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ORIGINAL PAPER

EVALUATION OF THE EFFECTIVENESS OF SELECTED PHOSPHATE PREPARATIONS IN THE EQUALIZATION OF SUBCLINICAL HYPOPHOSPHATAEMIA OF DAIRY COWS*

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Abstract

Subclinical types of deficiency diseases are at present the most common disorders in farmed cattle. Their diagnosis is extremely difficult and should be verified by biochemical blood tests. The most effective as well as the cheapest method of supplementing subclinical deficiencies is the administration of oral mineral preparations. The objective of this work has been to assess the effectiveness of mineral preparations in equalizing subclinical hypophosphataemia conditions in dairy cows during the postpartum and early lactation period. The study was conducted on 42 dairy cows of the Holstein-Friesian breed (HF) originating from three farms in the Lublin region, from 7 days to 5 weeks after calving, diagnosed with subclinical hypophosphataemia. The animals which were selected for the study were divided into three experimental groups, 10 cows in each group, based upon the mineral preparation applied, and into the control group (12 cows). The following parameters were determined in the blood serum of the cows: inorganic phosphorus content (Pi), glucose concentration (GLUC), total cholesterol (CHOL T) and total protein content (TP), the total bilirubin level (BIL T), creatinine (CREA), as well as the activities of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and creatinine kinase (CK). It was found that the most effective phosphate deficiency treatment was with sodium dihydrogen phosphate monohydrate, and that supplementation with this preparation allowed for the normalization of biochemical blood parameters. Long-term supplementation with increased doses of preparations containing phosphorus may lead to disruptions in the kidney and muscle functions, which is indicated by a significant increase in creatinine and CK activity levels. The therapeutic success of the treatment of subclinical deficiencies is dependent on the continuous monitoring of the health status of a herd due to the multietiological and multiform nature of metabolic diseases.

Keywords: dairy cows, hypophosphataemia, supplementation.

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INTRODUCTION

Phosphorus (Pi) takes part in the storage, conversion and release of energy, it participates in many enzymatic reactions, the transfer of genetic information, and in maintaining the acid-base balance of living organisms (Ternouth 1990, Karn 2001, Goff 2006). In cattle, phosphorus is necessary for microbial fermentation in the rumen (Puggaard et al. 2011). In blood plasma, 70% of phosphorus is present in its organic forms (phospholipids, phosphate esters, phosphoproteins), the remainder are inorganic forms of phosphorus, such as $H_2PO_4^-$ and HPO_4^{2-} ions (~ 50% of inorganic Pi), approximately 15% of inorganic phosphorus is protein-bound and 35% occurs in the form of Ca, Mg or Na compounds (Tenenhouse 2005).

Phosphorus deficiency can lead to muscular weakness and reduced bone mineralization, and, in some animal species, it causes disorders in the functioning of the immune system (Kegley et al. 2001). The normal level of Pi in cattle's blood should be within the limits of 1.05-2.76 mmol l^{-1} (Moore 1997, Goff 2006, Kaya et al. 2008). Hypophosphataemia is a disorder that commonly occurs in dairy cows during the periparturient period. Subclinical hypophosphataemia is the most frequently recognized type of this disorder.

The increased demand for phosphorus during gestation is caused by the growth of the foetus and, after parturition, by milk production. The symptoms of clinical hypophosphataemia include: decreased appetite and milk yield, lameness and weight loss. Additional symptoms may occur, such as bone and joint pain, frequent leg-to-leg crippling, unstable gait and difficulties standing. Over the course of the most severe manifestation of the disease, cows which suffer from phosphorus deficiency typically remain in the recumbent position and during attempts to lift a downer cow, muscle tremors, falls, tendon and muscle injuries and also bone fractures may occur (Shupe et al. 1988, Kaya et al. 2008, Kurek et al. 2010). However, during the course of the subclinical type of phosphorus deficiency, frequently the only sign that may be observed is a decrease in milk yield.

