LITHUANIAN VETERINARY ACADEMY

Danielius Starevičius

THE STUDY OF THE PROPHYLACTIC EFFECTIVENESS OF "KALCIFOSTILIS", AN ORALLY ADMINISTRATED PREPARATION IN PREVENTING HYPOCALCEMIA AND HYPOPHOSPHATEMIA IN DAIRY COW

Summary of doctoral dissertation
Biomedical sciences, veterinary medicine (12B)

Kaunas, 2009

The doctoral dissertation was arranged in 2005-2009 in Lithuanian Veterinary Academy

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Public defence of doctoral dissertation in Veterinary Science Council will take place at the Lithuanian Veterinary Academy I auditorium on 2nd of October 2009-06-22

Address: Tilžės str. 18, 47181 Kaunas, Lithuania.

The summary of the doctoral dissertation was sent on 2nd October 2009 according to the confirmed address list. The doctoral dissertation is available at the libraries of the Lithuanian Veterinary Academy and LVA Veterinary Institute.

LIETUVOS VETERINARIJOS AKADEMIJA

Danielius Starevičius

KARVIŲ HIPOKALCEMIJOS IR HIPOFOSFATEMIJOS PROFILAKTIKOS VEIKSMINGUMO, NAUDOJANT PERORALINĮ PREPARATĄ KALCIFOSTILĮ, TYRIMAI

Daktaro disertacijos santrauka

Biomedicinos mokslai, veterinarinė medicina (12B)

KAUNAS, 2009

Disertacija rengta 2005-2009 metais Lietuvos Veterinarijos Akademijoje.

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Disertacija bus ginama viešame Veterinarinės medicinos mokslo krypties tarybos posėdyje 2009 m. spalio 2 d. Lietuvos veterinarijos akademijos I auditorijoje.

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Disertacijos santrauka išsiuntinėta pagal patvirtinta adresų sąrašą 2009 spalio 2 d.

Disertaciją galima peržiūrėti Lietuvos veterinarijos akademijos ir LVA Veterinarijos instituto bibliotekose.

INTRODUCTION

Over the last forty years there had been a two-fold increase in a cow's milk yield due to purposeful selection. This resulted in the reduction of a cow's lifespan, as well as the reduced resistance to metabolic diseases (Ilahi et al. 2004; Oltenacu et al. 2005). The majority of metabolic diseases appear after calving. There is a change in the animal's hormonal balance, slowing down of dry matter intake from the digestive tract and the intensification of colostrum synthesis during calving. All those reasons determine the sudden reduction of calcium and phosphorus in the blood, whereas the cow's body does not have enough time to utilise the endogenous reserves (Goff, 2005; Kurosaki, 2007). Due to the lack of calcium and phosphorus in the body, the cells' membrane permeability changes and the muscle can not contract. Up to 10% of cows suffer from paresis during and after calving. Clinical hypocalcaemia usually causes the reduction of phosphorus, therefore more than 20% of cows suffering from paresis following calving, develop a downer cow syndrome (Menard-Thompson, 2007). Subclinical hypocalcaemia is difficult to diagnose, however it is found in more than 50% of cows, which are more than 5 years old, following calving (Reinhart et al., 2004). Cows suffering from clinical and subclinical hypocalcaemia, as well as hypophosphatemia, are more likely to develop mastitis, abomasum displacement and ketosis in the future.

Being aware of hypocalcaemia and hypophosphatemia's course mechanism and manifestation period presents opportunities to choose more flexible preventive tactics and to stop the reduction of calcium and phosphorus in the blood during calving. Moreover, with the presence of a variable amount of minerals in the blood, it would be correct to assume that preventive tactics should depend on the critical mineral reduction time during calving.

Even though there has been a fair share of research carried out abroad trying to establish the causes and the time course of hypocalcaemia and hypophosphatemia, nevertheless it is worth noting, that researchers still haven't completely established the pathogenesis of the diseases and their preventive measures (Klimienė, 2001; Thilsing-Hansen, 2002; Bushinsky, 2003; Reinhart et al., 2004; Goff, 2005; Kurosaki, 2007; Menard–Thompson, 2007). When giving mineral supplements in liquid or solid form to the cow during pregnancy, and especially during the last weeks of pregnancy, the increased amount of calcium and phosphorus, absorbed from the digestive tract, inhibits the absorption of said minerals from bones, which affects the metabolism postpartum (Agger et al., 1997).

In Lithuania's dairy cattle farms biochemical blood tests are rarely carried when cows are calving or at the beginning of a lactation period, therefore it is difficult to make a judgement on the prevalence

of subclinical hypocalcaemia and hypophosphatemia.

Presently, hypocalcaemia and hypophosphatemia's preventive measures, employed by the majority of cattle farms, are limited to giving dry cows an increased amount of phosphorus in feeds. During calving a cow's appetite diminishes, which causes a decrease in food consumption, also the aforementioned supplements are poorly absorbed. With scientific developments, mineral mixtures, which vary in composition and drug form and intended as a preventive measure, are constantly created to be easy to administer per os, to absorb quickly from the digestive tract and to replenish macroelements in the blood. We believe that the use of inexpensive orally administered mineral preparations in Lithuania would be effective, keeping in mind that productive cows sometimes develop parturition paresis due to the sudden reduction in the amount of calcium and inorganic phosphorus in the blood. Even though there is a great number of preparations, also varying in their composition, on offer to treat hypocalcaemia and hypophosphatemia, not all of them are effective and easy to use as a preventive measure. Therefore we believe it would be useful to develop an orally administered preparation, which would be effective as a preventive measure for metabolic disorders in cows, as well as develop its usage pattern.

To sum up, it is important to know the critical point in time when the amount of minerals in the blood of the cow falls below the physiological standard, and at which point the optimum result can be achieved when administering preparations orally.

Task and purpose of the study

Establishing the change dynamics of certain macroelements, glucose and parathormone in the blood of a pregnant cow. Developing a preparation to be used as a preventive measure for metabolic disorders in cows, and establishing its effectiveness based on the results of a biochemical and clinical study.

Goals:

1. Establishing the change dynamics of calcium, inorganic phosphorus, magnesium, glucose and parathormone in the blood of a cow during pregnancy, during calving and postpartum.
2. Establishing the change in the amounts of macroelements (Ca, Pn, Mg) in the blood serum of a cow after oral administration of *Kalcifostilis.*
3. Examining therapeutic and prophylactic effectiveness of *Kalcifostilis* to prevent hypocalcaemia and hypophosphatemia in cows.
4. Establishing prophylactic effectiveness of *Kalcifostilis* in cows that previously suffered from parturition paresis, brought on by clinical hypocalcaemia and hypophosphatemia.

Novelty and practical use

Kalcifostilis, an orally administered preparation for the prevention of mineral metabolism disorders in cows, was developed by the Experimental and Clinical Pharmacology Laboratory of the Lithuanian Veterinary Academy.

We researched the change in the amounts of calcium, inorganic phosphorus, magnesium, glucose and parathormone in the blood serum of a cow during pregnancy, during calving and postpartum. We also established those change parameters in the blood serum of a cow after the insorption of the component parts of the preparation. The obtained data allowed us to pinpoint the most critical period of time for developing clinical and subclinical mineral metabolism diseases, with relation to calving time. We established the optimum time to administer *Kalcifostilis* and developed its usage pattern. *Kalcifostilis* is manufactured by UAB Ruvera and used in practice.

MATERIALS AND METHODS

The blood for the research was taken from Lithuanian and German black and white cow breeds throughout the year. Table 1 describes the diet of the cows selected for the experiment.

Table 1. Cows' daily diet

Diet make-up kg	Dry cow	Lactating cow
Rapeseed meal	0,5	0,5
Soybean meal	-	1
Hay	3	2
*Haylage	12–19	15–22
Protein, vitamin and mineral supplement for cattle	0,15	0,22
Salt	-	0,08
Chalk	-	0,11
Wheat flour	1	2,5
Dry yeast	1	2
Energo KET	0,2	0,2

- during grazing period dry cows received 20-25 kg of cultivated grass feeds instead of haylage, while lactating cows received 15 kg.

The animals were classified into groups according to their age, previous cases of parturition paresis and the number of lactations. Prior to that, the cattle's documentation was studied (age, insemination date, diseases); the researched animals' anamnesis on calving, their condition postpartum (appetite, weight loss, muscle spasms, parturition paresis, the manifestation of apathy and its duration), other diseases, which manifested themselves afterwards (such as ketosis and laminitis) was also collected from cattle carers (the farm manager, yardmen and milkmen). The research was divided into 4 stages based on the goals at hand.

The methodology of establishing the dynamics of the amounts of calcium, inorganic phosphorus, magnesium, glucose and parathormone in the blood of a cow during pregnancy, calving and postpartum

The first test established how the amounts of Ca, Pn, Mg and glucose change in the blood serum during cow pregnancy, during calving and postpartum. 30 cows, divided into three groups, were selected for the experiment. 20 pregnant cows, as well as 10 non-pregnant, control cows were tested. The cows that didn't develop paresis after calving were classified as a test group I, whereas those that developed paresis postpartum were classified as a test group II. The control group was made up out of 10 non-pregnant cows.

