DYNAMICS OF CALCIUM, PHOSPHORUS AND MAGNESIUM IN COW SERUM FOLLOWING ORAL ADMINISTRATION OF KALCIFOSTILIS SOLUTION DURING AND AFTER PARTURITION

Algimantas Matusevičius*, Danielius Starevičius, Vytautas Špakauskas, Irena Klimienė,
Algis Černauskas, Saulius Petkevičius
Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania
*Correspondence to: A. Matusevičius, E-mail: amatusevicius@lva.lt, Tel./Fax: +370 37 363559

Abstract. The present study was undertaken to determine the prophylactic efficacy of a mix of calcium (Ca) and phosphorus (P) salts (Kalcifostilis) administered orally for replenishing of mineral deficit in post-parturient cows. Two experiments were performed to identify the most effective time for Kalcifostilis administration, to evaluate its prophylactic efficacy and to prepare the optimal scheme for use. Kalcifostilis was administered as a single oral dose of 500 ml gel-solution. In the first experiment, 65 pregnant third and fourth gestation dairy cows were divided randomly into 4 experimental (groups 1–4) and control group (group 5). Administration of Kalcifostilis was as follows: group 1 – at parturition (pt) and <6h post pt; group 2 – day before pt, at pt and <6 h post pt, group 3 – <6 h post pt, and group 4 – once daily every second day three doses pt and <6h post pt. In groups 1–2 significantly higher Ca and P levels compared to the controls ($p<0.05$) were observed at pt and post pt (2.12–2.25 and 1.75–1.84 mmol/l). In group 4, Ca and P serum levels were comparable to the physiological norm (1.79–2.04 and 1.29–1.44 mmol/l, respectively), but not prevented mineral deficit as 4 cows from 10 developed paresis. In controls before pt, on pt and day post pt levels of Ca and P in serum were reduced and after 1–6 days post pt returned to physiological norm.

For the second experiment 50 cows of fourth and fifth gestation, which previously had post-parturient paresis were randomly divided into two experimental (groups 1, n=20 and group 2, n=15) and control group (group 3, n=15). Administration of Kalcifostilis was as follows: group 1 – twice at 24h interval 1–2 days before pt and group 2 − at pt and <6h post pt. In group 1, 12 cows of 20 had no post-pt paresis (60% efficiency). Furthermore, in group 2, high prophylactic efficacy of 93.3% was reached because 14 cows of 15 had no post-pt paresis. In group 2, the probability of post-pt paresis was 6-fold lower compared to group 1. In the control (group 3), 13 cows of 15 developed post-pt paresis (87%).

In conclusion, the results showed that Kalcifostilis for the prophylaxis of paresis was most effective when administred on pt day and <6h post pt (efficacy 93.3–100%).

Keywords: pregnant cows, Kalcifostilis, calcium, phosphorus, magnesium.

KALCIO, FOSFORO IR MAGNIO KIEKIO KAITOS DINAMIKA KARVIŲ KRAUJYJE SUGIRDŽIUS KALCIFOSTILĮ VERŠINGUMO METU IR VERŠINGUMUI PASIBAIGUS

Algimantas Matusevičius, Danielius Starevičius, Vytautas Špakauskas, Irena Klimienė,
Algis Černiauskas, Saulius Petkevičius
Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas, Kaunas, Tilžės g. 18, LT-47181
tel. +370 37 36 35 59; el. paštas: amatusevicius@lva.lt

Santrauka. Šio darbo tikslas – nustatyti kalcifostilio profilaktinį efektyvumą sugirdant jį peroraliai veršingoms karvėms. Kalcifostilio sudėtyje yra 30 g kalcio chlorido, 4 g natrio dihidrofosfato, 5 g magnio chlorido, 0,5 g kalio chlorido, 0,02 g benzalkonio chlorido, 0,4 g karboksimetilceliuliozės, išgryninto vandens – iki 100 ml. Atlikti du bandymai, kurių metu nustatėme tinkamiausią kalcifostilio naudojimo laiką, profilaktinį efektyvumą ir optimalią naudojimo schemą. Kalcifostilis gelio pavidalu sugirdytas karvėms po 500 ml.