The most common causes of hypophosphataemia are: poorly balanced feed rations, Pi deficiency or an inappropriate Ca to Pi ratio in fodder, disorders in phosphorus absorption, disruptions in the hormonal control of the Ca/P metabolism, an overdose of Ca or Mg used for the treatment of milk fever. Hypophosphataemia often accompanies other periparturient disorders in cows, such as reduced appetite, ketosis, abomasal displacement, fatty liver, metritis, mastitis and most frequently hypocalcaemia.

The prevention of phosphorus deficiency in dairy cows is mainly based on proper balancing of the feed ration to the current stage of life of the animals and supplementation with mineral preparations containing phosphorus.

The aim of this work is to evaluate the effectiveness of the long-term administration of selected mineral preparations to counteract the subclinical states of hypophosphataemia during the postpartum and early lactation period, including those biochemical blood parameters that describe protein and energy metabolism as well as the functioning of parenchymal organs.

MATERIALS AND METHODS

The study was conducted on 42 dairy cows of the Holstein-Friesian breed (HF) within the age range of 4-6 years, from 7 days to 5 weeks after calving. The animals were selected from three farms in the Lublin region, for which the health status of the cattle was monitored through routine clinical and laboratory control tests and the milk quality and production yields were regularly inspected. At the peak of lactation, the milk yield at these farms was 35 kg of milk per day on average, and the animals were in healthy condition (BCS 3.5/5). In the groups examined, at the beginning of the study, the cows produced 25 kg of milk daily on average. This research study was approved by the Local Ethics Review Committee for Animal Experiments, permission number 19/2014.

The cows were kept in a tethered housing system and their diet was based on TMR. The composition of the food ration was designed for a cow with an average daily milk production yield of approximately 20 kg. Furthermore, each cow with a yield that exceeded 20 kg was given 1 kg of concentrated fodder per each additional 2 kg of milk. The composition of the fodder was the following: corn silage, haylage, hay, straw, cereal grains (barley, wheat, oats), protein concentrate, and complex mineral feed additive, in the form of feed mixture.

Animals were chosen for the study on the basis of being diagnosed with inorganic phosphorus deficiency (1.07 to 1.26 mmol l^{-1}) in the blood during routine tests on the 7th day after calving. In the medical history, the cattle owners had informed the researchers about deficient milk production from these animals. After diagnosing hypophosphataemia, the mineral preparations were introduced into the diet in order to compensate for the deficiency. Some animals were excluded from this study, such as those diagnosed with symptoms characteristic of periparturient disorders (downer cow syndrome or milk fever, injuries, teat losses, lameness) and also with postpartum changes to the reproductive organs (a disturbance in the involution of the uterus, uterine prolapse, vaginal injuries and vaginitis).

The control group (K) were 12 cows not diagnosed with any clinical symptoms or any significant mineral metabolism disorders in the past 12 months. In this group, the cattle were fed a standard mineral feed mixture for lactating cows that contained 19.5% Ca, 3.0% P, 7.5% Na, 6.0% Mg, 1.0% S and also Zn, Mn, Cu, I, Co and Se. The preparation was administered in the quantity of 0.1-0.4 kg/cow/day depending on the amount of milk produced.

The animals which were selected for the study were divided into three experimental groups, 10 cows in each group, based upon the mineral preparation applied.

In group I (beside standard mineral preparation as in the K group) sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) was used at a dose of 0.025 kg of P/cow/day administered *per os*. The second group (II) were the animals which were given 0.023 kg of P/cow/day, administered *per os* (as an additional mineral supplement) in the form of a complex mineral preparation, directed against Pi deficiency, containing calcium sodium phosphate ($CaNaPO_4$). Group III consisted of animals supplemented with an additional 0.016 kg of P/cow/day *per os* in the form of a complex mineral preparation containing calcium sodium phosphate.

For the purpose of the research, blood samples were always taken at the same time of day, in the morning, after milking and after the animal had passed a physical examination, for the first time one week after calving and, subsequently, after 2 and 4 weeks from the time of the first blood test.

The following parameters were determined in the blood serum: inorganic phosphorus content (Pi), glucose concentration (GLUC), total cholesterol (CHOL T) and total protein content (TP) and also the total bilirubin level (BIL T), creatinine (CREA), the activities of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and creatinine kinase (CK), with the use of a biochemical analyser BS-130 (Shenzhen Mindray Bio-medical Electronics Co., Ltd) and dedicated reagent sets (Cormay).

