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Effect of oral administration of calcium chloride gel on blood mineral concentrations and parturient paresis prophylaxis in cows

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Summary

The goal of the study was to evaluate resorbtion from the digestive tract and the clinical effect of a new calcium gel preparation, containing calcium chloride, kalium chloride, inorganic phosphorus, natrium dihydrophosphate, magnium chloride, carboxy-methylcellulose and benzoalkoniumchloride, for the prevention and treatment of parturient paresis in cows. Four experiments were carried out with 50 healthy and/or parturient paresis-prone cows that received the calcium gel preparation several times orally. The amount of calcium, inorganic phosphorus, magnesium and glucose was measured in the cows' blood serum using Eos-Bravo analyzer and Hospitex reagents; the amount of 25-OH vitamin D was determined by means of the imunofermentation (IFA) method. Orally administered calcium chloride gel preparation is well taken up in the cows' digestive tract, normalizes the amount of calcium, magnesium and inorganic phosphorus in cows' blood serum, as well as protects the cows from developing parturient paresis. The preparation must be administered twice before parturition with a 24 hour interval and its third dosage must be administered at the time of parturition.

Keywords: calcium, magnesium, inorganic phosphorus, cows, parturient paresis, hypocalcaemia

Calcium, magnesium and phosphorus ions are antagonists and metabolic processes among them are strongly interrelated. The lack of these ions and/or inappropriate proportions cause changes in biochemical processes in tissues and triggers severe metabolic diseases such as hypocalcaemia, hypophosphataemia, rachitis, osteodystrophy, tetany, paresis after calving (3, 13, 14, 18). Frequent cases of paresis of dairy cows after calving have been indicated by a number of researchers (1, 8, 17). It has been reported that the concentration of calcium in the plasma of cows with parturient paresis decreases to 1.41 ± 0.11 mmol/l, inorganic phosphorus to 0.66 ± 0.05 mmol/l, while the amount of magnesium is normal or increased (2, 17).

The last two to three weeks of gestation constitute an important phase of the lactation cycle for high producing dairy cows. One management objective for this phase is to provide a period of time prior to the onset of lactation during which the gradual adaptation to the early lactation diet can take place. Another objective is to stimulate homeostatic responses during late gestation in order to metabolically prepare the cow to adapt to the sudden changes that occur at parturition and the onset of lactation. A cow having a higher calcium turnover would be more resistant to severe hypocalcaemia at the onset of lactation than a cow with low turnover, even though prepartal plasma calcium concentration might be the same in both (22, 25).

During their first gestation cows rarely develop paresis, whereas the number of cases of paresis increases with the third or subsequent lactations (10). This is due to increasing productivity and slower compensatory mechanisms of older cows on account of hypocalcaemia.

Many strategies have been used to prevent cow paresis, including feeding a pre-calving diet containing a low Ca concentration (20), and supplementing Ca at calving (4).

Another way to prevent cow paresis is to administer various preparations containing calcium, magnesium and other salts (5) through parenteral routes. However, intravenous or percutaneous injections must be carried out by a veterinarian and, moreover, they cause stress to the animal (7). Therefore preference has been given to per-oral preparations of salts during recent years (6, 12).

When administered a couple of times before and after calving, these preparations prevent the development of

paresis in most cows. In addition, peroral administration can be performed by the owner of the animal and, unlike injections, makes it possible to avoid stress. Mineral materials contained in the preparation has a positive influence on the rumen microflora and can be acquired by the animal in a natural way. Many researchers have determined (9, 16, 24, 26) that the main cause of parturient paresis is a large amount of cations in cows' fodder. Not only do they state the significance of lack of calcium, phosphorus and magnesium but also the importance of the amount of potassium, anions, vitamins and hormones in the etiology of parturient paresis. Data referring to the ability of oral preparations containing calcium chloride and calcium propionate to maintain calcium homeostasis vary. Many authors (11, 29), who have conducted research on calcium chloride emulsion determined that when the emulsion was given to the cows 24 h and 12 h before calving, the amount of calcium in the blood increased. The cows that received this preparation did not develop parturient paresis, whereas 13% of the cows in the control group were affected by this disease after parturition. Authors who conducted research on the effect of emulsion containing calcium chloride and magnesium chloride (21, 28) determined that the preparation is safe, eases parturition and safeguards against parturient paresis. The highest calcium concentration in the blood was detected half an hour after administration of calcium propionate and calcium chloride. The effect was also influenced by the amount of solution and concentration of calcium in it (22). Administration of 10% calcium chloride emulsion protects against experimental and natural hipocalcaemia and parturient paresis. Liepa et al. (2000) demonstrated that compared to peroral administration prophylactic parenteral calcium preparations reestablish the amount of calcium faster.

