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ORIGINAL PAPER

LONG-TERM SUBCLINICAL COPPER DEFICIENCY AND IT IS INFLUENCE ON FUNCTIONS OF PARENCHYMAL ORGANS AND THE SERUM MACRO-ELEMENT DEFICIENCY IN DAIRY COWS*

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Abstract

Copper deficiency is a mineral management disorder increasingly often diagnosed in dairy cattle herds in Poland. Diagnosis of this disorder in herds can be very difficult as subclinical deficiencies may occur over long periods of time without clear clinical symptoms. Subclinical or atypical deficiencies that long remain untreated cause major disturbances in homeostasis. Information is scarce in the literature about the changes that occur in biochemical blood parameters in the course of atypical long-term subclinical copper deficiency. This study was conducted on a group of 80 cows from farms in the central part of the Lublin region in Poland. The animals were divided into four groups: three groups with Cu deficiency (two of these groups did not receive preventive supplementation, and the third one had oral copper supplementation administered) and a control group (clinically healthy cows). Blood for the tests was collected in the same period, 6 and 12 weeks after calving and a year after the previous tests. The results obtained in the groups where no special preventive measures had been taken showed a negative influence of copper deficiency on the functional condition of organs, especially the liver and pancreas (in the final stage of the study), and on blood phosphorus concentration. Such findings were not observed in the group supplemented with a copper-rich feed additive. In this group, after only a month of being supplemented with a preparation containing copper, a 15% increase in milk yield was observed. Meanwhile, the milk yield decreased in the remaining groups.

Keywords: hypocupraemia, cows, supplementation, liver, pancreas.

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INTRODUCTION

Copper (Cu) is one of the microelements necessary for maintaining the normal structure and functioning of many systems, namely the skeletal, haematopoietic, integumentary, nervous and immune ones (Frank et al. 2000, Sharma et al. 2005). It also participates in the processes of growth and development of animals (Tessman et al. 2001, Tiffany et al. 2002, Legleiter, Spears 2007, Sharma et al. 2008). In the serum, copper is bound mainly to ceruloplasmin (which transports about 95% of blood copper) and albumins (Linder 1991, Linder et al. 1998, Milne 1998). The liver acts as a storage organ for copper, but significant amounts of copper are also present in the plasma, erythrocytes and bone marrow. The biological role of copper is related mainly to the processes of oxidation and reduction; it is a co-factor for about 30 enzymes, such as cytochrome oxidase and superoxide dismutase, SOD (Spears 1999). Copper-dependent enzymes include: monoamine oxidase, MAO (Sharma et al. 2005) and polyphenol oxidase (tyrosinase) participating in the transformation of tyrosine into dihydroxyphenylalanine (DOPA) and catalysing the synthesis of melanin (Mastore et al. 2005). Copper deficiency reduces the activity of these enzymes and leads to neurological disorders, which result from the depressed synthesis of catecholamines (Zatta, Frank 2007). A lower copper concentration (hypocupraemia) leads to a significant lowering of vitamin A and E levels and to a depressed secretion of thyroid hormone (T_3 and T_4) in the organism (Sharma et al. 2003, 2005). Copper participates in the synthesis and conversion of these hormones (Abdollahi et al. 2013), and its low level depressed the secretion of tyrosine hydroxylase, which consequently leads to an inhibition of the thyroid hormone release factor (Yatoo et al. 2013). Disorders of copper management in an organism cause fertility problems, such as periodical infertility of females (lack of or silent rut), absolute infertility or pregnancy loss (Corah, Ives, 1991, Zatta, Frank 2007). Copper is absorbed from the digestive system, mainly in the small intestine. In ruminants, due to the numerous reactions taking place in the rumen, copper is absorbed at about a 5% level. Over 90% of copper is excreted with the faeces; while this mostly involves non-absorbed copper it also includes copper from the bile ducts, which is the main elimination route for this element.

Hypocupraemia is a frequent problem in herds of dairy cows, caused by small amounts of this element in the soil and feed, impaired absorption in the digestive system, and high demand during rapid growth and/or high milk yield (Suttle 1991, Gengelbach et al. 1994, Radostits et al. 2000). In dairy cows, diagnosed copper deficiency is most often of a secondary character. In such cases, the tests should include the levels of copper antagonists (molybdenum, sulphur, zinc, and iron) in a feed dose. Moderate deficiency is defined as a serum copper level between 4.71 and 9.26 μmol L^{-1}, while severe deficiency is a level below 4.71 μmol L^{-1} (Picco et al. 2004). The deficiency

is often accompanied by systemic symptoms, such as lack of appetite, pica, lower milk yield, lower weight gain and moderate anaemia (Mills et al. 1976, Sharma et al. 2005). Diagnosis of this disorder in herds can be very difficult as subclinical deficiencies may occur over a long period of time without clear clinical symptoms. Copper deficiency is often diagnosed accidentally, during screening tests or the treatment of other diseases.