The mathematical-statistical processing of data was performed using the Statistica 10.0 program. For calculating the significance of difference, the Mann-Whitney *U* Test was applied.

RESULTS

The results of the experiments are presented in Table 1. Within group I, during the supplementation with sodium dihydrogen phosphate monohydrate, periodic disturbances of appetite were no longer observed, in addition to which feed efficiency improved. There was a substantial increase in milk yield, which reached an average of 43 kg of milk per day on completion of the study. In groups II and III, feed efficiency improved and the average milk yield in these groups was 37 kg of milk per day by the end of the experiments.

In cows from all of the groups studied, at the commencement of the study, statistically significant values of the concentration of Pi, lower than those in the control group, were observed.

In group I, as a result of the supplementation with sodium dihydrogen phosphate monohydrate, a statistically significant increase in the concentra-

Table 1

Results of biochemical tests in cows

Group	Blood specimen collection	Parameters								
		Pn (mmol l^{-1})	CREA (μmol l^{-1})	BIL T (μmol l^{-1})	CHOL T (mmol l^{-1})	AST (u l^{-1})	TP (g l^{-1})	Gluc (mmol l^{-1})	GGT (u l^{-1})	CK (u l^{-1})
I	1	1.13±0.05^{++}	59.82±12.20	8.58±2.10	5.48±0.37^{++}	70.67±18.71^{ac}	63.71±3.00	2.82±0.28^{+}	22.20±5.27	85.07±10.46^{Aa}
	2	1.65±0.12**	97.24±16.61**	6.13±1.77*	4.05±0.58**	68.47±12.88	68.64±2.84**	3.28±0.32**	24.67±4.95	115.00±21.94**
	3	2.19±0.19^{**A}	126.12±19.83^{**Aa++}	4.69±1.63^{**A}	3.08±0.69^{**Aa}	64.13±12.08	70.33±2.99**	3.82±0.48^{**ABa+}	23.27±5.48^{ABa}	144.60±22.06^{**Aa++}
II	1	1.16±0.05^{++}	64.30±10.98	8.94±2.40	5.63±0.67^{++}	50.00±16.48^{b}	63.54±3.82	2.91±0.27^{+}	23.47±4.85^{+}	80.87±8.24^{ABa}
	2	1.45±0.09**	74.37±11.55	7.01±1.66	4.88±0.36**	64.60±11.85*	68.07±2.99**	3.36±0.29**	24.13±5.17	103.40±14.89**
	3	1.63±0.14^{**B}	104.19±17.70$^{**ABb++}$	4.85±1.59^{**A}	3.57±0.52^{**ABa}	73.27±10.09**	68.58±4.64*	3.89±0.27^{**Aa+}	20.93±4.32^{Aa}	119.13±25.06^{**ABb+}
III	1	1.19±0.07^{++}	68.72±12.32	9,92±2,31	5.68±0.70^{++}	67.40±10.91^{c}	65.71±4.57	3.07±0.43	25.33±4.84^{++}	70.60±11.27^{Bb}
	2	1.33±0.09**	94.35±25.86*	8.45±1.22*	4.62±1.15*	65.87±11.62	68.30±6.01	3.32±0.60	27.47±6.58	72.33±14.94
	3	1.41±0.09^{**C++}	86.99±19.87^{*Bb++}	8.25±1.36^{*B}	4.28±0.86^{**Bb+}	70.60±12.93	69.21±4.28	3.33±0.34^{Bb}	29.07±5.89^{Bb}	100.07±17.71^{**Bb}
K	1	1.89±0.37	47.68±27.10	6.81±4.86	3.25±1.30	66.88±24.42	65.33±3.43	3.58±0.69	17.31±4.38	82.75±45.85
	2	1.81±0.31	45.53±24.85	7.10±3.76	4.04±0.91	71.50±17.33	66.33±4.25	2.83±1.01	22.00±6.09	80.25±16.49
	3	2.01±0.60	52.67±19.64	6.51±3.61	3.19±1.08	71.17±10.51	67.63±6.57	3.03±0.86	24.17±5.15	80.25±26.23
Normal values of investigated parameters for cows		1.81 - 2.10	88.4 - 183.0	1.9 - 7.0	1.8 - 5.2	58 - 100	51 - 71	2.2 - 4.5	22 - 64	56 - 124