The first test group (I gr.) consisted of 14 cows that didn't develop parturition paresis. The cows' blood was taken to be tested on day 140 and 210 of pregnancy, 5-1 days before calving, during calving, as well as 0-6 hrs, 6-12 hrs and 1,2,6 and 10 days after calving.

The second test group (II gr.) consisted of 6 cows that developed parturition paresis. This group's blood was taken to be tested at the same time as that of the test group I. The blood of the control group was taken at the same time.

The establishment of the change dynamic in the amount of macroelements (Ca, Pn, Mg) in cows' blood serum after administering *Kalcifostilis*

The previous experiment established that during calving, as well as immediately after calving, the amount of calcium and inorganic phosphorus in the cow's blood drops considerably, and some cows develop paresis, which is why *Kalcifostilis*, an orally administered preparation, was created in the Experimental and Clinical Pharmacology Laboratory of the Lithuanian Veterinary Academy. It has the following components: calcium chloride ($CaCl_2 \cdot 6H_2O$) – 30.0 g, magnesium chloride ($MgCl_2\ 6H_2O$) – 5.0 g, potassium chloride (KCl) – 0.5 g, sodium dihydrogen phosphate ($NaH_2PO_4\ 2H_2O$) – 4.0 g, carboxymethyl cellulose – 0.3 g, benzalkonium chloride – 0.02 g, purified water –

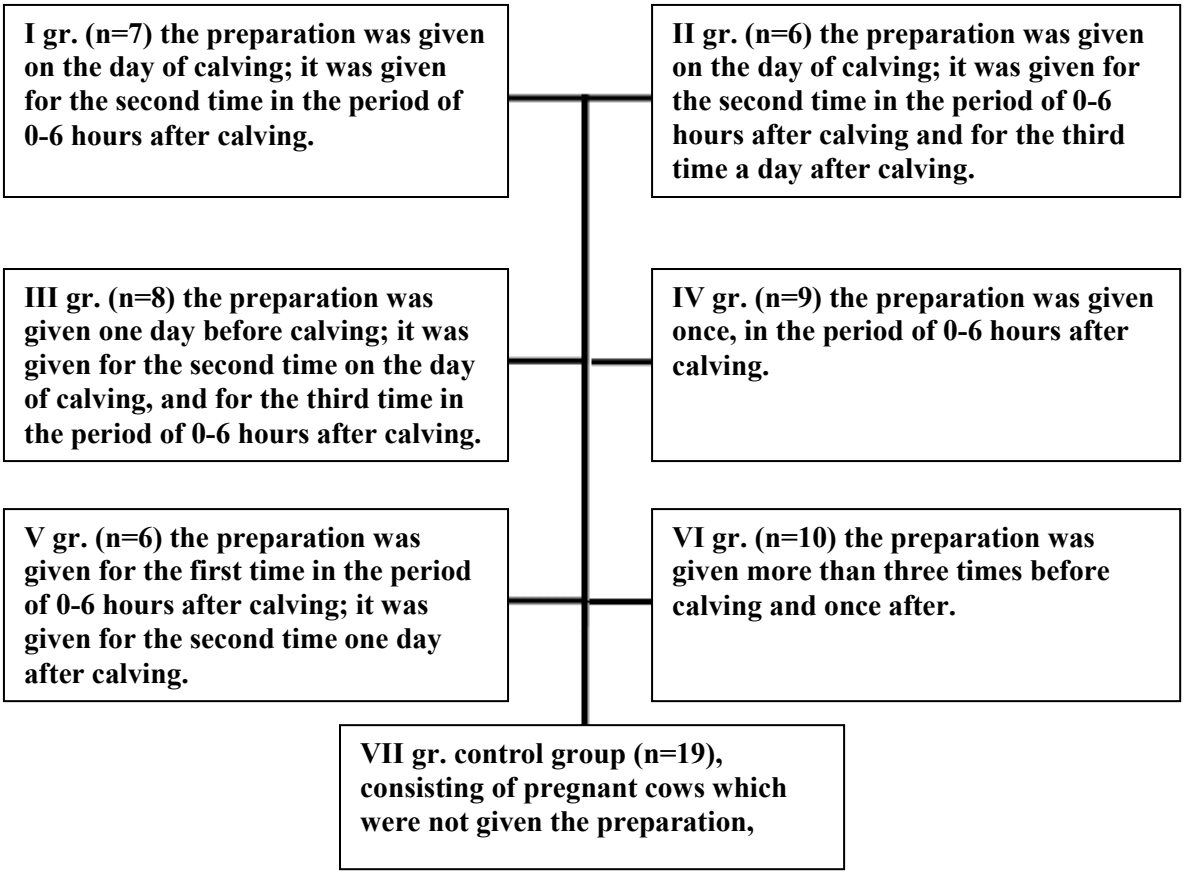
up to 100 ml. We established the effect of *Kalcifostilis* on the change dynamic of calcium, inorganic phosphorus and magnesium in the blood serum of a cow by conducting two experiments with clinically healthy cows.

15 clinically healthy, non-pregnant cows with a 3-4 years lactation period were selected for the first experiment. The animals were divided into a test group (n = 10) and a control group (n = 5). The test group were given 400 ml of the preparation twice at a 24 hour interval, whereas the control group were not given the preparation. The blood was taken before administering the preparation, as well as 3, 5 and 24 hours after the preparation was administered for the first time. The preparation was administered for the second time 24 hours after being administered for the first time, and blood was taken 3 hours after (27 hours after the first administering), 5 hours after (29 hours after the first administering) and 24 hours after (48 hours after the first administering).

Ten clinically healthy, 5-6 year old cows were selected for the second experiment. Six test group cows were given 500 ml of the preparation twice at a 24 hour interval, whereas 4 control group cows were not given the preparation. To reduce animal distress, the blood was taken less frequently after the first experiment established the time period, during which there was a greater change in the amount of the substances, which make up the preparation, in cows' blood. The blood was taken before administering the preparation, as well as 5 hours and 24 hours after; it was also taken 5 hours and 24 hours after the second administering (29 hours and 48 hours after the first administering respectively).

The methodology of establishing prophylactic effectiveness of *Kalcifostilis* in the prevention of hypocalcaemia and hypophosphatemia in cows

To establish the prophylactic effectiveness of *Kalcifostilis* we used 65 pregnant cows that had gone through 3-7 lactation periods. 46 test cows were classified into 6 groups according to when the preparation was administered in relation to calving. Picture 1 depicts the classification of cows into groups according to the schedule of administering *Kalcifostilis*.



Picture 1. The schematic classification of cows into groups in relation to when Kalcifostilis was administered

The previous experiments' results allowed us to establish that *Kalcifostilis* can be used as a preventive measure for hypocalcaemia and hypophosphatemia, which can occur during or after calving. We also established that mineral substances, present in the preparation, are easily absorbed from the digestive tract, increasing the amount of the mentioned substances in the blood. The blood of cows in all groups was taken 1-5 days before calving, during calving, as well as 0-6 hrs, 6-12 hrs, 1, 2, 6 and 10 days after calving.

The methodology of establishing the prophylactic effectiveness of *Kalcifostilis* for the cows, which suffered from parturition paresis in the past

The prophylactic effectiveness of the preparation against paresis was evaluated during the experiment. The farm cows that previously developed paresis of a varying degree of complexity after each calving were chosen for the experiment. 1-2 days prior to calving 17 cows were given 500 ml of the preparation twice at a 24 hour interval. Based on the fact that 10 cows didn't develop parturition paresis, whereas 7 did, two groups were formed: Group I (didn't develop paresis) and

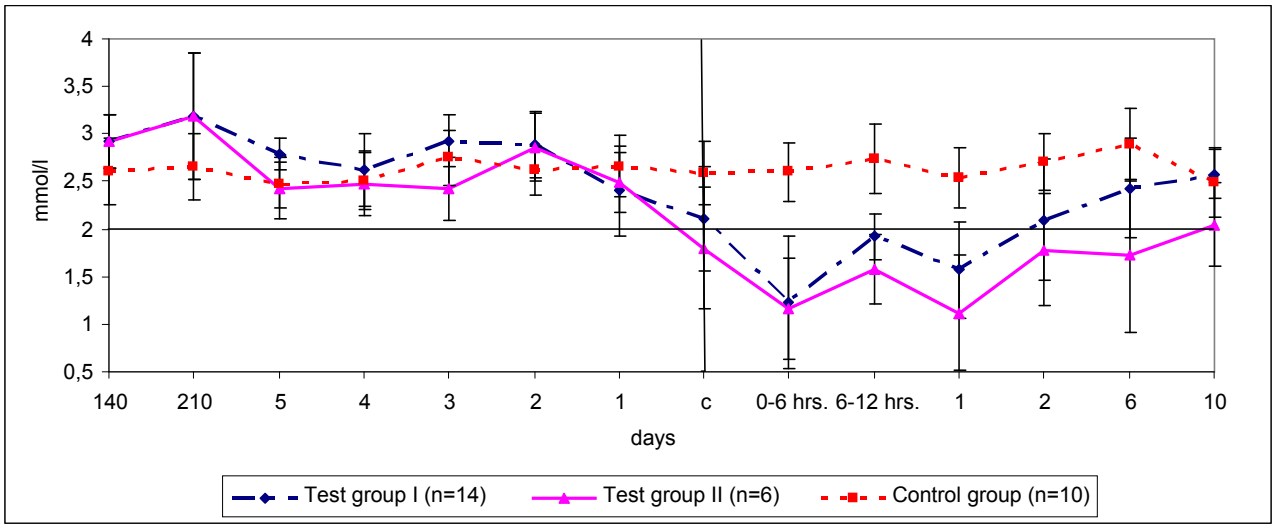
Group II (developed paresis). The blood was taken for testing before administering the preparation and 5 hours after administering it for the second time. The calving process, together with the cow's condition postpartum was observed.