Hypocupremic diarrhoea is often chronic in character; it is resistant to treatment and is not accompanied by inflammatory lesions in the digestive system. Increasing numbers of cases are reported with atypical symptoms, such as lack of appetite, drop in milk yield, or lameness. The diagnosis of copper deficiency is based on interview, clinical examination, assay of copper levels in the serum and hepatic tissue, and ceruloplasmin activity (Milne 1998). Disturbed copper homeostasis often has an asymptomatic course over long periods of time, and the absence of a strict relationship between serum copper concentration and the appearance of clinical symptoms hinders the diagnosis.

The aim of the study was to determine the influence of chronic subclinical copper deficiency on the functioning of the parenchymal organs, mineral metabolism and milking.

MATERIAL AND METHODS

The study included 80 dairy cows (HF breed, 60 with deficiency, 20 healthy as a control group) with the following parameters: age range: 3-4 years, body weight: 550-650 kg, good health condition, average or good nutritional status (BCS- 3.5) and average annual milk yield of 8.500 litres. The animals were in week 6 after calving and the study lasted to week 12 after calving in the following year. The farms were organized as tie-up stalls with the TMR feeding composed of: maize silage, maize grain, mixed grass haylage, hay, complete feeding stuff with protein content up to 24%, and home-made concentrate feed (on the basis of premixes, home-grown cereals, straw), with mineral and vitamin supplements in amounts corresponding to milk yield. Feed doses were adjusted to milk production, current physiological condition, age and weight of a given cow. The cows from which the study material was collected were under continuous veterinary control. Cows diagnosed additionally with significant phosphorus, calcium or magnesium deficiencies, or major changes in the functional parameters of the parenchymal organs, were excluded from the study. Any cows after difficult calving, with a damaged pelvis, muscles, or hooves, or an illness in the last post-partum period, were also subject to exclusion. The preventive measures included a widely available preparation containing an increased amount of copper sulphate and organic compounds of copper (chelates), 1.900 mg kg^{-1} of the additive, while a supplement used earlier contained copper sulphate pentahydrate 1.500 mg kg^{-1}.

On the basis of interviews with the farmers, clinical examination of cows and results of biochemical blood tests, the cows were divided into three study groups and a control group. Group I included cows which did not show any clinical symptoms, with a blood copper level lower than the assumed norm (Olech 2016). During the interviews, the owners reported periodical problems occurred with appetite. Group II included cows with periods of lowered appetite, rumination disorders, periodical diarrhoea, and in some cases also limb oedema, especially in the area of the ankle and wrist joints. Group III included animals with no diagnosed clinical symptoms or mild periodical appetite disorders and diarrhoea. In these cows, after the diagnosis of Cu deficiency on the basis of laboratory tests, the owners immediately decided to change the mineral additive for a market-available preparation with an increased copper content (1900 mg kg^{-1} mineral supplement), containing copper as organic chelates and sulphate (oral administration, 200 g per day). Group IV were clinically healthy cows, with no pathological disorders reported in the interviews concerning the dry period, post-partum period or study period, the copper levels of which were within the normal range for cattle.

The blood for the tests was drawn from the external jugular vein, placed in test tubes with a clotting agent and centrifuged at 3000 G in order to obtain the serum, which was then stored at –80°C until the analyses were performed. Each time, blood collection was preceded by a detailed interview and clinical examination. The evaluation included the method of feeding and type of feed, as well as dairy efficiency. The blood was collected four times, 6 and 12 weeks after calving (sampling 1 and 2, respectively), and in the same period a year after the first sampling (sampling 3 and 4). The sampling times were selected on the basis of previous clinical studies in cattle, which showed that this kind of deficiency during lactation was most frequently diagnosed in weeks 6 and 12 after calving. The copper (Cu) and zinc (Zn) concentrations were measured by atomic absorption spectrometry (ASA) on a spectrometer by Perkin Elmer – 4100. The following serum parameters were assayed: total calcium (Ca), inorganic phosphorus (Pi), total magnesium (Mg), total bilirubin (tB), creatinine (Cre), activity of aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGTP) and amylase (AMY). The tests were performed using a BS-130 Chemistry Analyzer by MINDRAY.