Pirmuoju bandymu 65 trečio ir ketvirto veršingumo karvės suskirstytos į keturias bandomąsias ir vieną kontrolinę grupes. Kalcifostilis panaudotas taip: I grupės karvėms pirmą kartą sugirdytas veršiavimosi metu, o antrą kartą – per pirmąsias 6 val. po veršiavimosi; II gr. – pirmą kartą sugirdytas likus dienai iki veršiavimosi, antrą kartą – veršiavimosi metu, o trečią kartą – po veršiavimosi praėjus 6 val.; III gr. karvėms – praėjus 6 val. po veršiavimosi ir IV gr. – tris kartus su dviejų dienų intervalu iki veršiavimosi ir ketvirtą kartą – praėjus 6 val. po veršiavimosi. I ir II grupės karvių kraujyje po veršiavimosi nustatytas statistiškai reikšmingai (2,12–2,25 ir 1,75–1,84 mmol/l) didesnis ($p<0,05$) Ca ir P kiekis palyginti su kontrolinėmis. IV gr. karvių kraujo serume Ca ir P kiekis buvo artimas fiziologinei normai, tačiau nepakankamai saugojo nuo mineralų trūkumo, nes keturios iš dešimties karvių susirgo pareze po veršiavimosi. Kontrolinėje grupėje prieš veršiavimąsi, veršiavimosi metu ir praėjus dienai po veršiavimosi karvių kraujyje Ca ir P sumažėjo ir fiziologinę normą pasiekė po 1–6 dienų.

Antrajam bandymui parinkta 50 ketvirtos ir penktos laktacijos karvių, po ankstesnių veršiavimųsi sirgusių pareze. Jos suskirstytos į dvi bandomąsias ir vieną kontrolinę grupes. Kalcifostilį davėme taip: I gr. (n-20) karvėms sugirdėme du kartus su 24 val. intervalu likus 2–1 dienai iki veršiavimosi; II gr. karvėms (n-15) sugirdėme veršiavimosi metu ir praėjus 6 val. po veršiavimosi. I gr. po veršiavimosi pareze susirgo 12 iš 20 karvių (60 proc.). II gr. kalcifostilis buvo

efektyvesnis – 93 proc., nes 14 karvių iš 15 pareze nesusirgo. II karvių grupėje galimybė susirgti pareze buvo 6 kartus mažesnė palyginti su I gr. ir kontrolinės grupės (13 iš 15 susirgo pareze) karvėmis (87 proc.).

Geriausias profilaktinis efektas (93,3–100 proc.) gaunamas, kai kalcifostilis sugirdomas karvei veršiavimosi dieną ir antrą kartą tuoj pat po veršiavimosi. Daugkartinis kalcifostilio sugirdymas iki veršelio atvedimo likus daugiau kaip trims dienoms netikslingas, nes slopina organizmo pasiruošimą paimti endogeninį kalcį bei fosforą iš kaulų ir neapsaugo nuo klinikinės bei subklinikinės hipokalcemijos ir hipofosfatemijos.

Raktažodžiai: veršingos karvės, kalcifostilis, kalcis, fosforas, magnis.

Introduction. Many metabolic disorders of cows occur after parturition (pt). In Lithuania, according to the State Food and Veterinary Service of Lithuania, more than 20.0% of cows develop post-pt paresis every year.

At the time of pt some changes in hormone balance take place, resorption of nutritional materials from the digestive tract becomes slower and intensive synthesis of colostrum starts. These changes cause sudden decrease of calcium (Ca) and inorganic phosphorus (P) in serum. On the other hand, the cow is not capable of complet exploiting its endogenic reserves of calcium (Goff et al., 2005; Kurosaki et al., 2007).

The shortage of Ca and P causes changes in cell membrane permeability impairing muscle contraction, which results in muscle paresis. Paresis is observed in 10% of pt and post-pt cows. Clinical hypocalcaemia often causes decrease of inorganic P, therefore more than 20% of post-pt cows with paresis develop recumbent cow syndrome (Menard and Thompson, 2007).

Subclinical hypocalcaemia is difficult to diagnose in a clinical way, although it is usually recognized during the first days after pt in more than 50% of cows with the third or subsequent lactations (Reinhart et al., 1988). Awareness of the mechanisms of hypocalcaemia and hypophosphataemia and the time they are most likely to manifest themselves allows us to choose more flexible methods of prophylaxis and prevent decreases of Ca and inorganic P in the blood of cows during and after pt.

Calcium chloride water solutions can be dissociated easily in the digestive tract; therefore Ca^{2+} can be easily moved into blood plasma by means of passive transport. The calcium chloride gel preparation do not resolve as fast as its analogue water solution, it causes less irritation to gastrointestinal mucosa and is less likely to provoke aspiration pneumonia (Thisling-Hansen et al., 2002).

Cows with small Ca deficit (< 4 g of the total amount of Ca contained in the body fluids) can be administered oral calcium solutions instead of intravenous injections. Moreover, prophylactic effect of calcium chloride solutions was observed when oral solutions were administered to parturient cows. Several studies have been carried out. However, there is no unanimous opinion regarding most appropriate time of mineral administration to the cows. Majority of producers suggest that 4 doses of such preparations should be administered. The first two administrations are recommended before pt, and the remaining two doses should be given immediately after and on the day following pt (Thisling-Hansen et al., 2002; Kurosaki et al., 2007).