During the second experiment 8 cows, previously suffering from parturition paresis, were given the preparation two days and one day prior to calving, as well as during calving. The blood was taken before administering the preparation, during calving, as well as 1, 2, 6 and 10 days after calving.

RESULTS AND DISCUSSION

The dynamics of calcium, inorganic phosphorus, magnesium, glucose and parathormone in the blood serum of a cow during pregnancy, calving and postpartum

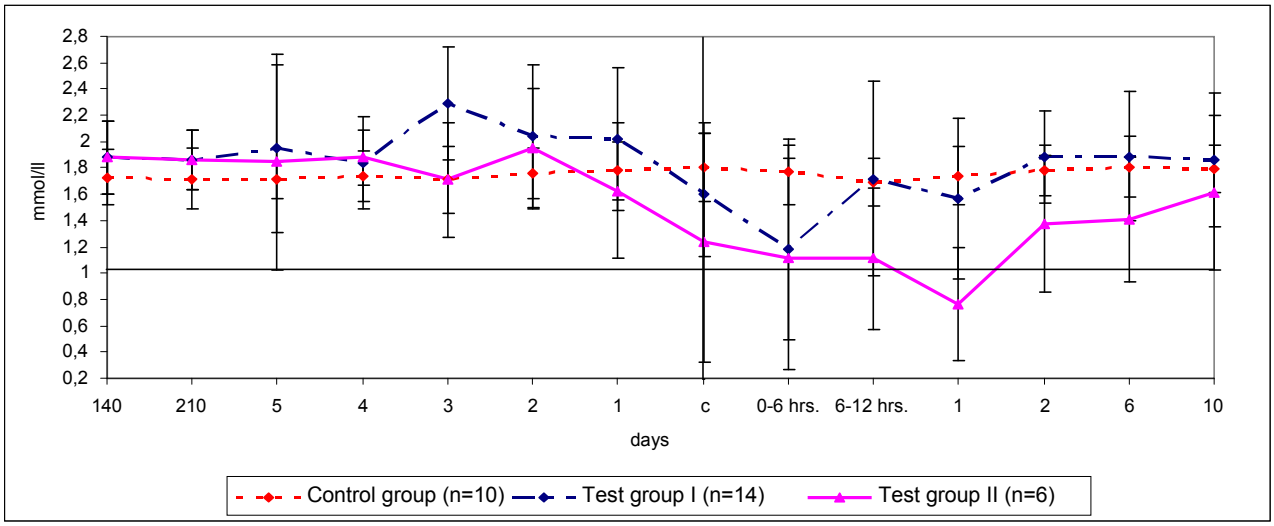
The data we collected suggests that often there is a disruption in mineral metabolism towards the end of a cow's pregnancy, during calving and in the first days following calving. During calving, as well as immediately after, the amount of calcium in the blood serum of a cow, regardless of the fact whether the cow developed paresis or not, starts to drop dramatically (up to 1.8-2.11 mmol/l, Picture 2). Other authors also established the reduction in the amount of calcium in the blood (up to 0.69-2.73 mmol/l) before, during and after calving (Goff, Horst, 1997; Goff, 2000; Larsen et al. 2001; Ruat, 2003). Goff, as well as the others (2002), established that hypocalcaemia develops postpartum due to increased calcium emanation with colostrum, as the cow, producing 10 litres of milk in one milking, loses about 23 g of calcium with milk. This is 9 times the average amount of calcium present in the blood. We established that the most critical time in relation to a cow developing paresis, is during calving and one day after. Currently we have established that the average amount of calcium in the blood of a cow suffering from paresis is 1.12±0.6 mmol/l, the amount of inorganic phosphorus – 0.76±0.43 ($p<0.05$, Picture 3). The amount of calcium in the blood of the cow, which doesn't develop paresis, goes back to normal on the second day after calving, whereas when a cow is suffering from paresis, the amount of calcium goes back to normal on day 10 after calving.



Picture 2. The change in the amount of calcium in the blood serum of a cow during pregnancy, calving and after

c* – calving day

The amount of inorganic phosphorus in the blood changed less than that of calcium. There was only a significant change in the amount of phosphorus (up to 0.76±0.43 mmol/l, Picture 3.) on the first day of lactation in the blood serum of those cows that developed parturition paresis. Two cows developed downer cow syndrome, which is associated with hypophosphatemia. It was established that if phosphorus amount in the blood serum of a cow, suffering from parturition paresis, is less than 0.9 mmol/l, the risk of developing downer cow syndrome is 12 times as high (Menard, Thompson, 2007), whereas if the amount of inorganic phosphorus in the blood of a dairy cow drops to less than 1.03 mmol/l, the cow will develop subclinical hypophosphatemia (Adams et al., 1996). During our experiments hypophosphatemia proved to be at its peak (0.76±0.43 mmol/l) one day after calving in the group of the cows that developed the illness. Subclinical hypophosphatemia was established in the blood of 23% of the cows that didn't develop the illness, and 80% of those that did. Productive cows mostly loose calcium and phosphorus with milk (1 kg of cow's milk contains 1.3 g of calcium and 1 g of phosphorus). When a cow is lactating or pregnant, or when there is a lack of the aforementioned elements in the feed, bone reserves are used. These reserves amount to 1.5-2 kg of calcium and 1-1.5 kg of phosphorus. With the disruption of calcium and phosphorus metabolism, the concentration of calcium and phosphorus in the blood serum, as well as Ca/P correlation, changes. We established that there was no change in calcium-phosphorus correlation for those cows that developed parturition paresis (up to 1.47:1). There was a reduction of Ca/P correlation for cows that didn't develop parturition paresis (up to 1:1).



Picture 3. The change of inorganic phosphorus in the blood of a cow during pregnancy, calving and after

c* – calving day

The amount of magnesium in the blood of the cows, both those that developed the illness and those that didn't, changed within the physiological standard – 0.78–1.44 mmol/l. Only when the levels of magnesium have been low for a longer period of time do the symptoms of clinical hypomagnesaemia appear (grass tetany).

We established that on average there were larger amounts of glucose (3.25–4.07 mmol/l) in the blood of a cow, which didn't develop paresis, on the day of calving, as well as one day after that. We think that the glucose increase was caused by the slowing down of insulin secretion due to smaller amounts of calcium present during that period (Horst et. al. 2003), as well as the increase of corticosteroids in the blood during calving, which encourages the breakup of glycogen (Tucker, 2000). There was a sudden decrease in glucose amounts postpartum (1.32±0.91) in the blood of those cows that developed paresis. We believe that clinical hypocalcaemia also causes the disruption in energy metabolism (Kelton et. al., 1998). Hypoglycaemia in those cows, which didn't develop paresis, peaked on day 6 of lactation, with a 1.87 mmol/l glucose amount in the blood on average, which is 15% less than that of a physiological standard. Hypoglycaemia in those cows, which developed parturition paresis, was even more acute – 1.44 mmol/l on average. We think that after calving, when there is an increase in lactogenesis, large amounts of glucose are used for lactose synthesis, and that causes the amount of glucose in the blood to decrease, which corresponds to the data, presented by Overton (1998).

In the course of our experiment PTH significantly increased during calving. Right before calving the hormone's concentration was 1.52±0.58 μmol/l, whereas during calving it increased to

9.12±4.39 μmol/l. Other scientists (Goff, Horst, 1997) established that PTH amount begins to increase 2 days before calving, with the largest amount present 12 hours after calving. With the reduction of calcium in the blood serum, there is an increase in PTH amount in the blood, as well as the increase in 1.25-$(OH)_2D$ synthesis, an increase in calcium insorption from the digestive tract and its release from the bones; there is also an increase in calcium reabsorption in the kidneys (Horst et al., 1994; Goff et al., 2002).

Summing up the results of the experiment, we can state that cows develop parturition paresis, downer cow syndrome, subclinical hypocalcaemia and hypophosphatemia because the amounts of calcium and phosphorus in the blood serum decrease dramatically right at the end of pregnancy, during calving and one day after calving.

The change in the amounts of calcium and inorganic phosphorus in the blood serum of a cow after administering *Kalcifostilis*

The results of the experiments show that calcium, inorganic phosphorus and magnesium, present in *Kalcifostilis*, get absorbed from the digestive tract and elevate the quantity of those macroelements in the blood of a cow. During the first experiment, after the first time of test group cows being given 400 ml of *Kalcifostilis*, the amount of calcium in their blood 3 hours later was 5% higher than that of control group cows; it also remained slightly higher up until the end of the experiment. The amount of inorganic phosphorus within the test group and control group remained similar, however after 24 hours it significantly increased within the test group (32% higher than that of control group cows); it too remained higher up until the end of the experiment. There was a tendency for the amount of magnesium to increase, however the difference proved unreliable. Due to the fact that the amount of calcium increased by an insignificant amount during the first experiment, and the animals tolerated the preparation well, we increased the dosage of the preparation to 500 ml for the second experiment.

