VARIOUS TYPES OF HYPOPHOSPHATAEMIA IN DAIRY COWS AND THE CLINICAL IMPLICATIONS DEPENDING ON THE INTENSITY OF THE DEFICIENCY

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Abstract

The paper aimed at defining the specific biochemical parameters, as well as the use of treatment methods, according to types and intensity of the clinical signs of non-complicated hypophosphataemia in cows. The investigations were carried out on 300 HF cows in 15 herds in the Lublin Region. The mean milk yield in the herds was 36.8 ±2.8 dl. The cows were 3-9 years old and had a similar diet ratio. The animals with hypophosphataemia symptoms in the early stadium (2-7 d) were divided into three groups according to the intensity of clinical signs whereas the control group consisted of cows with no clinical signs of the illness. The inorganic phosphorus (Pi), total Ca, total Mg, K, Fe, total protein, Cu, bilirubin, FFA, and creatinine concentrations, as well as AST and AP activity, were determined in serum and plasma.

The animals with markedly exhibited signs (recumbency and tremor) showed significantly decreased Pi, Mg, and FFA contents and simultaneously increased activity of AST and AP. In the group exhibiting minimal signs (group I), there were no significant disturbances in homeostasis except the low Pi concentration, so it was possible to use the specialised phosphorus preparations in the treatment. In the other groups (II, III), which exhibited more marked signs of the illness, it was necessary to improve the parenchymal organs functions in addition to phosphorus level normalisation.

Key words: dairy cows, hypophosphataemia, symptoms, biochemical parameters.

Phosphorus (P) belongs to the group of macroelements that have a key importance to bovine constitution. Together with calcium cations (Ca++), it takes part in many biological processes. 85% of the total pool of P is in the bones, being one of the most important elements needed in the normal functioning of the skeletal system (16). In the form of phosphates, it is included in nucleic acids, ATP, phosphocreatine, and phospholipids, as well as phosphoproteins of cellular membranes, both microsomal and mitochondrial. It takes part in glycolysis and gluconeogenesis processes and in keeping acid-alkaline balance in the body (16).

The contents of non-organic phosphates in physiological terms in dairy cows’ blood is different and range from about 1.05 to 2.76 mmol/L - i.e. from 3.35 to 8.55 mg/dl (2, 9, 16, 19, 22, 24). It causes great interpretation problems during the analysis of field sample scoring, which is the best to relate to referential scores gain in the same husbandry and production conditions.

Correct calcium-phosphorus (Ca/P) administration, assuring a proper level of these ions in the serum and congruent with current demand of the animal, depends on many endogenous and exogenous factors. The non-organic phosphorus level in the organism depends on the correct absorption of P ions, mainly in the small intestine, level of Na ions, correct excretion in urine, deposition and mobilisation in bones, and the administration of Ca in the diet. Side products of cereal and soya bean as alimentary supplements are a rich source of easily absorbable P. Keeping the homeostasis depends on complicated hormonal regulation in which parathormone (PTH), calcitonin (Calc), and active metabolic products of vitamins D and A play the main role (3, 8, 10, 16). In maintaining this homeostasis, parenchymatous organs such as the liver and kidneys are involved and their conditions reflect inter alia the activity of AST, ALP, GGT, GLDH, and the bilirubin level in the serum.