At the time of pt, the cow's appetite decreases, it consumes less feed and gets less minerals. Therefore during this period, in which the animal cannot exploit its endogenic resources of Ca and inorganic P, oral administration of Ca and P salt solutions is highly advisable, as they reabsorb fast from the digestive tract and can restore the resources of the minerals.

In our recent study it was shown that mineral substances of Kalcifostilis are well absorbed from the digestive tract and highest concentrations in serum were detected 3–5h after administration (Matusevičius et al., 2008). In addition, it was determined (Starevičius et al., 2007) that pt day and the first day after pt are the most critical time for paresis development. During these critical days, the concentration of Ca (1.34±0.45 mmol/l) and of P (1.11±0.1 mmol/l) in the blood fell below physiological norm, while the amount of parathormone (PTH) increased from 0.84–1.94 to 8.32–9.72 µmol/l, respectively. It is well known, that Ca, inorganic P and magnesium (Mg) salts have been widely used in veterinary practice for treatment and prevention of metabolic diseases. However, even today both treatment and prophylaxis of these diseases are complicated and it is not always clear when it is the best time to administer these supplements of minerals during pregnancy.

The present study was undertaken to determine the prophylactic efficacy of Kalcifostilis against hypocalcaemia and hypophosphataemia in pregnant cows. In addition, the aim of this study was to identify the most effective time for Kalcifostilis administration, to evaluate its prophylactic efficacy and to prepare the optimal scheme for use.

Materials and methods

The oral gel-solution Kalcifostilis composed of 30 g calcium chloride, 5 g magnesium chloride, 0.5 g potassium chloride, 4 g natrium dihydrogen phosphate, 0.02 g benzalconic chloride, 0.4 g carboxymethyl cellulose and up to 100 ml of purified water was developed.

Two experiments were undertaken to investigate the prophylactic efficacy of Kalcifostilis in March–April 2008 and January–March 2009. The first experiment on healthy cows was carried out at the animal farm of Practical Training and Testing Centre of Lithuanian Veterinary Academy. Seventy Lithuanian Black and White cows used in tests were pregnant third and fourth gestation with comparable insemination time and had a body weight of about 400 kg. In the first experiment, 65 cows were divided into 4 experimental (groups 1–4) and controls (group 5) according to administration time of Kalcifostilis (Table 1). Administration of Kalcifostilis was as follows: group 1 – during pt and <6 h after, group 2 – 1 day before pt, on the day of pt and <6 h post-pt,

group 3 – <6 h post-pt, and group 4 – 3 and more times before pt and once after. Kalcifostilis was administered as a single oral dose of 500 ml gel-solution. Serum samples were collected 5–1 days before pt, during pt and within 0–6 h after pt, also 1, 2, 6, 10 days after pt.

Table 1. **Scheme of Kalcifostilis administration in the first experiment**

group 1 (n=13) 1 − on pt*, 2 − <6 h post pt*	group 2 (n=13) 1 − day before pt*, 2 − at pt*, 3 − <6 h post pt*
group 3 (n=15) 1 − <6 h post pt*	group 4 (n=10) 1 − >3 times before pt* with 48 h interval 2 − <6 h post pt*
group 5 (n=14) controls	

*— parturition

For the second experiment, 50 cows of fourth and fifth gestation, which during the previous years had post-pt paresis of different severity, were divided into two experimental groups (group 1, n=20 and group 2, n= 15) and controls (group 3, n=15) (Table 2). According to insemination date and cows clinical features in regard to pt, 1–2 days before pt cows in group 1 were administered *per os* with 24 h interval 500 ml of Kalcifostilis. In group 2, cows were twice treated with 500 ml of Kalcifostilis on pt day and <6h post-pt. Blood samples were taken before administration of Kalcifotilis and 5 h after the second treatment. Parturition and general clinical features of the cows were observed. In both experiments control and experimental cows were fed diet based of hay, cornmeal silage, haylage, slices of sugar beet, barley meal, chalk and white salt. The cows had free access to water.

Table 2. **Scheme of Kalcifostilis administration in the second experiment**

group 1 (n=20) 1 − 1-2 days before pt* with 24 h interval	group 2 (n=15) 1 − at pt* 2 − <6 h post pt*
group 3 (n=15) controls	

*— parturition

Blood samples were taken from the *vena coccygea* and collected into disposable plastic anticoagulant-free tubes *Venoject*. Blood serum was separated by 10 min centrifuging at the speed of 2000 rotations per minute. Serum was placed into Ependorf tubes with caps. The remaining serum samples were frozen and kept at -20 °C temperature. Serum samples were simultaneously de-frozen and levels of Ca, Pn and Mg were measured using biochemical analyzer Eos-Bravo (Italy) and Hospitex diagnostic (Italy) reagents.