During the second experiment, 5 hours after administering the preparation the amount of calcium showed a bigger increase than during the first experiment; it was 15% higher in test group cows, compared to the increase in control group cows. The amount of inorganic phosphorus in the blood of test group cows was increasing 24 hours after administering the preparation for the first time; however the biggest difference in the amount of inorganic phosphorus between that of test group and control group cows was noticed after administering the preparation for the second time. It is possible that the amount of inorganic phosphorus in the blood increased because calcium, present in *Kalcifostilis*, suppressed PTH secretion, which in turn decreased phosphorus escape with saliva and urine. The amount of magnesium doesn’t change much, but it was added to the preparation so that it

could suppress the affect that calcium has on the heart. We believe that adding magnesium achieved this task, and none of the cows suffered from any cardiac activity disruption, which is often observed after injecting a cow with a larger amount of calcium chloride or calcium gluconate solution (Farningham, 1985). Looking at the experiment results achieved by Queen (et al., 1993), as well as our own results, we can state that calcium, inorganic phosphorus and magnesium present in the oral gel, are absorbed well, increasing the amounts of the aforementioned elements in the blood. The preparation, administered per os, is tolerated well and does not cause unwanted reactions. *Kalcifostilis* can be used for the prevention of hypocalcaemia and hypophosphatemia.

The prophylactic effectiveness of *Kalcifostilis* in preventing hypocalcaemia and hypophosphatemia

The data we obtained (Picture 4) showed that 5-1 days before calving the cows in all the groups exhibited calcium change within the physiological standard. The amount of calcium in the blood of those cows, which didn't get *Kalcifostilis*, the control group (VII gr.), begins to decrease during calving; hypocalcaemia manifests 0-6 hrs postpartum (1.14±0.69 mmol/l), as well as one day after (1.52±0.51 mmol/l). Within this group calcium amount goes back to normal on day six after calving.

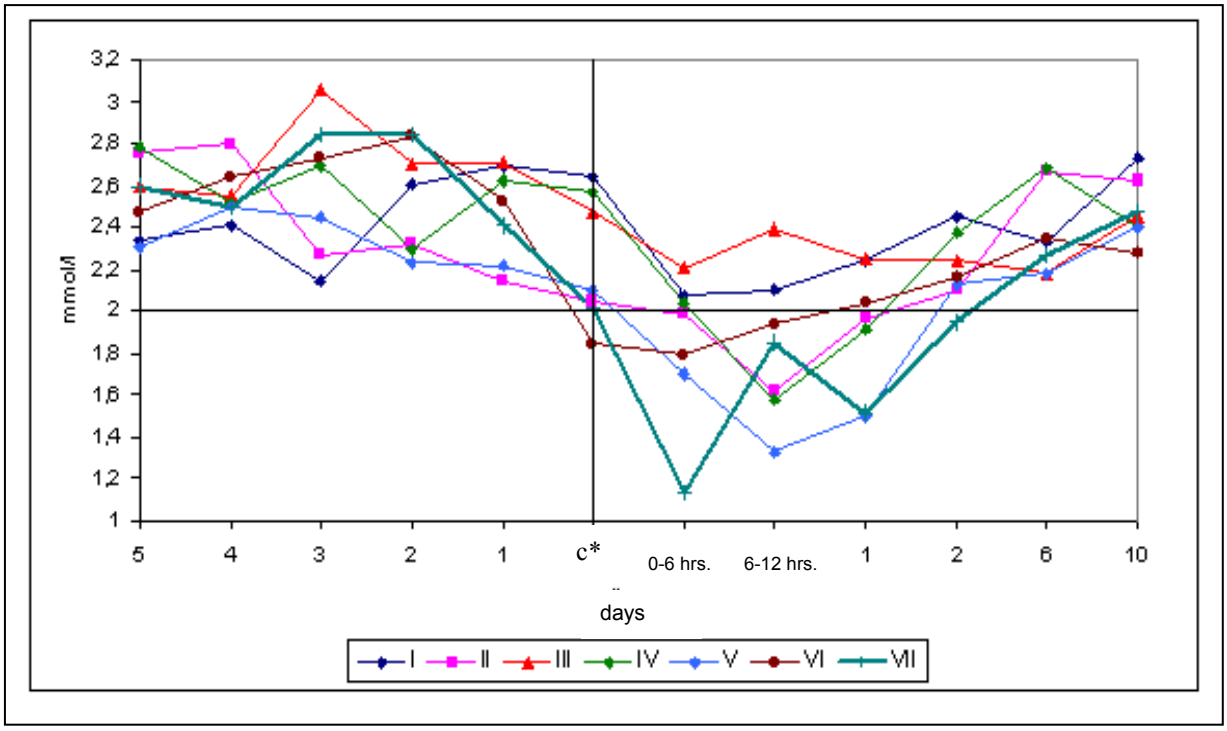
When administering *Kalcifostilis* during calving and immediately after (I gr.), as well as once more the next day (II gr.), between 0-6 hours postpartum and one day after, calcium amount changes within the physiological standard, or in some cases a short-term hypocalcaemia develops (II gr., in the period of 6-12 hours 1.62±0.44 mmol/l).

When giving *Kalcifostilis* one day before calving, during calving and once after, the amount of calcium in cows' blood changed within the physiological standard throughout the whole experiment, with the exception of one cow, which exhibited a drop in the amount of inorganic phosphorus below 0.9 mmol/l and developed downer cow syndrome. According to Goff, Horst (1993) some cows lack more than 10 g of endogenous calcium postpartum, and orally administered calcium salt solutions are not always effective; they are however effective when the lack of endogenous calcium is less than 9 g.

When giving *Kalcifostilis* only after calving (group IV and V, picture 4), the amount of calcium 0-6 hours, 6-12 hours and one day postpartum decreased much more than that of group I, II and III. According to Hernandez et al. (2004), after orally administering calcium chloride solution to cows only after calving, the amount of calcium in the blood serum decreased and there was little prophylactic value. By the time the cows are given the mineral salt solution, the clinical processes,

associated with calcium metabolism, have already been affected too much, and it becomes impossible to avoid hypocalcaemia and hypophosphatemia.

When giving *Kalcifostilis* more than three times before calving and once after (VI gr., Picture 4) the amount of calcium in the blood was at its lowest during calving. After calving, in a period of a day, the amount of calcium within the group increased and was changing within the physiological standard; however, a number of cows in the group developed paresis. Horst et al. (1994) established that when cows continuously receive the increased amounts of calcium before calving, the secretion of PTH and $1.25(OH)_2$ vitamin D is suppressed, therefore with the intensive start of colostrum synthesis there is no demineralisation of the bone tissue, nor calcium reabsorption in the kidneys.



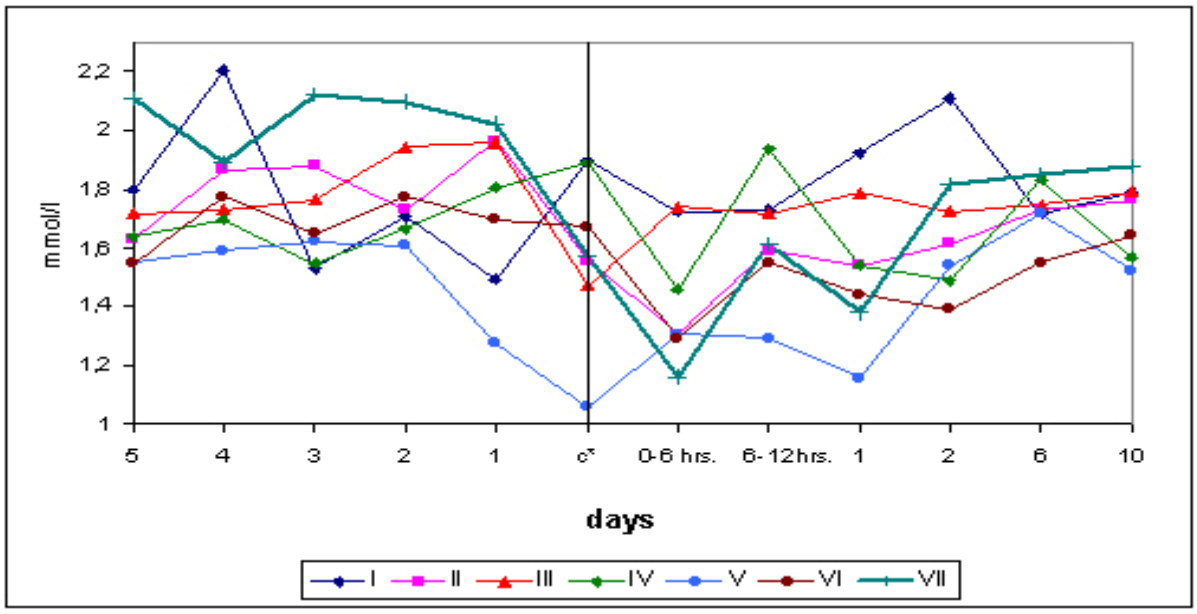
Picture 4. The change in the amount of calcium in the blood serum of a cow after administering *Kalcifostilis* at different points in time in relation to calving

c^* – calving day

In the course of our experiment we established that the amount of inorganic phosphorus in the blood of control group cows (VII gr.) changed similarly to calcium amounts. The amount of inorganic phosphorus in the blood of control group cows (VII gr.) decreased during calving (1.57±0.57), 0-6 hours after calving (up to1.16±0.74, Picture 5), as well as one day after (1.38±0.67). After

administering *Kalcifostilis* during calving and immediately after (I gr.), as well as the next day (II gr.), the postpartum decrease in the amount of inorganic phosphorus in the blood was short-term.