In summary, it can be stated that calcium, inorganic phosphorus and magnesium salts have been used in veterinary practice for many years for the treatment of animals with metabolic diseases. However, even today treatment of such diseases is not always successful.

The aim of the study was to determine the dynamics of changes in blood concentrations of the active substances contained in the new oral calcium gel preparation elaborated at the Lithuanian Veterinary Academy. The second goal of this study was to evaluate the prophylactic effect of this calcium gel preparation in protecting cows against parturient paresis.

Material and methods

Four experiments were carried out on 50 cows (age range 4-9 years). A gel preparation was of the following composition: calcium chloride ($CaCl_2 \times 6\ H_2O$) – 30.0 g, magnesium chloride ($MgCl_2 \times 6\ H_2O$) – 5.0 g, kalium chloride (KCl) – 0.5 g, natrium dihydrophosphate ($NaH_2PO_4 \times 2H_2O$) – 4.0 g, carboxymethylcellulose 0.3 g, benzalkonium chloride – 0.02 g, and up to 100 ml of distilled water. The preparation was orally administered to the test cows.

The first experiment was carried out on 15 clinically healthy post partal cows (age range 4-6 years). The cows were divided into the test group (n = 10) and the control group (n = 5). Cows in both groups were given feed consisting of hay, cornmeal silage, haylage, slices of sugar-beet and barley meal. Water was given to satiety. Cows in the test group were orally administered 400 ml of the gel preparation described above twice with a 24 h interval, while the cows in the control group did not receive the preparation. Blood samples were taken before administration of the preparation and then 3 h, 5 h, and 24 h after the first administration. Twenty four hours after the first administration, the cows were given the preparation repeatedly and blood samples were taken after 3 h (27 h after the first administration), 5 h (29 h after the first administration), and 24 h (48 h after the first administration).

The second test was carried out on 10 clinically healthy cows (age range 5-6 years). 500 ml of the preparation was orally administered to 6 cows in the test group with a 24 h interval. Four cows in the control group did not receive the preparation. To minimize the stress to the animals, blood samples were taken more rarely during those intervals which showed more substantial changes of the active substances of the preparation in cows' blood during the first test, i.e. before administration of the preparation and 5 h, 24 h after the administration, and 5 (29) h and 24 (48) h after the second administration.

The aim of the third study was to assess prophylactic efficacy of the calcium gel preparation against parturient paresis of cows. 1-2 days before the expected parturition, 500 ml of the preparation was orally administered with a 24 h interval to 17 cows that already had a history of parturient paresis after almost every parturition from 3 to 6 calving. Blood samples were taken before administration of the preparation and 5 h after the second administration. The parturition process and the clinical status of cows after parturition were observed.

During the fourth experiment the calcium gel preparation was orally administered to 8 cows with a history of parturient paresis during recent years. The administration of the preparation was carried out twice before calving: 2 days and 1 day respectively, and the third administration was carried out at the time of parturition.

Blood samples were collected into disposable plastic tubes. The separated blood serum was removed after the blood clot had been formed (but not later than after 4 h). The amount of calcium, inorganic phosphorus, magnesium and glucose in blood serum was measured in the laboratory using the Eos--Bravo analyser (Hospitex Diagnostics, Italy) and Hospitex reagents. The amount of 25-OH vitamin D was determined by means of the imunofermentation (IFA) method (Immundiagnostik, Austria).

The received data was processed by the statistical calculations program Prism 3. Arithmetical mean ($\bar{x}$) standard deviation (SD), and significance (p) were calculated. Significance was accepted at the $p < 0.05$.

Results and discussion

Dynamic of calcium, magnesium and inorganic phosphorus in the serum of healthy cows after oral administration of calcium gel preparation.

Experiment 1. Data in tab. 1 show that before receiving the calcium gel preparation, cows both in the test and control group had similar blood serum concentrations. 3 hours after oral administration of the calcium gel preparation, increased calcium blood serum concentration (tab. 1; $p < 0.04$) was recorded as compared to that

of the control group. Slightly higher calcium concentration in the blood serum of cows in the test group was determined also 5 h after the first administration, 3 h and 5 h after the second administration, when compared to the data before the administration of the calcium gel preparation; however, these differences did not reach statistical significance. The dynamics of magnesium blood serum concentrations of test cows was mild and the level of magnesium was similar to that of the control cows. The level of inorganic phosphorus in the blood serum of cows had a tendency to rise after the administration of the calcium gel preparation and its highest concentration was determined 24 h after the first administration. Its amount did not change much after the second administration of the calcium gel preparation compared to the level detected 24 h after the first administration. No undesirable side effects after oral administration of calcium gel preparation were observed. In summary, it can be noted that oral administration of calcium gel preparation is well tolerated by cows, supplies the animal's organism with additional mineral materials, and is easy to use.