The data on the levels of serum Ca, P and Mg in cows following oral administration of Kalcifostilis solution during and after pt were analyzed by analysis of variance (ANOVA). Comparison of the means of the main effects was performed using *Fisher's t*-test multiple method with a significance level of 0.05. The test was performed on the least square means.

The research was carried out in accordance with Good Clinical Practice and the Law of Animal Care, Housing and use of the Republic of Lithuania (Žin., 1997, Nr. 108-2728).

Results

The serum levels of calcium in all groups varied within physiological (Klimiene et all., 2005) norm (2.15 mmol/l) 1–5 days before pt (Fig. 1). In control cows, acute hypocalcaemia was recorded within the first 6 h after pt 1.14±0.69 mmol/l and after 24 h (1.52±0.51 mmol/l). In controls, the concentration of calcium returned to physiological norm (2.97±0.69 mmol/l) on the 6th day post-pt.

After treatment with Kalcifostilis during and <6 h post-pt, Ca serum level varied within physiological norm during and post-pt (group 1) with the corresponding mean values 2.32±0.67 mmol/l and 2.03±0.56 mmol/l and was significantly higher compared to controls ($p<0.05$). In group 1, statistically significantly higher serum concentration of Ca compared to controls was recorded on day 1 post-pt with mean value of 2.12±0.56 mmol/l ($p<0.05$). Cows in group 1 did not develop parturient paresis.

When Kalcifostilis was administered <6h post-pt (group 3, Fig. 2), the serum Ca level reduction was significantly lower compared to groups 1 and 2 (Fig. 2). When Kalcifostilis was administered before pt and <6h post-pt (group 2), the serum Ca level remained within physiological norm during the whole period of study and during the most critical time was statistically significantly higher ($p<0.05$) compared to controls. However, the level of P in serum decreased in one of the cows up to 0.9 mmol/l and the animal developed recumbent cow syndrome.

When Kalcifostilis was administered >3 times before and once post-pt (group 4, Fig. 1), the decrease of serum Ca level was most acute at the time and post-pt with the corresponding mean values 1.85±0.24 mmol/l and 1.79±0.26 mmol/l. One day later, the serum Ca level in this group varied within physiological norm. However, 2 cows from 10 developed paresis and 2 animals had the recumbent cow syndrome.

The changes of P level were followed by similar pattern of Ca serum levels in the control cows. The amount of Ca decreased in control group during pt, <6 h post-pt and 1 day post-pt to 1.57±0.57; 1.16±0.74 ($p<0.05$) and 1.38±0.67 mmol/l (Fig. 2).

When Kalcifostilis was administered during pt and immediately post-pt (group 1), the decrease of P in serum post-pt was short-termed, the mean value 1.54±0.5 mmol/l. One day post-pt, in group 1 a significantly higher level of P in serum 1.75 mmol/l±0.5 compared to controls

1.38±0.67 mmol/l was registered (p>0.05). In group 2, where Kalcifostilis was administered 1 day before pt, at pt and immediately post-pt significant decrease of P was recorded during pt 1.21±0.56 mmol/l compared to controls (p<0.05). Furthermore, in group 2 level of P increased to pre-calving level (1.72- 1.8 mmol/l). In group 3, where Kalcifostilis was used only post-pt the level of P decreased significantly compared to controls during pt, within 6h and 1 day post-pt to 1.42±0.49; 1.39±0.44 and 1.37±0.5 mmol/l, respectively.

>3 administrations of Kalcifortilis before and once post-pt (group 4) did not protect the cows from a decrease of serum P. During the experiment the recumbent cow syndrome was more frequently observed in the controls, also in groups 3 and 4 (Fig. 2).