Biochemistry results were statistically analyzed in Statistica 10.0 PL software, to calculate the mean (x) and standard deviation (SD). Significance of differences between average values was estimated by t-Student test, at the significance level of $p < 0{,}05$.

RESULTS

During the whole observation period of the clinical study, no deviations from baseline in groups I and II (week 6 after calving) were observed and the deficiency persisted. However, an average 10% decrease in milk yield was noted. On the other hand, in group III where a supplement with an increased copper content was administered, as early as the second test (week 12 of the lactation period) the owners reported the disappearance of periodical appetite disorders and loose stools. Simultaneously, a significant increase in milk yield (by about 15%) was observed in this group a year from the implementation of the copper deficiency prophylaxis. In the control group, the average milk yield after a year in the same study periods remained unchanged.

Initially, the lowest copper concentration was observed in group II; however a significant decrease in copper concentration during the observation period was noted only in group I (especially between sampling 3 and 4), reaching levels lower than in group II (Table 1). In group III, where an increa-

Table 1

Concentrations of mineral elements at each stage of the study

Group	Blood sampling	Cu (μmol L^{-1})		Zn (μmol L^{-1})		Ca (mmol L^{-1})		Pi (mmol L^{-1})		Mg (mmol L^{-1})	
		X	SD	*X*	SD	*X*	SD	*X*	SD	*X*	SD
I Without copper deficiency symptoms	1	7.6^{a*}	1.9	27.3^{a}	4.2	2.18	0.09	1.61	0.11	1.05	0.10
	2	6.8^{a}	1.4	25.8	6.1	2.09	0.07	1.59^{a}	0.08	1.04	0.07
	3	6.5^{a*}	2.1	26.7	4.8	2.15	0.04	1.57^{a}	0.06	1.11	0.09
	4	5.5^{a*}	1.6	26.1	5.4	2.08	0.08	1.49^{a}	0.08	1.14	0.06
II With clinical symptoms	1	6.9^{b*}	2.1	24.6^{b}	6.3	2.19	0.03	1.58	0.05	1.09	0.05
	2	6.5^{a}	1.8	25.3	6.6	2.11	0.09	1.53^{a}	0.04	1.12	0.07
	3	6.3^{a}	1.9	26.3	6.1	2.12	0.05	1.52^{a}	0.06	1.10	0.11
	4	5.9^{a*}	1.6	25.7	5.4	2.07	0.11	1.47^{a}	0.07	1.15	0.08
III Copper deficiency with supplementation	1	7.9^{a*}	2.3	21.2^{b*}	2.1	2.14	0.07	1.59	0.04	1.07^{*}	0.12
	2	11.1^{b*}	3.4	23.1	2.6	2.10	0.08	1.67^{ab}	0.06	1.01	0.14
	3	23.6^{b*}	3.9	27.9^{*}	2.9	2.17	0.07	1.66^{ab}	0.08	0.93	0.10
	4	26.4^{b*}	4.1	29.4^{*}	1.9	2.05	0.11	1.71^{b}	0.07	0.83^{*}	0.16
IV Healthy cows	1	14.3^{c}	2.2	27.3^{a}	5.2	2.17	0.04	1.77	0.08	1.04	0.09
	2	13.7^{c}	2.6	26.1	7.1	2.12	0.05	1.80^{b}	0.06	1.03	0.11
	3	14.9^{c}	2.4	25.8	4.9	2.14	0.03	1.74^{b}	0.08	1.12	0.07
	4	13.9^{c}	2.7	26.9	5.6	2.09	0.09	1.78^{b}	0.06	1.10	0.14

The significance of differences between mean values in different groups $^{a, b, c}$ at $p < 0{,}05$;
The significance of differences between mean values in the groups * at $p < 0{,}05$;
X – the mean;
SD – standard deviation.

sed copper supply was ensured, there was a gradual increase in the level of this element during the observation period, most visible between sampling 2 and 3, while at the termination of the study (after a year), copper concentration values were statistically higher than in the control group. While assessing the levels of the analysed microelements, it should be noted that the average concentrations of zinc in groups I and II did not change despite the decline in copper levels. Moreover, in group III, an increase in the copper concentration was accompanied by a distinct increase in the zinc level. However, this was not statistically significant. In the control group, despite minor fluctuations, the zinc concentration did not change significantly during the whole study period. At the same time, no statistically significant differences between group IV and the study groups were observed.