Data given are mean ± SD, *,** – the significance of differences between the first and subsequent blood tests within the subgroup, at $p < 0.01$ or $p < 0.001$; *a*,*b*,*c*, *A*,*B*,*C* – the significance of differences between the subgroups with the main groups at $p < 0.01$ or $p < 0.001$;
+, ++ - the significance of differences between the studied subgroups and the control group at $p < 0.01$ or $p < 0.001$;
Normal values of investigated parameters for cows (according to Oikawa, Katoh 2002, Tracz 2012, Olech 2014)

tion of Pi was determined after 3 and 5 weeks postpartum. In group II, after the use of a complex mineral mixture formulated to compensate for phosphorus deficiency, a statistically significant increase in Pi levels at the time of the second and third blood tests was achieved. In group III, a statistically significant increase in Pi concentrations was observed initially, although average phosphorus levels in the subsequent blood tests were found to be statistically significantly lower than in the control group. At the time of the third blood test, the concentration of Pi in the serum of group I cows was statistically significantly higher in comparison to the other groups and the Pi level in group II was statistically significantly higher than the average concentration of inorganic phosphorus in group III.

In the groups studied, it was observed that an increase in phosphorus levels was accompanied by a rise in the creatinine levels of the serum. The average total bilirubin concentrations at the time of the first blood test, in all the groups, were above the average concentration in the control group, and they exceeded the upper limit of reference standards. As Pi levels increased, a drop in the average BIL T concentrations was observed in all of the groups studied. Simultaneously in groups I and II, after 4 weeks of supplementation with phosphorus, BIL T levels decreased to reach normal values and, as well as this, they were lower than in the control group (Table 1).

The average cholesterol levels in all of the groups studied, at the time of the first blood test, were above the upper physiological norm and statistically significantly higher than the average for the control group. During the research, a significant drop in CHOL T levels occurred in all of the groups studied. After four weeks of additional phosphorus supplementation, the average cholesterol levels were within the physiological norm (Table 1). The average AST activities remained at normal physiological levels throughout the study. The total protein content was within the norm for the entire duration of the study. After 4 weeks of using mineral preparations, the average TP concentrations in all groups were slightly higher than in the control group. The highest total protein concentration was achieved by group I.

At the beginning of the study, the average glucose concentrations in all of the groups studied were lower than in the control group. During the experiment, the concentration of glucose was increasing and at the final stage of the study, it reached statistically significantly higher values in groups I and II than the average values in group III and the control group. The average GGT activities in the studied groups were statistically significantly higher than in the control group and this trend persisted until the completion of the experiments.

An evident increase in the average CK activity was observed upon supplementation and CK activity was statistically significantly higher than in the control group (Table 1). In group I, where the increase in Pi levels in the serum was the highest, there was simultaneously the highest CK activity, which was statistically significantly higher than in the other groups.

DISCUSSION

Subclinical types of deficiency disorders are extremely difficult to diagnose (Gerloff, Swensen 1996, Goff 2004, Odette 2005, Goff 2008, Reinhardt et al. 2011), so regular laboratory screening of a herd is necessary for the proper assessment of the health status of the cows (Odette 2005, Goff 2008, Kurek et al. 2014, Olech et al. 2014). The implementation of an appropriate treatment, at an early stage of the disease, lowers costs and improves the milk production efficiency of the cattle stock (Gerloff, Swensen 1996, Goff 2004, Odette 2005, Goff 2008, Reinhardt et al. 2011). The available literature is dominated by studies and descriptions of Ca level disruptions in the blood during the early postpartum period (Kurek, Stec 2005, Goff 2006, 2008, Kurek et al. 2014). Consequences of the occurrence of subclinical deficiencies of other elements have not been adequately researched and described, and there is a lack of publications concerning the systemic consequences of long-term treatment with high doses of supplements (Matusevičius et al. 2012, Kurek et al. 2014). At the present time, the feed additives that are most commonly used after the periparturient period are widely available, complex mineral-vitamin mixtures, which target at achieving the best milk yield at a particular physiological stage of life.