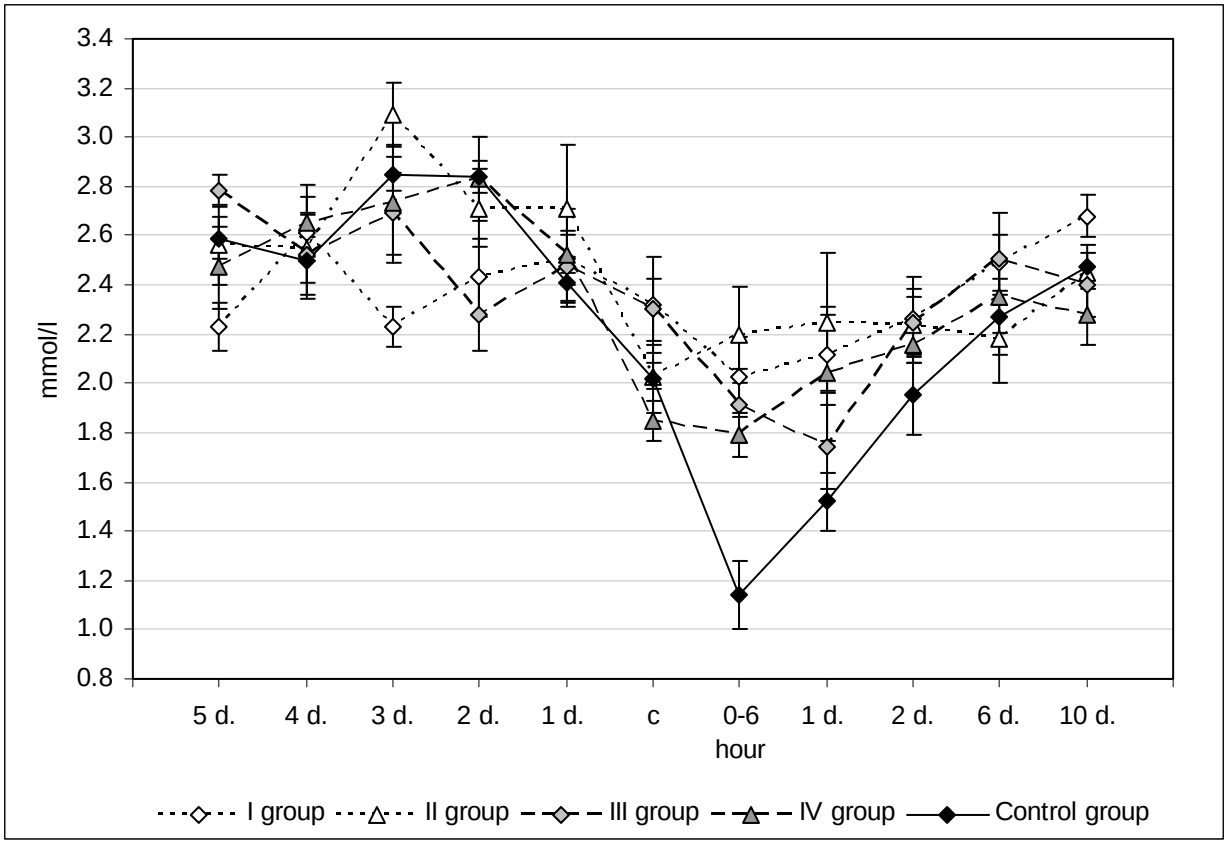

Fig. 1. **Variation of calcium in blood serum of cows after oral administration of Kalcifostilis at different times with regard to parturition time;** c – parturition day

The serum levels of Mg during the experiment varied within physiological norm. It had a tendency to increase during and post-pt, but there were no significant differences between groups.

The results of the second experiment have shown that before pt in all paresis-prone cows the level of serum Ca and P decreased 1.87±0.413 mmol/l and 1.31±0.163 mmol/l and Mg increased 1.39±0.166 mmol/l. Before administration of "Kalcifostilis", the first symptoms of paresis, such as muscle tremor and cold extremities were observed in 80 % of cows. In group 1, where cows were treated with "Kalcifostilis" 2 times before pt, 8 cows of 20 developed post-parturient paresis thus the prophylactic effect was 60% compared to controls. Post-pt cows have shown signs of hypersensitivity, excitability, fine tremors over the flanks and triceps, and display ear twitching and head bobbing. Furthermore, cows were obtund, anorectic, had a dry muzzle, subnormal body temperature, and cold extremities. However, 1–2 h after intravenous injections of 250 ml of 38 % calcium borogluconate salt, the animals were able to stand up, started to eat feed and to drink water. In group 1, the level of Ca compared to the data before administration of "Kalcifostilis", decreased from 2.15±0.425 mmol/l to 1.71±0.509 mmol/l, P from 1.41±0,261 mmol/l to 1.05±0.388 mmol/l (p=0.085). The cows which did not develop paresis had an elevated amount of Ca after administration of Kalcifostilis 2.44±0.346 mmol/l compared to the level before administration − 1.87±0.413 mmol/l (p=0.027). The level of P increased from 1.31±0.163 mmol/l to 1.54±0.461 mmol/l (p<0.05).