As far as calcium and magnesium are concerned, minor fluctuations in the levels of these elements in eeek 12 after calving were observed in comparison to the values from sampling 1 and 3, but they were not statistically significant (Table 1). No relationships between average concentrations of calcium, magnesium and copper were detected in any group. Throughout the study, calcium levels fluctuated between 2 and 2.25 mmol L^{-1}. No subclinical and clinical hypocalcaemia was detected either (Sobiech et al. 2010), which is important especially in the light of the information supplied in the interviews about periodically occurring clinical symptoms in the study groups (which could be related to low calcium levels). The average magnesium levels also remained within the physiological normal range during the whole study period (Winnicka 2008, Olech 2016). In Group III, a statistically insignificant decrease in magnesium levels was observed.

Findings on phosphorus are different. In groups I and II, a decrease in the copper concentration was accompanied by the lowering of phosphorus levels. The phosphorus concentration was the lowest at sampling 4, as was the case with copper, but it still remained in the lower values of the normal range (although significantly lower than in the control group). On the other hand, no significant changes in the concentration of this element were observed in groups III and IV.

The chosen parameters describing the functional condition of the parenchymal organs showed major disorders, especially in group II. The serum bilirubin level was the highest in group II, while in group I a gradual increase in its concentration was observed through the whole study period (Table 2). In both of these groups, at the end of the study, the upper limit of the physiological norm had been exceeded more than twice, and the increases were statistically significant in relation to both the group baselines and the control group values. In Group III, the concentration increase was not statistically significant.

The activity of GGTP in the groups which did not take the preventive preparations increased significantly, achieving the highest values in group II. In group III, a statistically significant, but much lower, increase in the

Table 2

Activity and concentrations of parameters measuring the function of the parenchymal organs

Group	Blood sampling	Total bilirubin (μmol L^{-1})		Creatinine (μmol L^{-1})		AST (U L^{-1})		GGTP (U L^{-1})		AMY (U L^{-1})	
		X	SD	*X*	SD	*X*	SD	*X*	SD	*X*	SD
I Without copper deficiency symptoms	1	7.18^{*a}	1.54	75.4	4.8	69.4^{*}	14.3	34^{*}	6	13^{*a}	3
	2	8.38^{a}	0.86	72.6	5.9	58.9	19.2	39	7	10^{a}	4
	3	13.34^{*a}	1.03	81.3	3.6	38.9	21.8	42	9	9^{a}	2
	4	14.71^{*a}	0.68	77.4	7.1	48.4^{*}	19.5	48^{*}	5	6^{*a}	5
II With clinical symptoms	1	8.72^{*a}	1.19	82.1	5.3	75.8	22.8	41^{*}	4	14^{*a}	3
	2	10.77^{b}	0.68	84.2	8.1	77.9	31.1	47	3	12^{a}	4
	3	13.00^{*a}	0.86	78.4	6.3	72.4	24.2	48	9	8^{*a}	2
	4	16.76^{*a}	1.37	80.1	5.2	69.3	12.7	56^{*}	11	5^{*a}	3
III Copper deficiency with supplementation	1	6.67^{*b}	1.19	73.4	7.1	48.5^{*}	23.2	31^{*}	4	14^{a}	4
	2	8.04^{a}	1.54	78.2	6.3	53.8	12.9	33	5	16^{a}	3
	3	8.89^{*b}	1.88	83.9	4.8	57.7	17.4	37	3	17^{b}	5
	4	10.09^{*b}	2.39	85.8	8.3	68.4^{*}	15.9	43^{*}	5	19^{b}	7
IV Healthy cows	1	2.74^{c}	2.05	83.7	3.5	55.8	11.9	19	3	26^{b}	6
	2	3.08^{c}	1.71	87.4	3.9	51.3	14.8	23	6	28^{b}	4
	3	3.59^{c}	1.71	81.2	4.6	64.9	19.3	17	2	25^{c}	5
	4	2.05^{c}	1.37	83.6	5.1	69.9	15.3	21	5	27^{c}	5

The significance of differences between mean values in different groups $^{a, b, c}$ at $p < 0{,}05$;
The significance of differences between mean values in the groups * at $p < 0{,}05$;
X – the mean;
SD – standard deviation.

GGTP activity was observed. In all study groups, its activity was significantly higher than in the control group.

The level of creatinine in all study groups and in the control group was lower than the assumed normal values during the whole study period (Winnicka 2008, Olech 2016). An increase in its level was observed only in group III, to which the copper preparation was administered (Table 2).