The decrease in the non-organic phosphorus level in dairy cows serum is nowadays one of the most common metabolic disorders in herds with both the intensive and extensive type of utilisation. Clinical manifestations of hypophosphataemia are of various character and intensification - from variable appetite and decrease in milk production to lameness and recumbent position. The most common reasons for the deficiency are: wrongly-balanced dose, simple deficiency of this macroelement in the alimentary dose, incorrect P/Ca ratio in feed, low absorption of phosphorus compounds...
available in the diet, and disorders of hormonal system, as well as administration of too big doses of Ca or Mg ions in treating other diseases like clinical hypocalcaemia (3, 8, 10, 16, 21, 27). It has become the reason for researchers’ interest in the most common cases of hypophosphataemia during the course of other diseases (3, 12, 22, 23, 30). The authors of these research publications emphasise that P level has in these cases the a vital influence on the formation of the clinical view, which is exactly seen for example in postpartum stroke (3, 12). Hypophosphataemia coexists with fatty liver disease in perinatal period in high-efficiency cows (15) and in sinister torsion of the rumen, which is the general factor of in appetite reduction (14). In the occurrence of the independent reduction in P level, we observe first of all the cases with very advanced clinical changes, manifested by recumbent position, great difficulty in standing up and problems with movement, painful bones and muscles, frequent demineralisation, and spontaneous bone breaks. To the less characteristic symptoms belong: loss of appetite and reduction in milk production (1, 9, 19, 26).

In our observations we found that essential for herd's productivity even a small dietary fault can induce the disease. It is necessary to adapt prophylactic and treatment procedures to changeable conditions in the herd.

In our observations we found that essential for proper therapeutic conduct is determining the character and degree of intensity of disorders and analysis of changing biochemical parameters (incidence in the case of hypophosphataemia without other coexisting pathological changes). It appears that depending on the kind and degree of the intensity of clinical manifestation of hypophosphataemia uncomplicated by other deficiencies, the biochemical profile looks different, which requires individual therapeutic attitude in every case. The aim of the undertaken research was defining the specific biochemical profile of the blood as well as the use of the treatment methods according to types and intensity of clinical signs of non-complicated hypophosphataemia in cows.

Material and Methods

Three thousand dairy cows from 15 herds in the Lublin Region were used in the 3-year study. The herds had from a few to a few dozen dairy cows (average 50), in good feeding conditions (non padded), and with milk yield about 30 kg per day in the culmination of lactation (average 36.8 ±2.8). All the animals were of pure blood HF or NCB with part of HF blood and were 3-9 years of age. For the unification of the research group, the examined cows were from the herds with similar kind of feeding based on maize or silage made from maize, hay silage, hay, cattle fodder with 18%-24% of proteins, fodder concentrate made by owners from their own cereals and premixes, straw and mineral-vitamin supplements used in amounts depending on milk productivity. Feed doses were prepared by nutrition specialists in accordance with milk yield, the cows’ condition, and current physiological status.

At the beginning of the research, the animals which exhibited very low Ca levels were eliminated from the investigations, because this could have had an effect on the clinical manifestation and status of the animals with hypophosphataemia. The animals with organic changes hindering movement (injury of the pelvis), and muscle and hoof disorders were eliminated, even if these cows showed a low P level. The affected animals treated longer than 2-7 d without effects were also excluded from the research.

Next, based on medical history data, the intensity of the disease symptoms, and the results of clinical examination, the animals with laboratory-confirmed hypophosphataemia were divided into four groups. Group I consisted of 20 cows with uncharacteristic and poorly-expressed clinical signs. In anamnesis, the owners emphasised decreased or periodically-changed appetite, decrease in milk production, uncertain movements in freestanding cow barns or difficulties when standing up. Group II comprised 18 cows with a mean intensity of symptoms, mainly in the motor system, both stressed in anamnesis, and observed in clinical examinations. In this group, the prevailing symptoms were: various types of lameness, laboured standing up, palpation pain in various parts of the legs, in some cases oedema of joints and muscles, and the characteristic shifting of weight from foot to foot. Appetite and thirst was retained or only slightly smaller than normal. Group III included 14 cows which during examination were usually in a recumbent (lying position), and after being forced to assume a standing position they showed muscle tremble all over the body and an immediate need to lie down. In spite of such clear advanced symptoms, most of the cows did not manifest reduced appetite and thirst, or a significant decrease in milk production.
The control group (group IV), consisted of 20 cows from farms where no health problems had been noted six months before the research started, and which were in the middle of lactation (on the average from 7 d to one month after partum).

Blood samples were taken from the external cervical vein from each animal during the first veterinary intervention after the initial diagnosis. Before blood sampling, a detailed anamnesis and clinical examination were made, as well as the evaluation of the feeding system, the kind of given feed, and milk production.

