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Studies on the aetiology and treatment of hypophosphataemia developed naturally in cattle from Van region of Turkey*)

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Summary

The study investigated the aetiology and treatment of cattle with symptoms of lameness, stiff gait and bone fractures in the Van region of Turkey. The study was performed on 200 diseased and 50 healthy cattle aged between 1-8 years old, of both sexes. Blood samples for haematological and biochemical analysis were taken once from the healthy animals and twice from the diseased animals. Fodder samples were also collected. Total protein levels and P values of the diseased animals before treatment compared to the healthy control animals were lower while ALP and Ca were higher. After treatment an increase in the total protein ($P < 0.05$) and P ($P < 0.001$) was observed, and a decrease in ALP ($P < 0.001$), and Ca ($P < 0.05$). Phosphorus concentrations in the fodder of the diseased animals were significantly lower. Analysis of the haematological, biochemical parameters of the animals and their fodder showed typical changes for hypophosphataemia. The diseased animals were treated by receiving unorganic P and vitamin D_3 injected intramuscularly and mineral suplementation which was administered orally. The animals were observed for 30 days. After treatment, the symptoms observed in the diseased animals disappeared gradually as well as pica symptoms. Supplementing P to the animal's fodder is vital in order to avoid such problems in the future.

Keywords: cattle, hypophosphataemia, aetiology, diagnosis, treatment

Hypophosphataemia is a disorder characterised by weight loss, altered hair structure, abnormal posture, and lameness. The disorder developes slowly and stealthly. Furthermore, anorexia, collapsing knees, pain in the bones and joints, hesitant walking, lameness, frequent shifting from foot to foot while standing, preferential lying down, difficulty in standing up, spontaneous fractures in the vertebrae, pelvic, rib and long bones may develop (1, 4, 9, 12, 16, 27-29). Bones are demineralized and cortical surfaces are rough, soft and fragile (5, 12, 14, 28, 29). Furthermore, reduction in the bone density and cortical thinness have also been reported (1, 7, 12, 14, 29). Softened bones, sponge like structures, widening in the bone marrow hole, pale colour, and decrease in bone density have been reported in the histopathology of the bones in animals with hypophosphataemia (7, 28).

Although clinical symptoms may signal hypophosphataemia, definitive diagnosis is made after determining inorganic P levels in blood serum, bone, rumen fluid, food, faeces, saliva and urine samples. Furthermore, Ca, Mg, and total protein concentrations should also be determined (3, 13, 16, 31). While the normal serum inorganic P level in cattle is about 4-5 mg/dl, when it is deficient this level may decrease to less than 1 mg/dl. Liver enzymes such as alanine aminotranspherase (ALT), alkaline phosphatase (ALP) and aspartate aminotranspherase (AST) concentrations have also been reported to change. Determining the concentrations of other minerals and trace elements (Zn, Cu, Mg, Mn, Na, K, Cl) may help to assertain diagnosis (3, 13).

Treatment should be started by amending rations. In simple cases only P supplementation is necessary, while in severe cases other medical treatments should also provided. A full recovery in severe deformations of bone and joints is almost imposible (6, 27). In our region, animals may very often be observed to have clinical syptoms like those mentioned for hypophosphataemia. Although the problem causes important economical losses in the Van region, there have

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been no studies on this disorder's aetiology and treatment. Therefore, the aim of the present study was to investigate the aetiology and treatment of cattle in this region exhibitng symptoms of lameness, hesitant walking, uneasy movement as well as fractures in the long bones and hips.

Material and methods

Animal material. The study investigated a total of 250 cattle from several different villages aged between 1-8 years old (mainly under 5 years old), of various breeds and sex. The study was carried out over a 2 years period. Since the problem arose at the end of winter and begining of spring, sampling during this 2 year period was only carried out between February-May. Fifty animals were healthy (control group), and 200 were naturally sick animals (treatment group) which had altered hair structure, collapsing knee joints, hesitant walking, lameness, shifted from foot to foot while standing, refusal to stand up when lying down, were hunchbacked, and had pica, pelvic and femur fractures (fig. 1).

Blood samples. In the study, blood samples were taken once from animals of the control group and twice from animals of the treatment group. The first blood samplings were done before treatment, and the second 30 days after treatment. Fodder samples of the ill animals (dried grass, wheat straw, clower straw and wheat bran) were also collected for mineral analysis. According to anamnesis all the fodder had been prepared from the plants grown in this region.