As far as AST is concerned, only statistically insignificant fluctuations within the physiological norm in this parameter were observed (Winnicka 2008, Olech 2016), and the average values in the study groups were close to those in the control group.

In groups I and II, featuring lowered copper levels with no supplementation administered, the activity of amylase in the study period was below the lower limit of the norm, and during the observation of the copper deficiency a statistically insignificant but distinct decline in its activity was noted. At the same time, the values of amylase noted in these groups were signifi-

cantly lower than in the control group (Table 2). In group III, taking an additive with an increased copper content, a statistically insignificant increase in the amylase activity was observed. In the final stage of the study, the average activity of the enzyme in this group was still lower than in the control group.

DISCUSSION

A decrease in the copper concentration was much more rapid in the peak lactation (the period between sampling 1 and 2 and the period between sampling 3 and 4). However, this may have been caused by a larger consumption of mineral elements during the peak lactation in the course of long-term deficiency. Unfortunately, there is no information in the literature about the course of untreated copper deficiency during the peak lactation. The concentrations obtained during sampling 4 in groups I and II were significantly lower than in the control group and visibly lower than the concentrations reported in the studies of other authors for healthy cows (Spolders et al. 2010). In group III, in which copper supplementation was administered, the greatest increase in the serum copper level was observed between sampling 2 and 3, i.e. at the longest interval between sampling times. However, the high copper values in the final stage of the study (week 12 of lactation) show that over-extended use of preparations with an increased content of chosen elements may lead to exceeding the physiological norms, or even to poisoning (Perrin et al. 1990, Bradley 1993, Johnston et al. 2014). In the group receiving supplementation (group III), it was recommended to replace half of the preparation with the mineral and vitamin additive used earlier. While assessing the levels of the microelements studied, it should be noted that the average concentrations of zinc in groups I and II did not change despite the decrease in copper levels. Some authors state that concentrations of copper and zinc in the blood serum are mutually dependent, that is a decrease in the copper concentration correlates with an increase in the zinc level, and vice versa (Osredkar, Sustar 2011). Moreover, in group III, the increase in the copper concentration was accompanied by a visible increase in the zinc level.

The tendency of GGTP to rise observed in our study was also reported by Kupczyński, Chudoba-Drozdowska (2002) and Cozzi et al. (2011). However, their studies were devoted to cows at the beginning of lactation, receiving no supplementation. It shows that long-term and untreated copper deficiency causes functional liver disorders, which in the future may lead to the culling of animals from a herd. On the basis of the research, it may be concluded that long-term copper deficiency leads to impaired functioning of the liver and pancreas, as well as significant changes in milk yield. On the other

hand, using additives supplementing copper requires monitoring changes in the biochemical blood parameters.

Interesting relationships were also observed between the concentrations of copper and phosphorus and the pancreatic amylase function in groups I and II. The values were similar to those observed by other authors during the lactation period in groups III and IV (Hadžimusić, Krnić 2012, Djokovic et al. 2014). Changes in the phosphorus concentration during copper deficiency may indicate a correlation between these elements in the body, but there is no information about this in the literature, thus further research is necessary. However, the current results suggest that an inorganic phosphorus assay should be included in the biochemistry tests performed in the course of chronic copper deficiency, as it is possible that the deficiency of this macroelement influences the development of clinical symptoms during copper deficiency. These observations (in groups I and II) require a broader study because it may be assumed that diarrhoea typical of copper deficiency in cattle (Tessman et al. 2001, Tiffany et al. 2002, Legleiter, Spears 2007, Sharma et al. 2008) is related not only to the lowered activity of copper-dependent enzymes, but also to a degraded pancreatic secretory function. At present, studies in this field have only demonstrated a relationship between copper deficiency and pancreatic malfunction and lowered amylase activity in rats and rabbits (Alvarez et al. 1989, Dubick et al. 1989, Fields, Lewis 1997). As for cattle, there is no information in this field in the available literature.

CONCLUSIONS

The study showed that even a subclinical but lasting copper deficiency significantly disturbs the vital functions of dairy cows, leading to a decreased milk yield and, as a consequence, a shorter productive life of these animals. The average milk yield observed in group III, receiving copper supplementation, seems to confirm the need to use preventive preparations even in cases of subclinical copper deficiency, as the milk yield decreased for those animals whose owners did not take such steps (groups I and II). The difference between the groups was about 25%. Following the presentation of the research results at the end of the study, the owners who underestimated the role of copper supplementation immediately decided to introduce some form of copper supplementation (individual intramuscular injections, copper licks, or ready mineral preparations with increased copper content).

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