After administration of Kalcifostilis (group 2) on pt day and 5-6 h post-pt, 1 cow of 15 cows had paresis, while 14 cows showed weak clinical symptoms of paresis, such as muscle tremor and cold extremities. The amount of Ca after administration of Kalcifostilis in serum of cows without symptoms of paresis decreased from 2.78±0.0,121 mmol/l to 2.13±0.432 mmol/l (p=0.03), and

of P from 1.90±0.425 mmol/l to 1.59±0.254 mmol/l ($p>0.05$) compared to the initial data, but the level of Ca and P ranged within physiological norm. In control (group 3) 13 cows from 15 developed post-pt paresis (87%).

However, in case of paresis-prone cows 2 treatments with Kalcifostilis before pt (group 1) were efficient (60%) as 8 cows of 20 had post-pt paresis. After administration of Kalcifostilis on pt day and 5–6 h post-pt (group 2) only 1 cow of 15 had post-pt paresis. Possibility that cows which received Kalcifostilis on pt day and 5–6 h post-pt (group 2) will have postparturient paresis was 6- fold lower compared to cows where preparation was administered before pt (group 1).

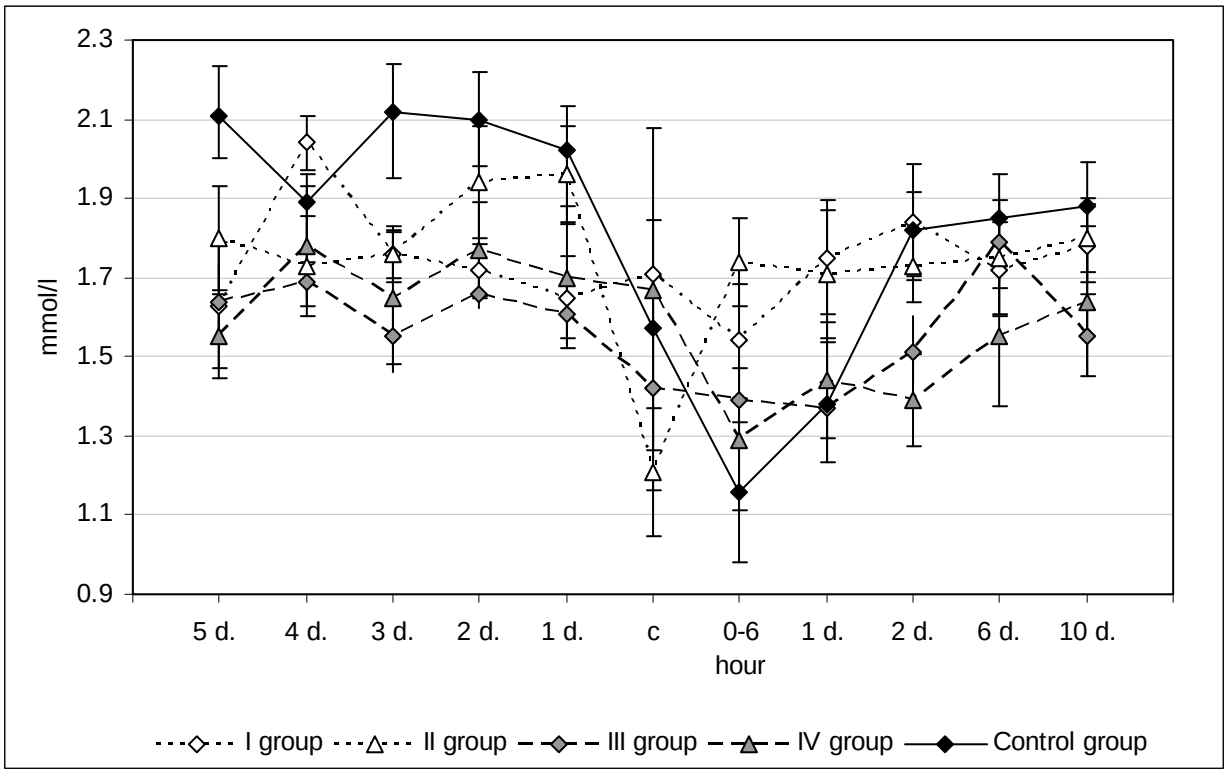

Fig. 2. **Variation of inorganic phosphorus in blood serum of cows after oral administration of Kalcifostilis at different times with regard to parturition time;** c – parturition day