It was noticed that the amount of inorganic phosphorus within the first group one day after calving was reliably larger ($p \geq 0.05$) than that of control group cows. Group III cows, which were given the preparation one day before calving, during calving and immediately after, exhibited a significant drop in the amount of inorganic phosphorus in the blood during calving (1.47±0.24 mmol/l); later on, the amount of phosphorus in the blood restored to the level that was prior to calving (1.72–1.8 mmol/l on average). Those cows that were given *Kalcifostilis* after calving (IV gr.) and once the next day (V gr.) exhibited a significant drop in the amount of inorganic phosphorus in the blood during calving (V gr. 1.06±0.21 mmol/l Picture 5), 0-6 hours postpartum, as well as the next day. The decrease in the amount of inorganic phosphorus couldn't be avoided within group VI cows, which were given *Kalcifostilis* at least three times before calving and once after. PTH removes phosphorus from the body and increases calcium amount, therefore quite often hypocalcaemia and hypophosphatemia manifest at the same time. 3.8-28% of cows that develop parturition paresis can not stand up after being injected with intravenous calcium salts solution; this condition is known as downer cow syndrome (Menard-Thompson, 2007). The syndrome manifested itself in some of the control group cows (VII), as well as those, that were given *Kalcifostilis* only after calving (IV and V), or at least three times before calving and once after (VI).



Picture 5. The change in the amount of inorganic phosphorus in the blood serum of a cow after administering *Kalcifostilis* at various points in time relating to calving

c* – calving day

The amount of magnesium changed within the physiological standard in all of the groups. There was a tendency for its increase during calving and after, however, no significant change was established.

During our experiments we noticed a tendency for the increase in the amount of glucose during calving and immediately after in all of the groups, however, all the groups developed hypoglycaemia on the second day (<2.1 mmol/l). The amount of glucose in the blood went back to normal the fastest in those cows that were given *Kalcifostilis* during calving and immediately after, as well as the next day (I and II gr.). This establishes the positive effect of *Kalcifostilis* on the energy metabolism.

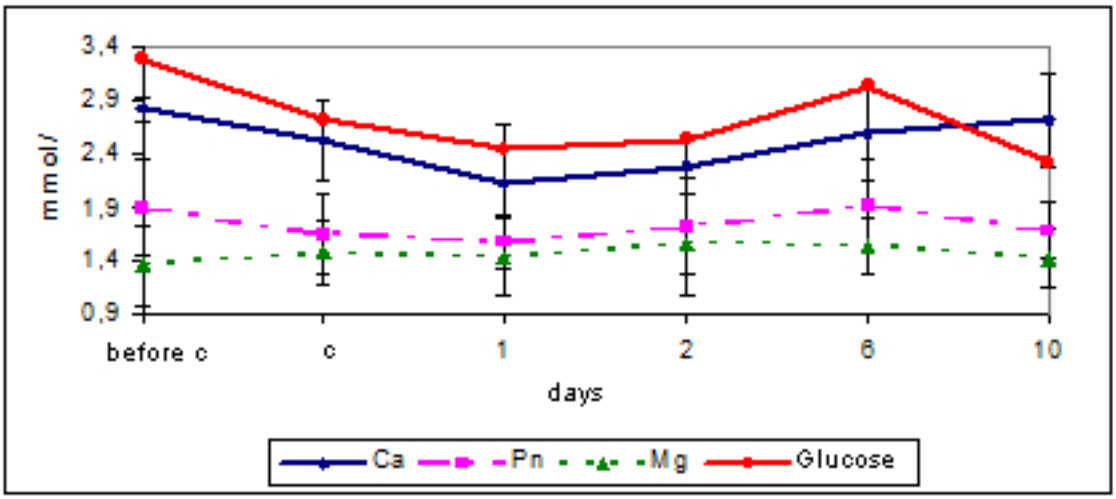
To sum up the results of this stage of the research, we can see that when administering *Kalcifostilis* during calving, immediately after, as well as the next day, the change in the amount of calcium and inorganic phosphorus is insignificant, and none of the cows from group I or II developed parturition paresis. When administering *Kalcifostilis* only after calving, two group IV cows developed paresis and later on - downer cow syndrome, three group V cows developed paresis, which in our opinion happened because clinical mineral metabolism processes had already begun by then.

When administering *Kalcifostilis* at least three times before calving and once after, clinical hypocalcaemia and hypophosphatemia became impossible to avoid. With the start of colostrum synthesis, the cows that get large amounts of calcium before calving don't have enough time to adapt to the need of increased amounts of calcium and phosphorus.

The effectiveness of *Kalcifostilis* as a preventive measure for hypocalcaemia and hypophosphatemia in those cows that had previously suffered from parturition paresis

The data we obtained suggests that the amount of calcium and phosphorus in the blood of the cows that developed paresis during calving and after significantly decreases. When giving *Kalcifostilis* twice before calving to those cows that previously suffered from parturition paresis, it was impossible to avoid paresis, however all the cows became healthy again after being given calcium preparations. Due to the fact that the cows did not get *Kalcifostilis* after calving, the prophylactic effect was insufficient; however the cows' condition was easier to treat, resulting in quicker recovery.

When an animal gets older, the number of active osteoclasts decreases. Cows, which are calving for the first time, very rarely develop clinical hypocalcaemia. When the animal gets older, the amount of PTH receptors in the tissue, as well as the number of cells in the intestines, sensitive to 1.25-dixydroxy vitamin D, gets smaller, therefore less calcium is absorbed from the bone and the feed (Horst, 1990).



Picture 6. The amount of calcium, inorganic phosphorus, magnesium and glucose in the blood of a cow, which suffered from parturition paresis in the past, after administering *Kalcifostilis*

When administering *Kalcifostilis* twice before calving and once during calving to the cows that previously suffered from paresis, the amount of calcium and inorganic phosphorus tended to decrease; however, it went back to normal after a day (Picture 6). None of the cows during the experiment developed paresis.

To sum up the results of this stage of the research, *Kalcifostilis*, when given twice before calving and once during calving, was effective in preventing hypocalcaemia and hypophosphatemia in cows, suffering from paresis in the past.

CONCLUSION

1. Establishing the change in the amount of calcium, inorganic phosphorus and parathormone in the blood of a cow at the end of the pregnancy, during calving and after calving, allows to diagnose hypocalcaemia and hypophosphatemia at an early stage, as well as choose the most optimal time for administering *Kalcifostilis*.

2. The macroelements' change during pregnancy shows that the most critical time for developing hypocalcaemia and hypophosphatemia is the first day after calving, when the amount of calcium and inorganic phosphorus falls (up to 1.2±0.6 mmol/l and 0.76±0.43 mmol/l respectively) below the physiological standard.

3. There is a decrease in the amount of calcium in the blood of a cow on the day of calving, at the same time there is an increase in the amount of parathormone (from 1.15±0.35 to 9.12±4.39 μmol/l), which encourages the absorption of the aforementioned mineral from the bone.

4. After administering *Kalcifostilis* right before calving, as well as once or twice after, the amount of calcium and inorganic phosphorus in cows' blood, compared to that of the cows which weren't given the preparation, significantly increased ($p<0.05$); as a result, those cows didn't develop paresis.

5. Multiple administering of *Kalcifostilis* earlier than three days before calving suppresses the absorption of endogenous calcium and does not prevent clinical and subclinical hypocalcaemia and hypophosphatemia.

PROPOSAL

1. *Kalcifostilis* should be given to cows on the day of calving, immediately after calving, as well as one day after that.
2. *Kalcifostilis* should be given to pregnant cows no sooner than two days before calving.
3. In order to establish subclinical hypocalcaemia and hypophosphatemia in cows, the blood sample must be taken for biochemical testing on the day of calving, as well as two days after calving.

LIST OF PUBLICATIONS

1. Starevičius D., Matusevičius A., Špakauskas V. "Correlation between the level of Ca, P, Mg, glucose and parathormone in blood and the symptoms of postnatal paresis in cows". Veterinarija ir Zootechnika. ISSN 1392-2130. T. 38 (60). 2007. P. 68–73.
2. Matusevičius A., Špakauskas V., Černauskas A., Klimienė I., Starevičius D. "Effect of oral administration of calcium chloride gel on blood mineral concentrations and parturient paresis prophylaxis in cows". Medycyna Weterynaryjna. PL ISSN 0025-8626. 2008, 64 (6). P. 773–777.

