels to 1.2 mmol/L in the serum of cows suffering from milk fever. The results of our investigation show a statistically significant decrease of calcium and phosphorus (to 1.89 ± 0.46 mmol/L and 0.71 ± 0.24 mmol/L, respectively) and increase of magnesium and sodium levels in the serum of cows suffering from milk fever. The correlation index between calcium and phosphorus level is 0.6993 in the serum of such cows. The same conclusion was drawn by Marten (1995), who considers that the composition of feedstuffs influences the levels of calcium, phosphorus, magnesium, sodium and potassium in the serum. Thus, alteration of proportions or deficiency of these macronutrients can lead to milk fever and osteomalacia.

According to our data, higher levels of potassium were detected in the serum of cows of groups III and IV and cows suffering from osteomalacia ($p < 0.05$).

Normal levels of potassium (on average 4.15 ± 0.62 mmol/L) were found in the serum of cows suffering from milk fever. These data do not conform to some reports (Distl *et al.*, 1989; Chandler, 1997) that potassium levels in the serum and feedstuffs influence milk fever in cows.

Our results are in agreement with data (Scholz, 1990; Leonard, 1992) showing that prolonged deficiency of calcium (osteomalacia) leads to the disorder of cell membrane permeability, which results in elimination of potassium from cells to the blood. According to our results, there is no reason to suggest that the increased levels of

potassium and sodium in the blood serum of cows may serve as a cause for milk fever or osteomalacia. This conclusion does not conform to the opinions of other authors (Golf and Horst, 1997, 1998; Ewy and Lutz, 1997; Grohn *et al.*, 1998). In our opinion, the alteration of sodium and potassium levels (compared to physiological norms) in the serum of sick cows might be the result of hypocalcaemia, hypophosphataemia and hypomagnesaemia due to the antagonistic origins of relevant macronutrients, which is supported by the estimated correlation between these macronutrients in the serum.

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