Discussion

Our study demonstrated that Kalcifostilis had a significant effect for protection of cows against post-pt paresis. It was shown, that cows had a tendency to develop post-pt paresis when the level of serum Ca and P was below 2 mmol/l and 1 mmol/l, respectively. This is in concert with a previous studies (Goff, 2000; Larsen et al. 2001; Ruat, 2003) indicating a decrease of Ca in serum before, during and post pt. Goff et al. (2005) determined that serum hypocalcaemia develops post-pt because of an increased amount of Ca in colostrum, as a cow loses about 23 g Ca and 10–15 g P (1 kg cow milk contains 1.3 g Ca and 1 g P; 1 kg of colostrum 2.3 g and 0.9 g, respectively), excreting 10 liters of colostrum during one milking. This amount is 9 fold higher than the total amount of Ca in serum. Therefore it is advisable to use prophylactic means and assure that decrease of Ca and P in serum could be minimal until creation of compensatory mechanisms which allow these minerals to be adopted from the bones. Administration of Kalcifostilis on pt day and <6 h post-pt, i.e. at the most critical time, normalizes the amount of Ca and P in serum of cows. Preparation Kalcifostilis was developed and used *per os* to serve this purpose in Lithuanian cattle farms. Our experimental data revealed that oral administration of Kalcifostilis both at the time of pt and <6h post-pt (group 1), as well as 1 day before pt, on pt day and <6h post-pt (group 2) protected cows from developing paresis. The amount of Ca in serum of these cows during critical period (i.e. on pt day and post-pt) remains higher compared to untreted controls and varies within physiological norm. More than 3 times administration of Kalcifostilis before pt and once post-pt (group 4) failed to protect from paresis; even though the amount of Ca and P in serum varied within physiological norm, 2 cows from 10 have shown recumbent cow syndrome.

The amount of P in serum decreased after pt of cows, which had a severe decrease of Ca (groups 3, 4, and 5). Due to the fact that parathormone (PTH) eliminates P from the body and enlarges the amount of Ca, hypocalcaemia is frequently accompanied by hypophosphataemia. When cows with pt paresis were injected with Ca salt solution and were not able to stand up, the recumbent cow syndrome was diagnosed. This condition was often related to hypophosphataemia (Menard and Thompson, 2007). During our study this

syndrome was observed in control cows (group 5), also in cows which received Kalcifostilis only post-pt (group 3) or >3 times before pt and once post-pt (group 4). As the amount of Ca in blood decreased, PTH level increased (Bandzaitė et al., 2005) and it stimulated elimination of P with urine and saliva. Therefore, even though the amount of Ca is restored, lack of P remains, which triggers muscle cell necrosis (Goof, 1998). Martz et al., (1999) claimed that hypercalcaemia and large amounts of Ca in cow feed suppressed resorption of phosphates from the digestive tract, therefore premature oral administrations of large amounts of calcium chloride suppressed adaptive mechanisms of Ca and P metabolism. It is also possible that the amount of P increased in blood because large concentration of Ca contained in Kalcifostilis, which suppressed PTH secretion and the decreased amount of PTH slowed down separation of inorganic P with urine and saliva (Goff, 2000).

PTH activates vitamin D $1.25(OH)_2$, which facilitates acquisition of Ca from feed in 24 h after the increase of PTH. Within 48 h, an enlarged amount of PTH triggers osteoclast transformation and their adhesion to the surface of bone tissue, when bone matrix is removed and Ca becomes free. In case of paresis these processes develop much slower (Reinhardt et al., 1988). In older cows the number of active osteoclasts is reduced. The fewer osteoclasts, the weaker their response to PTH, which results in a lower amount of Ca resorbed from bones to blood (Goff, 2000). Along with cow's age the amount of PTH receptors in tissues diminishes; the intestine also counts a smaller amount of 1.25 dihydroxivitamin D sensitive cells, therefore the digestive tract can absorb a smaller amount of Ca from feed (Horst et al., 1994). These reasons determine that an enlarged amount of vitamin D and PTH in the blood of cows with pt paresis stimulates separation of endogenic Ca and P into blood. To assure that PTH system is constantly active, dry cows' feed must not contain more than 20g Ca/day. However, maintaining such a ratio is rather difficult. Therefore Goff and Horst, (1994); Hernandez et al., (2004) suggested that at the time of pt cows should be given Ca salts *per os*. Goff and Horst (1993) claims that when cows after parturition lack more than 10 g endogenic Ca, oral Ca solutions are not always helpful, whereas when the shortage is less than 9 g Ca these solutions have proved to be effective. Hernandez et al., (2004) points out that oral administration of calcium chloride solution only after pt results in a diminished level of Ca in blood and the prophylactic effect is insufficient. We reproduced similar data during our experiment when Kalcifostilis was administered only after parturition (group 3). It is possible that timing for supplementing the animal's organism with mineral salts *per os* is too late and such deficit can be cured only by intravenous injections of Ca solutions. >3 oral administrations of Kalcifostilis before and once post-pt (group 4) did not protect cows from parturient paresis. Horst et al., 1994 claims that when cows receive enlarged amounts of Ca before parturition time their PTH and $1.25(OH)_2$ vitamin D secretion is suppressed. Cows which received oral Kalcifostilis on pt day and post-pt (group 1) did not develop paresis (Fig. 2), whereas when Kalcifostilis was administered on the day before pt, on pt and post-pt (group 2), one case of recumbent cow syndrome was recorded.