In order to obtain the serum, blood was sampled into test tubes without anticoagulants; to get plasma; test tubes with sodium heparin were used. The sampled material was kept cool for not longer than 3-5 h and after that was subjected to initial laboratory processing. After centrifugation, the material was kept at -70°C till the analysis was completed.

The following parameters were determined in the serum: the content of inorganic P (Pi) with Cormay diagnostic kit using Spectrophotometer UV/VIS Marcel S 330, total Ca, total Mg, Cu, and Fe, using spectrophotometry atomic absorption with Perkin Elmer–4100 apparatus, K using AVL 988-4, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) using ALPHA DIAGNOSTICS apparatus and an Epoll-20 photometer, glutamate dehydrogenase (GLDH) with Randox diagnostic kits using Marcel S 330 spectrophotometer, total protein (TP) with Cormay diagnostic kit and the contents of total bilirubin by Jedrasik and Cleghorn method using Marcel 330 apparatus. The plasma content of free fatty acids (FFA) was determined using the Front and Dol method and creatinine contents using colorimetry and spectrophotometer UV/VIS Marcel.

The results were shown in the form of arithmetical means ± standard deviation (SD) and subjected to statistical analysis using the Statistica 6.0 PL program (StatSoft Inc. 2003). Statistical differences were calculated according to the Student t-test for non-parametrical values, with significance level at P<0.05.

**Results**

The studies revealed that the serum level of P in all the examined groups was significantly lower than in the control group and referential values taken as the norm for cows (29). Mean levels of Pi were also statistically significantly lower in the experimental groups compared with the control group, in which the level was 4.9 mg%. The lowest and statistically-significant Pi level values were found in group III compared with the other two groups (Fig. 1).

Total Ca levels in all groups with clinical manifestations (groups I, II, and III) were lower than generally-accepted standards (29) and statistically differed from those in the control group (Fig. 2).

The highest normal mean Mg values were observed in cows with the most advanced clinical symptoms (group III) and these values were statistically significantly higher in comparison with the control group (Fig. 3).

AST activity increased in the cows from groups II and III, and the difference was significant. In these groups, an increase in AP activity in comparison with groups I and IV, and with the accepted standards (29), was also found; however, this difference was statistically significant only in comparison with the means in groups I and IV (Fig. 4).

Fig. 1. The concentration of selected macro- and microelements in the studied groups.
abcd – P<0.05; ABCD – P<0.01 significance of differences between means values within the groups.
Fig. 2. The mean activity of AST and ALP in serum.
abd – P<0.05; ABCD – P<0.01 significance of differences between means values within the groups.

Fig. 3. The mean values of FFA concentration in particular groups.
P<0.05; ABD – P<0.01 significance of differences between means values within the groups.
The highest average content of total bilirubin was noted in group III but also in group II the mean values were above the upper normal range (Fig. 5). The highest average FFA content was observed in group III and the difference was statistically significant compared with the other groups in which mean values were within the normal range.

Similarly, the highest mean creatinine values were found in group III but was not above the upper physiological normal range; the statistically significant difference was between group III and the other groups (Fig. 6).

The highest average Cu content in all experimental groups was lower than the mean contents in the control group, but only the values of group III were significantly different (Fig. 7).

The mean activities of GLDH and ALT and mean contents of K, Fe, TP, and carbamide were normal in the experimental and control groups (29).

**Discussion**

The regulation of systemic Ca/P management involves parenchymal organs such as the liver and kidneys, where the active forms of vitamin D are synthesised. All the observed symptoms in particular experimental groups have been also described by other authors (1, 9, 19, 26) in publications related to disorders of Ca/P metabolism. In none of those publications did the authors attempt to analyse the described hypophosphataemia cases, in particular ensuing clinical changes.