SANTRAUKA

Mūsų gauti tyrimų duomenys parodė, kad veršingumo pabaigoje, veršiavimosi metu ir pirmomis dienomis po veršelio atvedimo dažnai sutrinka mineralinių medžiagų apykaita. Sutrikus kalcio ir fosforo apykaitai, sumažėja jų koncentracija kraujo serume, pakinta kalcio ir fosforo santykis. Veršiavimosi metu ir po veršelio atvedimo nesusirgusių pareze ir susirgusių karvių kraujo serume kalcio kiekis pradėjo staigiai mažėti (iki 1,8–2,11 mmol/l). Kalcio kiekio sumažėjimą kraujyje (iki 0,69–2,73 mmol/l) prieš karvių veršiavimąsi, veršiavimosi metu ir po veršelio atvedimo nustatė ir kiti autoriai (Goff, Horst, 1997; Goff, 2000; Larsen et al. 2001; Ruat, 2003). Goff ir kiti (2002) nustatė, kad apsiveršiavus hipokalcemija karvėms išsivysto dėl padidėjusio kalcio išsiskyrimo su krekenomis, nes karvė, duodanti 10 litrų pieno vieno melžimo metu, su pienu netenka apie 23 g kalcio. Šis kiekis yra 9 kartus didesnis už bendrą kalcio kiekį kraujyje. Mes nustatėme, kad kritiškiausias periodas karvėms susirgti pareze yra veršiavimosi metas ir pirma diena po veršelio atvedimo. Karvės susirgo klinikine parezės forma praėjus vienai parai po veršelio atvedimo, kai kalcio kiekis buvo vidutiniškai 1,12±0,6 mmol/l, o neorganinio fosforo – 0,76±0,43 ($p<0,05$). Klimienė (2001) nustatė, kad pareze po veršelio atvedimo sergančių karvių kraujyje kalcio buvo 1,39, neorganinio fosforo – 1,1 mmol/l. Subklinikinė hipokalcemija pasireiškia, kai po veršelio atvedimo kraujo serume būna 1,37–2,00 mmol/l kalcio (Reinhart et al., 2004). Mūsų tyrimo metu nustatyta, kad subklinikinė hipokalcemija pirmą parą po veršelio atvedimo buvo 75 proc. nesusirgusių ir 100 proc. susirgusių karvių kraujyje. Nesusirgusių pareze po veršelio atvedimo karvių kalcio kiekis kraujyje tampa normalus antrą, o susirgusių – dešimtą dieną po veršelio atvedimo.

Fosforo kiekis kraujo serume kito mažiau nei kalcio. Fosforo kiekis gerokai sumažėjo (iki 0,76±0,43 mmol/l) pirmą laktacijos dieną tik pareze po veršelio atsivedimo susirgusių karvių kraujo serume. Nustatyta, kad jei karvių, susirgusių pareze po veršelio atvedimo, fosforo kiekis kraujo serume yra mažesnis nei 0,9 mmol/l, rizika susirgti gulinčios karvės sindromu padidėja 12 kartų (Menard, Thompson, 2007), o neorganinio fosforo kiekiui melžiamų karvių kraujyje nukritus žemiau 1,03 mmol/l (Adams et al., 1996) pasireiškia subklinikinė hipofosfatemija. Mūsų bandymų metu hipofosfatemija ryškiausiai pasireiškė po veršelio atvedimo praėjus vienai parai (0,76±0,43 mmol/l) susirgusių karvių grupėje. Subklinikinė hipofosfatemija tuo laiku nustatyta 23 proc. nesusirgusių ir 80 proc. susirgusių karvių kraujyje.

Produktyvioms karvėms daugiausiai kalcio ir fosforo pašalinama su pienu (1 kg karvių pieno yra apie 1,3 g kalcio ir 1 g fosforo). Karvės įsisavina iš pašarų apie 40 proc. kalcio ir apie 70 proc. fosforo (Bruder et al., 2001). Laktacijos, veršingumo metu ir trūkstant šių elementų pašaruose,

naudojamos rezervinės kaulų atsargos. Šios atsargos karvių kauluose sudaro 1,5–2,0 kg kalcio ir 1,0–1,5 kg fosforo. Šių atsargų pakanka pagaminti 1500 kg pieno. Fosfatų koncentracija kraujyje kinta pagal fosforo kiekį racione – kuo daugiau fosforo yra racione, tuo daugiau jo absorbuojama į kraują iš virškinamojo trakto, o didelis kalcio kiekis pašaruose slopina fosforo rezorbciją (Martz et al., 1999). Hipokalcemija sergančių karvių kraujo serume nustatytas padidėjęs PTH kiekis (Špakauskas ir kt., 2006), kuris slopina fosforo rezorbciją iš virškinamojo trakto bei skatina jo išsiskyrimą su šlapimu ir seilėmis.

Sutrikus kalcio ir fosforo apykaitai, sumažėja jų koncentracija kraujo serume, pakinta Ca/P santykis. Nepakitusį kalcio ir fosforo santykį nustatėme susirgusioms karvėms pareze po verševimosi (iki 1,47:1). Nesusirgusių pareze karvių Ca/P sumažėjo (iki 1:1).

Magnio koncentracija mažai kito. Ir sveikų ir susirgusių karvių ji kito fiziologinės normos ribose – 0,78–1,44 mmol/l. Tik ilgesnį laiką esant mažam magnio kiekiui kraujo serume, pasireiškia klinikiniai hipomagnemijos simptomai (ganyklinė tetanija) (George et al., 1995). Magnio koncentracija kraujyje priklauso nuo magnio kiekio pašaruose ir gali kur kas labiau kisti negu kalcio koncentracija (Chester-Jones et al., 1990).

Kalcis, fosforas ir magnis yra antagonistai, bet jų apykaitos procesai glaudžiai susiję. Svarbų vaidmenį jų valdyme turi prieskydinės liaukos hormonai, daug lemia virškinamojo trakto, inkstų, kepenų ir kitų organų būklė. Sutrikus organizmo rezorbcijos ir išskyrimo pusiausvyrai, dėl šių elektrolitų stokos arba netinkamo santykio pakinta biocheminiai procesai audiniuose, gyvuliai gana dažnai suserga mineralinių medžiagų apykaitos ligomis (Marx et al, 2000; Goff, Horst, 2003).

Nesusirgusių karvių kraujyje veršiavimosi dieną ir vieną parą po veršelio atvedimo nustatėme vidutiniškai didesnius gliukozės kiekius – 3,25–4,07 mmol/l. Manome, kad gliukozės kiekis padidėjo todėl, kad šiuo laikotarpiu sumažėjęs kalcio kiekis slopino insulino sekreciją (Horst et. al. 2003), be to, besiveršiuojančioms karvėms padidėja kraujyje kortikosteroidų kiekis, skatinantis gliukogeno skaidymą (Tucker, 2000). Pareze susirgusių karvių kraujyje gliukozės kiekis po veršelio atvedimo staigiai sumažėjo (1,32±0,91). Manome, kad dėl klinikinės hipokalcemijos sutrinka ir energijos apykaita (Kelton et. al., 1998). Prasidėjus laktacijai, dideli gliukozės kiekiai sunaudojami pieno laktozės sintezei, todėl po paros gliukozės koncentracija sumažėjo ir nesusirgusių karvių kraujyje. Stipriausia hipoglikemija, vidutiniškai 1,87 mmol/l, t. y. 15 proc. mažiau nei fiziologinė norma, pasireiškė 6-tą laktacijos parą nesusirgusių karvių kraujyje, o pareze po veršelio atvedimo susirgusių hipoglikemija buvo dar stipresnė – vidutiniškai 1,44 mmol/l. Manome, kad po veršelio atvedimo suintensyvėjus laktogenezei dideli gliukozės kiekiai sunaudojami pieno laktozės sintezei, todėl gliukozės kiekis kraujo serume sumažėja – tai atitinka Overton (1998) pateiktus duomenis.

Mažesni gliukozės kiekiai nustatyti ir laktuojančių neveršingų karvių kraujyje – tai atitinka Drackley (2001) pateiktus duomenis.

Kalcio ir fosforo apykaitos procesai yra sudėtingi. Svarbų vaidmenį jų valdyme turi kalciferolis, prieskydinės liaukos hormonas ir vitaminas D. Parathormonas kalcio ir fosforo kiekį serume reguliuoja per kauluose, žarnose ir inkstuose esančius receptorius (Horst et al., 1997; Bruder et al., 2001; Deftos, 2001). Mūsų bandymo metu PTH daugiausia padidėja per veršiavimąsi. Kiti mokslininkai (Goff, Horst, 1997) nustatė, kad PTH kiekis pradeda didėti likus 2 dienoms iki veršelio atvedimo ir didžiausias šio hormono kiekis buvo nustatytas praėjus 12 val. Prieskydinės liaukos sekrecinės ląstelės turi mažus kiekius iš anksto susintetinto hormono ir jos sugeba reaguoti į mažą kalcio koncentracijos pasikeitimą, sparčiai pakeisdamos hormonų sekrecijos greitį (Drezner, 2002). Sumažėjus kalcio kiekiui kraujo serume, padidėja PTH kiekis ir 1,25-$(OH)_2$D sintezė, suintensyvėja kalcio rezorbcija iš virškinimojo trakto ir mobilizacija iš kaulų bei stimuliuojama kalcio reabsorbcija inkstuose (Horst et al., 1994; Goff et al., 2002). Gauti tyrime rodmenys sutampa su Malz C. ir Meyer C. (1993) ir Goff, Horst (1997) paskelbtais duomenimis, kad pieno gamybai sunaudojami dideli kalcio kiekiai ir, negaunant pakankamai kalcio su pašaru, aktyvinama PTH sekrecija, kad padidintų kalcio kiekį kraujyje, pasisavinant jį iš kaulų.