Tab. 1. Dynamics of calcium, magnesium and inorganic phosphorus in blood serum from healthy cows ($\bar{x}$ ± SD)

Group significance	Before oral administration of the preparation	Hours after the first peroral administration of the preparation			Hours after the second peroral administration of the preparation		
		3	5	24	3	5	24
Dynamics of calcium concentration in cows' blood serum (mmol/l)							
Test	2.33 ± 0.09	2.41 ± 0.09*	2.40 ± 0.12	2.32 ± 0.05	2.41 ± 0.07	2.41 ± 0.06	2.29 ± 0.12
Control	2.32 ± 0.11	2.29 ± 0.10	2.36 ± 0.18	2.28 ± 0.04	2.39 ± 0.20	2.38 ± 0.18	2.25 ± 0.08
Dynamics of magnesium concentration in cows' blood serum (mmol/l)							
Test	1.22 ± 0.30	1.12 ± 0.27	1.47 ± 0.24	1.10 ± 0,41	0.70 ± 0.17	1.10 ± 0.17	1.13 ± 0.19
Control	1.21 ± 0.32	1.02 ± 0.36	1.00 ± 0.23	0.91 ± 0.14	0.51 ± 0.22	0.94 ± 0.18	1.13 ± 0.54
Dynamics of inorganic phosphorus concentration in cows' blood serum (mmol/l)							
Test	1.67 ± 0.28	1.76 ± 0.38	1.69 ± 0.06	2.19 ± 0.45	2.08 ± 0.36	1.90 ± 0.13	2.03 ± 0.31
Control	1.75 ± 0.18	1.84 ± 0.22	1.70 ± 0.20	1.50 ± 0.27	1.28 ± 0.09	1.70 ± 0.18	1.73 ± 0.50

Explanation: * – $p < 0.05$ data compared to initial data

Since the small changes in the concentration of mineral ions in the blood sera were observed during the first set of tests and cows tolerated the calcium gel preparation well, we decided that for the second set of experiments the dosage of every single administration of the calcium gel preparation should be enlarged up to 500 ml.

Experiment 2. A statistically increased level of calcium as compared to the data prior to the administration was recorded 24 h after a single dose as well as 5 h (29 h) and 24 h (48 h) after administration of the second dose ($p < 0.05$; tab. 2).

The amount of magnesium had a tendency to increase in the test group as compared to the level registered before the administration of the calcium gel preparation, but the difference did not reach statistical significance. Concentrations of inorganic phosphorus in the serum of test cows after administration of the calcium gel preparation were higher at all time-points measured as compared to initial data and the respective data of the control group. Any significant differences of vitamin D concentrations in cows' blood serum. However, we observed a tendency for a higher amount of vitamin D in the blood serum of test cows 24 h after administration of the calcium gel preparation as compared to the data received prior to the administration. Nevertheless, after the second administration such an increase was not registered. Therefore, we consider that to include vitamin D into the calcium gel preparation is not useful. Intramuscular injections of vitamin D might be more practical.

In conclusion it was started demonstrated that the mineral materials contained in the calcium gel preparations are easily absorbed by the digestive tract and supplement the amount of mineral materials in cows' body.

Prevention of parturient paresis in parturient paresis prone cows by oral administration of calcium gel preparation

Experiment 3. Data in tab. 3 illustrate that in the period just before parturition all parturient paresis prone-cows had a reduced amount of calcium and inorganic phosphorus compared to the physiological norm, while the amount of magnesium exceeded the physiological norm. After oral administration of the calcium gel preparation containing mineral materials, the amount of cal-

Tab. 2. Changes of concentrations of calcium, magnesium, phosphorus and 25-hydroxyvitamin D in the blood serum of healthy cows ($\bar{x}$ ± SD)