Conclusions

In conclusion, the results from this study indicate that prophylaxis of paresis is most effective when Kalcifostilis is administered orally at pt and the second dose is given immediately post-pt but not <6 h after, (efficacy 93.3–100%). Multiple administrations of Kalcifostilis for >3 days before pt are not recommended, as they suppress the readiness of the organism to adopt endogenic Ca from bones and do not protect from clinical or subclinical hypocalcaemia and hypophosphataemia.

Acknowledgements

The study was supported by the Lithuanian Science Foundation, Grant Nr. T-60/08. We are grateful to Ms. Ramunė Paulikienė for editorial assistance.

References

1. Bandzaitė V, Klimienė I, Špakauskas V, Šilkūnaitė J (2005) Parathormono, kalcitonino ir vitamino D įtaka mineralinių medžiagų kiekiui sveikų ir sergančių karvių kraujo serume. Vet Med Zoot 31: 5–9.

2. Goff JP, Horst RL (1993) Oral administration of calcium salts for treatment of hypocalcemia in cattle. J Dairy Sci 76: 101–108.

3. Goff JP, Horst RL (1994) Calcium salts for treating hypocalcaemia: carrier effects, acid-base balance, and oral versus rectal administration. J Dairy Sci 77: 1451–1456.

4. Goff JP (1998) Macromineral disorders of the transition cow. Vet Clin North Am Food Anim Pract 20: 471–494.

5. Goff JP (2000) Pathophysiology of calcium and phosphorus disorders. Vet Clin North Am Food Anim Pract 16: 319–337.

6. Goff JP, Sánchez JM, Horst RL (2005) Hypocalcemia: biological effects and strategies for prevention. Nutrition Conference The University of Tennessee, p. 6.

7. Hernandez J, Risco CA, Elliot JB (2004) Effect of oral administration of a calcium chloride gel on blood mineral concentrations, parturient disorders, reproductive performance, and milk production of dairy cows with retained fetal membranes. Am J Vet Res 65: 1071–1076.

8. Horst RL, Goff, JP, Reinhardt, TA (1994) Calcium and vitamin D metabolism in the dairy cow. J Dairy Sci 77: 1936–1951.

9. Klimienė I., Špakauskas V., Matusevičius A. Correlation of different biochemical parameters in blood sera of healthy and sick cows. Vet. Res. Commun. 2005. 29(2). P.95–102.

10. Kurosaki N, Yamato O, Mori F, Imoto S, Maede Y (2007) Preventive effect of mildly altering dietary

cation-anion difference on milk fever in dairy cows. J Vet Med Sci 69: 185–192.

11. Larsen T, Moller G, Belelio R (2001) Evaluation of clinical and clinical chemical parameters in periparturient cows. J Dairy Sci 84: 1749–1758.

12. Lietuvos Respublikos gyvūnų globos, laikymo ir naudojimo įstatymas Nr. 8-500 (1997) Valstybės Žinios 108: 2728.

13. Martz FA, Belo AT, Weiss MF, Belyea RL (1999) TrueSbsorbtion of calcium and phosphorus from corn silage fed to nonlactating, pregnant dairy cows. J Dairy Sci 82: 618–622.

14. Matusevičius A, Špakauskas V, Černiauskas A, Klimienė I, Starevičius D (2008) Effect of oral administration of calcium chloride gel on blood mineral concentrations and parturient paresis prophylaxis in cows. Med Weterynaryjna 64: 773–777.

15. Menard L, Thomson A (2007) Milk fever and alert downer cow. Does hypophosphatemia affect treatment response? Can Vet J 48: 487–91.

16. Reinhardt TA, Horst RL, Goff JP (1988) Calcium, phosphorus, and magnesium homeostasis in ruminants. Vet Clin North Am Food Anim. Pract 4: 331–350.

17. Ruat M (2003) Pharmacology of the extracellular calcium, ion receptor. Therapie 58: 395–401.

18. Starevičius D, Matusevičius A, Špakauskas V (2007) Kalcio, fosforo magnio ir gliukozės kaita veršingų ir apsiveršiavusiių karvių kraujyje. Vet Med Zoot 38: 68–73.

19. Thilsing-Hansen T, Jorgensen RJ, Ostergaard S (2002) Milk fever control principles: A review. Acta Vet Scand 43: 1–19.

Received 11 November 2011
Accepted 7 June 2012