Gauti tyrimų rezultatai rodo, kad *Kalcifostilyje* esantis kalcis, neorganinis fosforas ir magnis rezorbuojasi iš virškinamojo trakto ir papildo minėtų makroelementų kiekį karvių kraujyje. Lyginant su kontrolinės grupės karvėmis kalcio kiekis bandomų karvių kraujyje pirmo bandymo metu buvo didesnis 5 proc., antrojo – 15 proc. Šis skirtumas pasireiškė todėl, kad antro bandymo metu buvo sugirdyta didesnė preparato dozė. Neorganinio fosforo kiekis bandomųjų karvių kraujyje labiausiai didėjo praėjus 24 val. po pirmos preparato dozės sugirdymo, o didžiausi neorganinio fosforo kiekių skirtumai tarp bandomosios ir kontrolinės grupės karvių nustatyti po antros preparato dozės sugirdymo. Tikėtina, kad neorganinio fosforo kiekis kraujyje galėjo padidėti todėl, kad *Kalcifostilyje* esantis kalcis slopino PTH sekreciją, o sumažėjęs PTH kiekis mažino fosforo išsiskyrimą su seilėmis ir šlapimu (Goff, 2000). Magnio kiekis mažai kinta, bet kadangi jis yra kalcio antagonistas, tai pagrindinė jo veikla yra švelninti greitą kalcio poveikį širdžiai. Manome, kad magnis šią funkciją gerai atliko ir nė vienai karvei po preparato sugirdymo nenustatyta širdies veiklos sutrikimų, kas dažnai pastebima po greito ir didesnių kalcio chlorido ar kalcio borogliukonato tirpalų sušvirkštimo į veną (Farningham, 1985). Vadovaujantis Queen (et al., 1993) gautais tyrimų duomenimis ir mūsų tyrimų rezultatais, galima teigti, kad peroraliniame gelyje esantis kalcis, neorganinis fosforas, magnis gerai rezorbuojasi ir karvių kraujo serume padidina jų kiekius.

Sugirdžius preparato per os, jis gerai toleruojamas ir nesukelia nepageidaujamų reakcijų. Preparato sudėtyje esantis kalcio chloridas rūgština kraujo terpę, tai skatina kalcio atsipalaidavimą iš kraujo albuminų ir jis geriau pasisavinamas organizmo ląstelių (Fenwick, 1994). *Kalcifostilis* tinkamas naudoti hipokalcemijos ir hipofosfatemijos profilaktikai.

Subklinikinė hipokalcemija ir parezė po veršelio atvedimo pasireiškia, kai karvė išskiria daug kalcio su pienu, o su pašaru gauna nepakankamą jo kiekį, nes tuo metu kompensaciniai mechanizmai, kurių metu kalcis paimamas iš kaulų, dar nepradėję veikti (Goff, 2005). Špakauskas su bendraautoriais (2006) nustatė, kad veršiavimosi metu kalcio kiekis kraujyje sumažėja, o PTH sekrecija suintensyvėja. PTH aktyvina vitaminą D $1,25(OH)_2$, kuris tik praėjus 24 val. padidina kalcio pasisavinimą iš pašaro virškinamajame trakte. Padidėjęs PTH kiekis kraujyje 48 valandų laikotarpiu sukelia osteoklastų transformaciją ir prisitvirtinimą prie kaulinio paviršiaus, o jie, skaidydami kaulinį matriksą, atpalaiduoja kalcį. Sergant pareze po veršelio atvedimo minėti procesai vystosi dar lėčiau (Reinhardt et al., 1988). Norint, kad PTH sistema būtų nuolat aktyvi, užtrūkusios karvės su pašaru turi gauti ne daugiau kaip 20 g kalcio per dieną. Tačiau tokį racioną sunku sudaryti (Reinhardt et al., 1988; Jørgensen, Thilsing-Hansen, 2000, 2001; Thilsing-Hansen, Jørgensen 2000; Jørgensen et al., 2001; Wilson, 2001). Todėl Jonsson (1970), Pehrson (1989), Schultken (1992), Goff (1994), Hernandez (et al., 2004) pasiūlė veršiavimosi metu karvėms suduoti lengvai įsisavinamų, peroralinių kalcio druskų preparatų, siekiant papildyti organizmo rezervus. Švedų mokslininkai Ringarp (1967), Jonsson (1970) siūlo tik kalcio chloridą su hidroksiceliulioze sugirdyti likus vienai dienai iki veršiavimosi, veršiavimosi metu ir vieną ar du kartus po jo. Mūsų gauti duomenys parodė, kad sugirdžius *Kalcifostilio* (jo sudėtyje yra kalcio chlorido, dikalciofosfato, magnio chlorido, kalio chlorido, karboksimetilceliuliozės, benzalkonio chlorido, išgryninto vandens) du kartus iki veršelio atsivedimo ir kartą po jo, kalcio ir neorganinio fosforo kiekis karvių kraujyje, nors ir svyravo fiziologinės normos ribose, nuo parezės po veršelio atvedimo kaip reikiant neapsaugojo. Goff, Horst (1993) teigimu, kai kurioms karvėms po veršelio atsivedimo trūkstant daugiau kaip 10 g endogeninio kalcio, peroraliniai kalcio druskų tirpalai ne visada veiksmingi, o kai trūksta mažiau kaip 9 g endogeninio kalcio, peroraliniai mineralų preparatai veiksmingi. Hernandez et al. (2004) teigimu, sugirdžius kalcio chlorido tirpalo tik po veršelio atvedimo kalcio kiekis karvių kraujo serume sumažėjo ir profilaktinis efektas buvo mažas. Panašūs duomenys gauti sugirdžius *Kalcifostilio* tik po veršelio atvedimo (IV ir V karvių grupės). Galbūt tokiu metu sugirdžius mineralinių medžiagų tirpalų klinikiniai kalcio apykaitos procesai jau būna per daug komplikavęsi ir juos galima pakeisti tik švirkščiant kalcio preparatų į veną. Sugirdžius *Kalcifostilio* daugiau kaip tris kartus iki veršelio atvedimo ir kartą po jo, karvės nuo parezės po veršelio atvedimo nebuvo neapsaugotos. Horst et al. (1994) nustatė, kad karvėms, prieš

veršiavimąsi nuolat gaunančioms padidintus kalcio kiekius, slopinama PTH ir $1,25(OH)_2$ vitamino D sekrecija. *Kalcifostilio* gavusios karvės veršiavimosi dieną ir vieną (I gr) ar du kartus (II gr.) po veršelio atvedimo pareze nesusirgo. Minėtų grupių karvėms hipokalcemijos nenustatėme (I gr.) arba ji buvo nežymi ir trumpalaikė (II gr.).

Tyrimų metu nustatėme, kad neorganinio fosforo kiekis kraujyje po veršelio atvedimo sumažėjo toms karvėms, kurioms buvo gerokai sumažėjęs ir kalcio kiekis. PTH fosforą šalina iš organizmo, o kalcio kiekį didina, todėl dažnai su hipokalcemija pasireiškia ir hipofosfatemija. 3,8–28 proc. pareze po veršelio atvedimo susirgusioms karvėms sušvirkštus intraveninių kalcio druskų tirpalų, jos neatsikelia. Tokia būklė vadinama gulinčios karvės sindromu ir dažniausiai siejama su hipofosfatemija (Menard-Thompson, 2007). Mūsų tyrimų metu šis sindromas pasireiškė kai kurioms kontrolinės grupės karvėms (VII) ir karvėms, kurioms *Kalcifostilio* buvo sugirdyta tik po veršelio atvedimo (IV ir V) arba daugiau kaip tris kartus iki veršelio atvedimo ir kartą po jo (VI). Galima teigti, kad sugirdžius *Kalcifostilio* tik po veršelio atvedimo, karvėms jau buvo prasidėjusi klinikinė hipokalcemija. Sumažėjus kalcio kiekiui, padidėja kraujyje PTH kiekis (Špakauskas ir kt., 2006), kuris skatina neorganinio fosforo išskyrimą su šlapimu ir seilėmis, todėl toms karvėms, nors kalcio kiekis ir atsistato, tačiau fosforo vis tiek trūksta, o tai sukelia raumenų ląstelių nekrozę (Goff, 1998). Martz et al. (1999) teigimu, hiperkalcemija ir didelis kalcio kiekis pašare slopina fosfatų rezorbciją iš virškinamojo trakto, todėl per anksti sugirdytos ir didelės kalcio chlorido dozės slopino neorganinio fosforo ir kalcio apykaitos adaptacinius mechanizmus.