Group	Before oral administration of the preparation	Time after the first administration		Time after the second administration	
		5 hours	24 hours	5 (29) hours	24 (48) hours
Calcium (mmol/l)					
Test	2.25 ± 0.09	2.52 ± 0.40	2.57 ± 0.21*	2.57 ± 0.12*	2.48 ± 0.04*
Control	2.32 ± 0.03	2.13 ± 0.47	2.47 ± 0.10	2.42 ± 0.22	2.21 ± 0.30
Magnesium (mmol/l)					
Test	1.05 ± 0.13	1.14 ± 0.06	1.34 ± 0.31	1.45 ± 0.51	1.21 ± 0.03
Control	1.13 ± 0.054	1.27 ± 0.07	1.25 ± 0.04	1.31 ± 0.01	1.28 ± 0.10
Inorganic phosphates (mm/l)					
Test	1.98 ± 0.44	2.00 ± 0.20	2.26 ± 0.51	2.21 ± 0.37	2.28 ± 0.48
Control	2.00 ± 0.16	2.00 ± 0.10	2.14 ± 0.20	1.80 ± 0.55	1.84 ± 0.22
25-OH vitamin D (mm/l)					
Test	21.45 ± 9.83	22.62 ± 9.18	25.4 ± 11.93	22.42 ± 7.18	20.90 ± 3.86
Control	19.10 ± 3.62	20.88 ± 4.87	16.80 ± 4.28	18.00 ± 2.29	18.40 ± 1.69

Explanation: * – as in tab. 1.

Tab. 3. Dynamics of calcium, magnesium, and inorganic phosphorus concentrations in the blood serum of cows that developed parturient paresis and cows that resisted the disease after two oral administrations of the calcium gel preparation ($\bar{x}$ ± SD)

Cow group	Before oral administration of the preparation			24 h after the first administration and 5 h after the second		
	Ca mmol/l	Mg mmol/l	P mmol/l	Ca mmol/l	Mg mmol/l	P mmol/l
Group 1 resisted parturient paresis	1.91 ± 0.43	1.35 ± 0.16	1.35 ± 0.16	2.44 ± 0.37*	1.23 ± 0.08	1.54 ± 0.49
Group 2 developed the disease	2.12 ± 0.42	1.23 ± 0.24	1.40 ± 0.27	1.74 ± 0.52	1.33 ± 0.22	1.07 ± 0.39

Explanation: * – as in tab. 1.

cium in the blood serum of cows which resisted parturient paresis (group 1) rose significantly as compared to the data prior to administration (tab. 3; $p < 0.027$). There was a trend for a higher concentration of inorganic phosphorus, but the difference was not statistically significant.

Initial symptoms of parturient paresis were diagnosed before administration of the calcium gel preparation. Two doses of calcium gel preparation (500 ml each) were administered to animals with the interval of 24 h. Parturition proceeded well in all cows. However, 7 cows developed parturient paresis (group 2), while 10 were resistant to the disease (group 1). Considering this factor, the cows were divided into two groups. The amount of calcium 5 h after the second calcium gel administration as compared to the data prior to the administration decreased from 2.12 ± 0.42 mmol/l to 1.74 ± 0.52 mmol/l, inorganic phosphorus from 1.40 ± 0.27 to 1.07 ± 0.39 mmol/l, and the amount of magnesium did not change in the serum of sick cows (group 2). The cows that resisted the disease (group 1) had an increased concentration of calcium from 1.91 ± 0.43 to 2.44 ± 0.37 mmol/l and inorganic phosphorus from 1.35 ± 0.16 to 1.54 ± 0.49 mmol/l after administration of the preparation as compared to the data prior to administration.

In summary, the authors can affirm that this calcium gel preparation of mineral materials is an effective means to protect pregnant cows against parturient paresis. Ten (59%) out of 17 paresis-prone cows were protected from the disease by oral administration of calcium gel preparation just before the time of parturition. Moreover, even the 7 cows who developed paresis completely recovered after a single intravenous calcium borogluconate injection. Interestingly, it can be seen that paresis-prone cows develop parturient paresis when the serum concentration of calcium is below 2 mmol/l and inorganic phosporus – below 1.1 mmol/l (tab. 3).

Experiment 4. During this experiment cows received two oral administrations of the calcium gel preparation within the final two days of parturition with the 24 h interval and once on the day of parturition. These cows did not develop parturient paresis, although mild symptoms of paresis such as muscle tremors and cold extremities were diagnosed before calving. All cows selected for the tests previously had parturient paresis and when repeated medication was required their treatment was difficult and complicated. Oral administration of this calcium gel preparation protected cows against parturient paresis.

Data presented in tab. 4 show that even though the preparation was administered twice before calving and once again at the time of parturition, the amount of calcium on the day of parturition and on the first and second days after parturition was statistically significantly decreased ($p < 0.05$; tab. 4), whereas the amount of phosphorus slightly decreased and the amount of magnesium had a tendency to increase. Serum concentrations of calcium and phosphorus were within the limits of the physiological norm and apparently it was sufficient to protect the cows from developing parturient paresis. It can be concluded that two administrations of the calcium gel preparation before calving plus one dose at the time of parturition supplement the amount of calcium and phosphorus in cows' blood and protect them from the illness.