In the case of Pi deficiency, the prevention strategy described above may, on numerous occasions, worsen the deficiency disorder. When using a complex mixture for supplementation we increase the levels of all elements (which may sometimes compete with phosphorus) contained within the mixture. The modern approach to prophylaxis and the treatment of deficiency diseases involves selecting appropriate methods, the choice of which depends on the scale of the deficiency, the physiological stage and the type of systemic disorder as confirmed by laboratory tests. In the tested cows, the most apparent changes in blood phosphorus levels were recorded in the group which was administered sodium dihydrogen phosphate monohydrate. The beneficial effects of supplementation with this compound were described by Cheng et al. (1998). In the other groups studied, the results were not so satisfactory.

When evaluating the effects of long-term supplementation with phosphorus on the biochemical parameters of the health status of cattle, it was noted that high levels of bilirubin observed at the beginning of supplementation dropped to normal values during treatment with phosphate preparations. Similar results were obtained by Cozzi et al. (2011) and Piccione et al. (2012). In our research, we noted that the concentration of creatinine reached its lowest values 7 days after calving, but through supplementation with Pi and an increase in the quantity of milk produced, the creatinine levels increased. Piccione et al. (2012) noted that the blood creatinine level dropped as lactation progressed and it reached its peak values shortly after calving. At the initial stage of our study, the cholesterol concentrations for animals diagnosed with phosphorus deficiency were much higher than the concentrations reported by other authors at the same physiological stage in healthy animals (Darul, Kruczyńska 2005, Filipejová, Kováčik 2009, Cozzi et al. 2011, Kessler et al. 2014). As our experiment continued, these concentrations decreased in all of the groups studied, although many authors reported an increase in serum cholesterol levels as lactation progressed (Darul, Kruczyńska 2005, Piccione et al. 2012, Kessler et al. 2014). On completion of the study, the levels of cholesterol were close to the values reported by other authors for healthy animals at the same physiological stage (Cozzi et al. 2011, Piccione et al. 2012), which indicates that phosphorus deficiency affects the systemic energy metabolism, especially CHOL T concentration. As the experiment progressed, a rise in the average glucose levels was observed, same as in the studies by other authors concerning cows in which supplementation was not used (Kupczyński, Chudoba-Drozdowska 2002, Filipejová, Kováčik 2009, Joksimović-Todorović, Davidović 2012). The concentrations of total protein increased during the study within the physiological norm. The highest increase was noted for the group in which phosphorus levels increased the most. Phosphorus participates in the synthesis of proteins and amino acids (Ternouth 1990), which explains the increase in the total protein content over the course of supplementation with phosphorus compounds. In our studies, AST activities increased in groups II and III but a drop in AST activity was observed in group I. Kupczyński and Chudoba-Drozdowska (2002) reported an increase in AST activity in the early lactation period. These results contradict the studies by other authors (Filipejová, Kováčik 2009, Cozzi et al. 2011), although they are in agreement with the results for group I. The available literature lacks data that explains the fluctuations in AST levels during the early lactation period, so further studies are required to understand this phenomenon. During the study, no significant changes in GGT activities were observed, which is not consistent with the published works by other authors. Kupczyński and Chudoba-Drozdowska (2002), Filipejová and Kováčik (2009), Cozzi et al. (2011) noted an increased GGT activity in the early lactation period. CK activities increased during our study and this finding agrees with data published by Cozzi et al. (2001), who observed an increase in this parameter during early lactation. Increased values in CK activity indicate muscle damage (Oikavy, Katoh 2002).

CONCLUSIONS

1. The most effective supplement for equalizing Pi deficiencies is sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), and supplementation with this compound has positive effects on biochemical blood parameters which describe lipid metabolism and liver functions.

2. Compensating for phosphorus deficiency also lowers cholesterol and total bilirubin levels in the blood serum of cows.

3. Long-term supplementation with high doses of preparations containing phosphorus compounds may lead to disruptions of kidney function and a significant increase in creatinine and CK activity levels.

4. Prolonged use of high doses of phosphate preparations does not have a beneficial effect on the liver's functional status.

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