Nustatėme, kad magnio kiekis karvių kraujyje veršiavimosi metu turi tendenciją didėti, o didesnius magnio kiekius besiveršiuojančių karvių kraujyje nustatė ir Adams et al. (1996). Karvių organizme magnis yra kalcio antagonistas, nes jie tarpusavyje konkuruoja dėl prisitvirtinimo vietos ląstelėje, o magnio jonai pakeičia kalcio jonus ant membraninių receptorių ir surišimo vietų fermentuose (Niemack, 1985). Todėl, sumažėjus kalcio kiekiui organizme, magnio kiekis padidėja. Tyrimų metu gauti duomenys parodė, kad magnio kiekis karvių kraujyje po veršelio atvedimo didėja, o kalcio – mažėja. Mažesnius kalcio ir magnio kiekius nustatėme tik toms karvėms, kurioms *Kalcifostilio* buvo sugirdyta iškart po veršelio atvedimo ir antrą kartą kitą dieną.

Mūsų gauti duomenys parodė, kad gliukozės kiekis karvių kraujyje veršiavimosi dieną ir 0–12 val. laikotarpiu po veršelio atvedimo turėjo tendenciją didėti. Gliukozės kiekio padidėjimą ir insulino kiekio sumažėjimą, kai karvėms pasireiškė hipokalcemija, nustatė ir Horst et. al. (2003). Ši kalcio ir gliukozės kiekio kraujyje priklausomybė mūsų tyrimuose ryškiausiai nustatoma karvėms, kurioms *Kalcifostilio* buvo sugirdyta tik po veršelio atvedimo. Subnormalų gliukozės kiekį karvių kraujyje nustatėme antrą dieną po veršelio atvedimo. Šeštą ir dešimtą dieną gliukozės kiekis kraujyje buvo fiziologinės normos ribose tų karvių, kurios *Kalcifostilio* gavo veršiavimosi dieną ir

iškart po veršelio atvedimo bei kitą dieną,. Manome, kad karvėms, kurioms hipokalcemija nepasireiškė arba ji buvo trumpalaikė, apetitas nesutriko ir virškinamasis traktas bei kepenys geriau funkcionavo. Taip pat tos karvės greičiau kompensavo energijos trūkumą. Gliukozės kiekis, kuris sunaudojamas pieno gamybai laktacijos pradžioje, stipriai viršija karvės organizmo galimybes ja apsirūpinti, todėl šiuo laikotarpiu gali pasireikšti ketozė. Karvėms, kurioms *Kalcifostilio* buvo sugirdyta vieną ar du kartus tik po veršelio atvedimo, dešimtą dieną nustatyti mažesni gliukozės kiekiai. Kelton et. al. (1998) teigimu, karvėms, sirgusioms klinikine hipokalcemija, ketozė pasireiškia 9 kartus dažniau nei nesirgusioms. Pagal mūsų tyrimų duomenis, sugirdžius *Kalcifostilio* veršiavimosi dieną ir kartą ar du po veršelio atvedimo pareze karvės nesusirgo, o šeštą ir dešimtą dieną tokių karvių gliukozės kiekis kraujo serume kito fiziologinės normos ribose. Todėl galima teigti, kad tinkamas šio preparato panaudojimas teigiamai veikia karvių energijos apykaitą.

Gyvuliui senstant, kauluose mažėja aktyvių osteoklastų kiekis. Pirmaveršės karvės labai retai suserga klinikine hipokalcemija. Kuo mažesnis aktyvių osteoklastų kiekis, tuo mažesnis atsakas į PTH, tuo mažiau iš kaulų mobilizuojama kalcio (Goff, 2000). Didėjant karvės amžiui mažėja PTH receptorių audiniuose, o žarnose 1,25-dihidroksivitaminui D jautrių ląstelių skaičius, todėl iš pašaro virškinamajame trakte pasisavinama mažiau kalcio (Horst, 1990). Šios priežastys lemia, kad pareze po veršelio atvedimo sergančių karvių kraujyje nustatomi didesni vitamino D ir PTH kiekiai (Špakauskas ir kt., 2006). Taip pat nustatyta, kad trečios ir vėlesnių laktacijų karvės veršiavimosi metu išsiskiria daugiau estradiolio 17 β, kuris slopina kalcio pasisavinimą iš kaulų (Kurosaki et al., 2007). Remiantis tyrėjų gautais duomenimis, taip pat veterinarijos gydytojų ir gyvulių augintojų patirtimi, galima teigti, kad karvėms senstant ir vieną kartą pareze po veršelio atvedimo susirgusioms karvėms yra didelė rizika susirgti pareze po kito veršelio atsivedimo. Bandymų metu nustatėme *Kalcifostilio* profilaktinį efektyvumą apsaugant ankstesnio veršiavimosi metu klinikine hipokalcemija susirgusias karves.

Mūsų gauti duomenys rodo, kad veršiavimosi metu karvių kraujyje gerokai sumažėja kalcio ir fosforo kiekiai. Karvėms sugirdžius *Kalcifostilio* du kartus iki veršiavimosi, nuo parezės visiškai apsaugoti nepavyko, bet visos susirgusios karvės pasveiko vieną kartą sušvirkštus kalcio preparatų. Daugelis tyrėjų (Jönsson, Pehrson 1970, Pehrson et al., 1989, Goff et al., 1996, Oetzel, 1996, Agger et al., 1997) nurodo suduoti pirmąsias peroralines kalcio druskų tirpalų dozes vieną arba du kartus likus 24–12 val. iki veršelio atvedimo, o po jo dar kartą arba du kartus 24 valandų laikotarpiu. Kadangi bandymo metu karvės *Kalcifostilio* negavo per veršiavimąsi metu ir po veršelio atvedimo, profilaktinis efektas buvo nepakankamas, bet buvo lengvesnis gydymas ir gyvuliai greičiau pasveiko.

Ankstesniais metais sirgusioms pareze po veršelio atvedimo karvėms, sugirdžius *Kalcifostilio*

du kartus prieš veršiavimąsi ir veršiavimosi metu, jos pareze nesusirgo. Parezei po veršelio atvedimo, kai prasideda intensyvi krekenų sintezė, būdingas staigus kalcio kiekio kraujo serume sumažėjimas (Goff et al., 1997). Iki veršiavimosi likus 2–1 parai kraujotaka pieno liaukoje stipriai suintensyvėja. Suintensyvėjusi kraujotaka turi įtakos ir dideliam mineralinių medžiagų patekimui į krekenas (Davis et al., 1979). Karvių krekenų litre yra 1,7–2,3 g kalcio ir 0,9 g fosforo, o laktacijos pradžioje iš organizmo kasdien paimama apie 20–30 g kalcio ir 10–15 g fosforo pienui gaminti. Karvių kraujyje cirkuliuoja 3 g kalcio ir 1–2 g fosforo, o audinių skystyje – 8–9 g kalcio ir 4 –7 g fosforo, todėl veršiavimosi metu produktyvių karvių kalcio ir fosforo kiekis kraujyje sumažėja (Goff, 2005). Besiveršiuojančiai karvei sugirdžius *Kalcifostilio*, lengvai įsisavinamos jame esančios kalcio ir fosforo druskos papildo minėtų mineralų rezervus organizme bei apsaugo nuo klinikinės hipokalcemijos ir hipofosfatemijos.

IŠVADOS

1. Kalcio, neorganinio fosforo ir parathormono kitimo nustatymas karvių kraujyje veršingumo pabaigoje, veršiavimosi metu ir po veršelio atvedimo leidžia nustatyti hipokalcemiją ir hipofosfatemiją ankstyvoje stadijoje ir prognozuoti tinkamiausią *Kalcifostilio* sugirdymo laiką
2. Nustatyta makroelementų kaita veršingumo metu rodo, kad kritiškiausias momentas susirgti hipokalcemija ir hipofosfatemija yra pirma diena po veršelio atvedimo, kai kalcio ir neorganinio fosforo kiekis sumažėja (atitinkamai iki 1,2±0,6 mmol/l ir 0,76±0,43 mmol/l) žemiau fiziologinės normos.
3. Veršiavimosi dieną sumažėjus kalcio kiekiui karvių kraujyje tuo pačiu metu parathormono kiekio padidėjimas (nuo 1,15±0,35 iki 9,12±4,39 µmol/l) skatina minėto mineralo pasisavinimą iš kaulų.
4. Sugirdžius *Kalcifostilio* prieš pat veršelio atvedimą ir kartą arba du po jo, kalcio ir neorganinio fosforo kiekis karvių kraujyje, palyginti su preparato negavusių karvių, ženkliai padidėjo ($p<0,05$) ir jos nesusirgo pareze.
5. Daugkartinis *Kalcifostilio* sugirdymas iki veršelio atvedimo likus daugiau kai trims dienoms slopina endogeninio kalcio pasisavinimą ir neapsaugo nuo klinikinės bei subklinikinės hipokalcemijos ir hipofosfatemijos.

PASIŪLYMAI

1. *Kalcifostilio* karvėms reikia sugirdyti veršiavimosi dieną, iškart po veršelio atvedimo ir po vienos dienos.
2. Pradėti naudoti *Kalcifostilį* veršingoms karvėms ne anksčiau kaip likus iki veršiavimosi dviem dienoms.
3. Karvių subklinikinei hipokalcemijai ir hipofosfatemijai nustatyti kraujo mėginius biocheminiams tyrimams reikia imti veršiavimosi dieną ir dvi dienas po veršelio atvedimo.

TRUMPOS ŽINIOS APIE DOKTORANTĄ

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