Haemoglobin, total protein, albumin, glucose, AST, ALT, ALP, CK and P concentrations were determined by commercial kits using photometers (Boehringer 5010). Serum Zn, Cu, Fe, Mg, and Mn concentrations were determined by atomic absorbsion spectrophotometer (Unicam 929), and serum Ca levels were determined by (Celile 1100 series) spectrophotometer. Total leukocyte, erythrocyte counts and PCV values were determined manually.

Fodder samples were analysed in relation to P according to the literature (15) at the department of Soil of the Faculty of Agriculture.

Treatment. Sick animals received unorganic phosphorus (Fosfotonik®-Topkim, Turkey) at 24 mg/kg body weight for 20 days, once a day intramuscularly (im). Furthermore, for the same duration, ionised Depomin® (Vetaş-Turkey) 200 cc daily, Vit D (Devit-3® Deva-Turkey) at a dose of 500.000-1000.000 IU were applied twice. The sick animals were observed for 30 days.

Statistical analysis. Values obtained from control and sick animals before and after treatment were analysed by student-t test using SPSS packet programme (22).

Results and discussion

This study was carried out on cattle of various ages and breeds exhibiting symptoms of hypophosphataemia. Maintenance and nutritional conditions play a vital (4, 28) in the development of hypophosphataemia.

The animals used in the present study were kept in conditions of insufficient and unbalanced feeding, and during pasture extra fodder was not given to the animals according to anamnesis. Clinical symptoms observed in the animals used in the present study had similar symptoms of hypophosphataemia reported by other researchers (3, 16, 27, 28) (fig. 1) In this study only statistically significant labaratory parameters were discussed.

In the present study, animals showing clinical symptoms had 1.6 ± 0,12 mg/dl serum P concentration (tab. 1). Clinical, haematological, biochemical, trace element concentrations and fodder analysis revealed

Fig. 1. A sick animal before treatment

Fig. 2. The same sick animal after treatment

Tab. 1. Influence of treatment on parameters studied ($\bar{x}$ ± sb)

Parameters	Control group (n = 50)	Sick animals (n = 200)	
		Before treatment	After treatment
PCV (%)	34.9 ± 1.18	31.5 ± 0.52 *	31.6 ± 0.66
Total protein (g/dl)	8.43 ± 0.08	7.31 ± 0.09 ***	7.46 ± 0.10[a]
ALP (IU)	263 ± 24.2	665 ± 48.4 ***	374 ± 17.9[c]
Calcium (mg/dl)	7.71 ± 0.15	8.76 ± 0.32*	8.28 ± 0.23[a]
P (mg/dl)	3.79 ± 0.24	1.60 ± 0.12***	3.81 ± 0.99[c]
Cu (mg/L)	2.68 ± 0.22	2.04 ± 0.15*	2.09 ± 0.12

Explanations: statistical significance between control and before treatment group, * – P < 0.05, ** – P < 0.01, *** – P < 0.001; statistical significance of diseased animals before and after treatment (a – P < 0.05, b – P < 0.01, c – P < 0.001) that the disorder observed in the study was hypophosphataemia. Gartner et al. (11) suggested that if serum P concentration decreases to less than 2.01 mg/dl, the condition may be considered to be hypophosphataemia. Similarly, Jubb et al., (14) reported young cattle with clinical hypophosphataemia having 1.58 mg/dl serum P values. Furthermore, plasma phosphorus concentration under 2.1 mg/dl were considered to be at a critical level and suggested hypophosphataemia (25). Other researcher (16) found that in cattle fed a low phosphorus diet serum phosphorus concentration were reduced to 2.1 mg/dl, and continued to fall to 1.5 mg/dl after they gave birth. In the case of young cattle in particular, serum inorganic P concentrations were reported to be directly affected by P intake (9, 16). Serum P concentrations in the hypophosphatemic cattle obtained in the present study were in agreement with the other findings in studies of hypophosphatemic animals (5, 9, 11, 14, 16, 25, 28).

In the present study, inorganic doses of P at 24 mg/kg were given intra muscularly for 20 days, and a 200 cc solution containing 72 g/l P orally for the same period. After treatment it increased to 3.81 ± 0.9 mg/dl, and this increase was statistically significant ($P < 0.001$) (tab. 1). After the treatment began, the clinical signs mentioned above began disappearring, as for instance, spontaneously developed fractures such as pelvic, long bones and ribs, and pica (fig. 2). Jubb and Crough, (14) gave super juice containing 9% P, and 8.5 kg bear yeast (11 g daily) to treat cattle with clinical hypophosphataemia, and found that recovery began a week later. Two weeks later, hesitant walking was reported to disappear. They suggested that animals having clinical signs of hypophosphataemia should receive 12-15 g P daily. Therefore, the amount of P used in the present study was considered to be sufficient, and in agreement with the findings of Jubb and Crough (14).