It was shown that calcium, inorganic phosphorus and magnesium, contained in the oral calcium gel preparation, easily resolve in blood and normalize the amount of calcium and phosphates in blood. Changes in magnesium levels are small, but magnesium is the antagonist of calcium and is therefore necessary to buffer cardiotoxic activity of calcium. Apparently magnesium was efficient in this respect and not a single case of cardiac disorder was recorded after oral administration of the calcium gel preparation, whereas intravenous injections of larger doses of calcium chloride are often followed by cardiotoxic side effects.

These data show that after several oral administrations of the calcium gel preparation before parturition, the preparation resolves well from the digestive tract.

Tab. 4. Dynamics of blood serum calcium, magnesium, and inorganic phosphorus after three oral administrations of calcium gel ($\bar{x}$ ± SD)

Before oral administration of the preparation	After oral administration of the gel (days after parturition)				
	On the day of parturition	1	2	6	10
Amount of calcium, mmol/l					
2.81 ± 0.12	2.51 ± 0.37*	2.13 ± 0.54*	2.29 ± 0.26*	2.58 ± 0.44	2.71 ± 0.44
Amount of inorganic phosphorus, mmol/l					
1.90 ± 0.45	1.65 ± 0.37	1.58 ± 0.25	1.72 ± 0.45	1.91 ± 0.43	1.68 ± 0.27
Amount of magnesium, mmol/l					
1.36 ± 0.38	1.47 ± 0.29	1.44 ± 0.36	1.55 ± 0.47	1.53 ± 0.26	1.42 ± 0.28
Amount of glucose, mmol/l					
3.28 ± 1.08	2.71 ± 2.22	2.44 ± 0.65	2.53 ± 0.54	3.02 ± 1.43	2.32 ± 0.88

Explanation: * – as in tab. 1.

Calcium chloride decreases blood pH which dissociates calcium from blood albumins and calcium becomes more accessible to the body cells. The authors demonstrated a significant increase of the blood serum concentrations of calcium and phosphorus in cows that resisted paresis after oral administration of the calcium gel preparation. The level of calcium and phosphorus in the blood sera of cows that developed paresis declined, and magnesium level insignificantly increased. This is due to a disturbed balance among calcium, magnesium and other mineral materials. It can be partly explained by the fact that symptoms of acidosis were diagnosed to these cows before parturition and calcium could not become albumin-free in an acid environment.

Some authors (15) point out that an increased level of calcium was recorded in the blood serum of the Holstein cows, which received orally the calcium propionate 12 hours before the expected parturition, during parturition and 12 hours after it, as compared to the data received from the cows in the control group. The above mentioned preparation reduced the incidence of parturient paresis subclinical hypocalcemia. According to other authors (26), when, 24 and 48 hours after parturition, 54 g of calcium chloride gel was orally administered to cows with retained fetal membrane, there were no changes of calcium, magnesium and phosphorus levels as compared to the control group. When before and after delivery cows received Ewokal (company Suomen Rehu) twice, which contains calcium and magnesium salts, parturient cows did not develop paresis or retained fetal membrane (6). Cows develop parturient paresis when the calcium concentration in blood serum is below 1.5 mmol/l and inorganic phosphorus – 1.2 mmol/l. The first day after calving is the most critical time to develop parturient paresis (27).

It was demonstrated that during this period calcium and phosphorus levels in cows' blood decline considerably. Oral administration of mineral salts in the form of the calcium gel preparation before and during parturition protects those cows that have previously had parturient paresis against the disease. Paresis-prone cows must be given the preparation 1-2 days before the expected day of parturition and at the time of parturition.

The preparation must be administered *per os* twice before the time of parturition with a 24 hour interval and once more at the time of parturition. Since the administration of this preparation is easy it can be performed by a single person. While administering the calcium gel preparation the cow's head must be raised in order to avoid that a part of the preparation will be wasted in case a cow will lower its head. The thick consistence of the gel preparation is easier for cows to swallow, there is no danger of its access to the airway; moreover, when already in the rumen and the intestine it does not irritate the mucous membrane.

In summary, it was claimed that the authors' calcium gel oral preparation, which contains calcium, inorganic phosphorus and magnesium, is well taken up from cows' digestive tract, normalizes the level of calcium, magnesium and phosphorus in the blood serum of cows and protects them from parturient paresis.

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