Most authors think that typical symptoms of hypophosphataemia occur when the level of Pi is lower than 2–2.1 mg% (9, 13, 17, 24). Such concentrations were observed in our studies in cows with serious and typical symptoms of P deficiency (groups II and III). In
group I, where the mean value of Pi was higher, uncharacteristic symptoms or those weakly manifested were dominant. These symptoms have probably not been taken into consideration in other research works. It is worth noticing that these symptoms are quite common and in spite of high P level its mean values were considerably lower than those in healthy animals, which results in the occurrence of very late clinical symptoms of this type of P deficiency. Simultaneously, Ca concentrations in serum were lower than those in the publication of Kaya et al. (19) but are in accordance with the observations of other authors (3, 9) who notice that the attempts of the organism to compensate the Ca level can lead to the development of hypophosphataemia as in the case of postpartum paralysis.

The growing activity of AST and AP above the physiological range has been recorded by many authors (6, 24); however, in our studies it was not so high and essential as in other research publications (19). It confirms the hypothesis that frequently the cause of this condition lies in coexisting diseases, and relatively low levels of average activity are connected with the elimination from our research of animals with advanced changes in the motor system, decubitus ulcers, and illness without treatment for longer than two days. The increase in AST activity in farm animals may also be connected with muscle damage, while the increased AP activity is observed in higher osteoblastic activity and elevated remodelling of the bones. However, this activity is decreased in a rare disease of hypophosphatasia, as well as in hypothyroidism.

Serum bilirubin content as a specific indicator of biliary duct diseases, hepatic cellular failure, and disorders of the red blood cell system, is commonly used in the evaluation of the hepatic condition. Its increase is usually connected with the accelerated disintegration of red blood cells, disorders of haemoglobin disintegration, and during anorexia and stress in young animals, or for example by P poisoning. However, hyperbilirubinemia-connected diseases are always accompanied by changes in other hepatic indicators (GLDH, transaminases). In the studied cases of spontaneous bovine hypophosphataemia it was difficult to explain the elevation of total bilirubin and FFA concentrations above the normal range (6, 24) in spite of the lack of evidence of coexisting liver diseases. This condition may suggest that the animals had problems in the past but the symptoms were not noticed by their owners or that the animals in a lying position had a limited access to feed, especially high-energetic. The decrease in Cu concentration in serum in experimental groups is in accordance with the results of Kaya et al. (19). However, it is difficult to agree with these authors’ suggestion that this interesting phenomenon is connected only with feeding and it needs further studies.

Cows with the most advanced signs of the onset of the disease, in addition to a decrease in P level, have already changes in hepatic activity indicators, early symptoms of disorders in muscle tissue, and developing energy deficiency, resulting in the using by the cows’ reserves of their own fat. It may lead to deep and irreversible changes in health status if the therapy is not started. The obtained data indicate that even in the group with mild and uncharacteristic symptoms (group I), a decrease in P content in serum was as serious as in the alimentary tract, and usually-exclusively dietetic measures were ineffective and the disturbances required intensive treatment. Concurrently, the absence of necessary changes in other parameters of Ca/P restricts its use in treatment to only a few phosphoric preparations administered orally or in intramuscular injections. Such procedures would not eliminate the stress connected with the treatment especially in freestanding cow barns where the use of parenteral preparations prevails. The benefits are a shorter treatment period and lower costs. The diagnosis of the deficiency situation (hypophosphataemia) with insignificant symptoms of the disease gives the possibility of an early start to effective prophylaxis and the suppression of the disease development. The contents of P in the liver tissue decreases significantly in the early lactation period as a result of the reduced volume of hepatic cell cytosol and the P content in cytosol (15). Supplementation of P in the form of sodium phosphorate (orally, intravenously, or in combination with calcium gluconate), causes an increase in Pi level shortly after administration but later it is difficult to keep the normal level in the serum (5). Pi content in plasma is a precise bio-accessibility indicator of P administered in feed (21). Its serum content may be distorted by the improper preparation of the blood for analysis (haemolysis) (25). Hypophosphataemia may be the cause of haemoglobinuria syndromes, decrease in glicolyzed haemoglobin, and ATP synthesis (28).

References