The results obtained in the present study following treatment were lower than the same findings of other authors (9). This suggests the significance of inefficient feeding as well as P deficiency in the consumed fodder. Furthermore, the occurrence of hypophosphataemia in grazing cattle is important and pastures should be fertilized with phosphate fertilizer and P additives should also be used in the feed, which did not take place in the present study (tab. 1).

Phosphorus concentration in plants is at a maximum level if it is not controlled by moisture and temperature. But if moisture and maturation are limited in the plant then its' concentration is reduced. There is a close correlation between plant and soil P (16, 17). Mineral composition in the plants is affected by plant and soil factors such as pH, drainage, fertilization, plant type, maturity of fodder, and reactions between minerals (2, 17).

P concentrations in the fodder (dried grass, wheat straw, clover straw and wheat bran) used for feeding the animals were analyzed and found to be very low

Tab. 2. P concentrations of the fodder used for feeding of the sick animals

Type of fodder	Reference Value g/kg (23)	Inorganic phosphorus		
		Min-Max (g/kg)	Mean ($\bar{x}$) (g/kg)	Mean ($\bar{x}$) (%)
Dried grass	2.2	0.6-2.0	1.010	0.101
Wheat straw	0.4	0.10-0.28	0.184	0.018
Clover straw	2.4	0.2-2.2	1.020	0.102
Wheat bran	12	1.5-6.2	3.130	0.313

(tab. 2). Phosphorus concentrations in the fodder grown in this region were 0.1% in the dried grass, 0.0184% in the wheat straw, 0.102% in the clover straw, and 0.313% in the wheat bran. The concentrations obtained in this region were lower than other author's findings for the same type of fodder (10, 17, 23). When the present study was performed, there was a drought in the region. In such cases, phosphates cannot dissolve in soils with high pH values, and thus plants cannot benefit from undissolved phosphates. For the same reason, plant maturation does not develop properly and therefore P concentration in the plants is affected (2, 4).

When haematological parameters of control and sick animals were compared, only PCV values were found to be significantly different ($P < 0.05$). When PCV values were compared, they were significantly lower ($P < 0.05$) in the diseased animals (tab. 1). This difference could be due to nutrition, protein deficiency, and anorexia as reported in literature (26, 30). Total protein values before treatment were significantly low ($P < 0.001$) in sick animals compared to the same values obtained from control animals, but these same values increased significantly ($P < 0.05$) after treatment (tab. 1). The increase in the total protein values following treatment is believed to be due to good appetite, nutrition, and increased benefits from fodder as reported in literature (18, 26, 30).

ALP concentrations in healthy animals (263 ± 24.2 IU) and in sick animals (665 ± 48.4 IU) before treatment differed significantly ($P < 0.001$) (tab. 1). In bone disease, bone-originated ALP activity increases 20-25 times due to the increase in osteoblastic cell activation (19, 32). In the present study ALP was significantly higher ($P < 0.001$) in sick animals, but decreased significantly ($P < 0.001$) after the treatment. This was due to the regeneration processes occurring in the bones after P treatment, and confirms the findings of other researchers (19, 24, 32).

Calcium values (8.76 ± 0.3 mg/dl) of the sick animals were higher before treatment ($P < 0.05$) compared to the same values obtained from control animals (7.71 ± 0.1 mg/dl). Espinoza et al. (9) fed grazing cattle with different concentrations of P, and found that cattle fed with low concentration of P had higher Ca concentrations when compared to cattle fed with medium or high P. Similar findings were reported by Kiatoka et al. (18). Moreover, Call et al. (8) reported that high serum Ca concentration is related to low serum P concentration. These findings were also supported by Shupe et al. (28). In the present study, these values decreased after the treatment and this is in agreement with other researcher's findings (9, 18, 28). Furthermore, while serum copper concentrations in healthy animals and sick animals before treatment differed significantly ($P < 0.05$) (tab. 1), McDowell and Conrad (20) reported critical Cu values for cattle as 0.65 ppm. Other researchers (21) reported critical Cu values for cattle as 1.08 ppm for the summer season, and 0.9 ppm for winter-spring seasons. In the present study, Cu concentrations in diseased animals were lower ($P < 0.05$) compared to values obtained from control animals (tab. 1). This result is in agreement with findings of other researchers (20, 21). The difference observed in relation to Cu most probably was due to nutritional deficiency.

As a result, locomotor disorders observed in these cattle were due to hypophosphataemia. This was due to the low amount of P in the fodder consumed by these animals. They responded to P treatment well, and to protect animals from such disorders developing, P should be added to their fodder.

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