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ORIGINAL PAPER

HEMATOLOGICAL PARAMETERS IN DAIRY COWS WITH COPPER DEFICIENCY*

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Abstract

The aim of this research was to determine how hematological parameters develop in the course of various levels of copper deficiency in cattle. Animals suffering from copper deficiency were compared to cows undergoing routine health checkups on farms, in which no deficiency of this microelement was found. The study was carried out on three farms in the central Lublin region, and included 80 HF breed dairy cows, aged from 3 to 6 years, at the peak of lactation. The animals were divided into a total of 4 groups – 3 groups at different levels of copper deficiency and 1 group of healthy animals. For the groups of animals studied, a decrease in the parameters of the red blood cell system was found in all the three groups as compared with the control group. The number of erythrocytes in all of the groups was within reference limits; however, the erythrocyte counts were statistically significantly lower ($p < 0.01$) than in the control group. A statistically significant decrease in the Hb concentration, as well as Hct and MCH values below the reference standards were reported. The MCV parameter was statistically significantly lower ($p < 0.01$) than in the control group. In the examined groups, a statistically significant decrease ($p < 0.01$) in the number of leukocytes was observed as compared with the control group, as well as an increase in the number of neutrophils in groups II and III as compared with the control group. A decrease in the number of lymphocytes also resulted in statistically significant lower numbers ($p < 0.01$) relative to the control group. The results indicate that a hematological examination provides valuable indications, especially with regards to subclinical Cu deficiency. Normocytic, hypochromic anemia was reported in the examined animals. A decrease of Cu levels in the serum disturbs the ratio of neutrophil and lymphocyte populations in the blood.

Keywords: cows, hypocupremia, hematology, anemia.

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INTRODUCTION

With regard to its concentration in living organisms, copper (Cu) is the third most prevalent trace element, following iron (Fe) and zinc (Zn). Cu is a component or an activator of many enzymes. This element is a core component of the active centers of proteins necessary for iron homeostasis, such as ceruloplasmin and hephaestin. Ceruloplasmin plays a significant role in the process of Fe metabolism, and catalyzes the reaction of Fe 2+ iron oxidation to Fe 3+, which enables it to bind with transferrin and be transported in the blood plasma. Copper, being embedded in ceruloplasmin, is necessary to mobilize iron from the liver and transport it to the bone marrow, where it is used for erythropoiesis. In the case of copper deficiency, iron is stored in the liver, and its availability is reduced. Hephaestin (copper--containing ferroxidase) acts as an agent facilitating the transport of iron from enterocytes into the blood stream. Its absence in the organism leads to hypochromic microcytic anemia and the accumulation of iron in the intestinal epithelium (Smart et al. 1981, Wu et al. 2006, Wierzbicka, Gromadzka 2014, D'Angelo 2016). Copper plays a role in the absorption, mobilization and transport of iron to the bone marrow. It is necessary for the production and maturation of erythrocytes in the bone marrow, as well as for the synthesis of heme through the activation of ferrochelatase. It also affects the maintenance of internal homeostasis and many immune system functions dependent on phagocytosis (through neutrophil activity and their phagocytic capacity). The phagocytic capacity of neutrophils increase as a result of copper supplementation in goat kids, while deficiencies of Cu in the diet intensify neutrophil accumulation in the pulmonary microcirculation (Solaimam et al. 2007, Soch et al. 2011, Mohammed et al. 2014, D'Angelo 2016).

Copper deficiency (hypocupremia) may be primary or secondary. Primary deficiencies occur in areas devoid of copper in soil and when there is a low content of this element in feed. Secondary deficiencies, however, stem from the presence of Cu antagonists in water or feed. It has been documented that a high concentration of molybdenum, sulfur, iron and zinc in the diet reduces the absorption of copper from the gastrointestinal tract (Menzir, Dessie 2017, Kurek et al. 2017).

Literature data provide little information concerning hematological changes in the case of copper deficiency in cattle. In humans, the influence of copper on the hematopoietic system is not clear but it has been studied in detail, and the topic itself is widely discussed. The most common consequences of hypocupremia in humans are normocytic, macrocytic or microcytic anemia and neutropenia. As a consequence, copper deficiency contributes to disturbances in hemoglobin synthesis and the proper course of erythropoiesis. Copper deficiency is also accompanied by leukopenia with neutropenia, although to date its causes have not been fully explained (Harless et al.

2006, Halfdanarson et al. 2008, Mohammed et al. 2014). In animals with copper deficiency, neutrophilia was observed (Karimbakas et al. 1998).

In the subclinical course of cattle deficiency diseases, hematological tests are not usually performed for economic reasons, being limited exclusively to a biochemical panel, which does not fully reflect the current state of internal homeostasis. In cases of more advanced deficiency conditions, hematological tests are done if the treatment is not effective, i.e. after a few or even several weeks from diagnosing deficiency. This makes it difficult to correctly assess the hematological changes as copper preparations administered during treatment affect red and white blood cell parameters.

The aim of the research was to determine how hematological parameters develop in the course of various levels of copper deficiency in cattle. The results obtained in cows with various levels of copper deficiency were compared with the results obtained from clinically and laboratory healthy animals, from herds under constant veterinary supervision.

MATERIAL AND METHODS

The research was carried out in three farms in the central Lubelskie region, and included 80 HF breed dairy cows, aged from 3 to 6 years, with an average milk yield of about 6000 liters (SD = 0.7) during lactation preceding the examination period and a BCS condition of 3 points on a 5-point scale. The cows examined were between day 70 and day 100 of their lactation period. The farms used a similar feeding system (TMR containing corn silage, corn, hay silage, grass silage, hay, straw, granules, homegrown grain, and feed additives with a protein content from 18 to 24%, premixes and mineral-vitamin supplements). Feed rations were based on the milk yield, current physiological state, age and body weight of the cows. The animals were under constant veterinary care; periodically, biochemical blood tests were performed. Before every blood sampling, a detailed interview with the owner of the animals and clinical examination of the animals were carried out; the feeding patterns and quality of the fodder and milk yield were also assessed. Additionally, an interview about the prophylaxis and management of herd was conducted as well. Only those animals in which we excluded parasitic diseases, infectious diseases, injuries, and any kind of bleeding (both from the integuments and the gastrointestinal or urinary tract) were qualified for the study. The study did not include animals with severe labour and disturbances during the perinatal period. No additional preventive preparations were used in the cows qualified for the study, and no supplementation of copper was administered. The animals were divided into a total of 4 groups – 3 groups with different levels of copper deficiency and 1 group of healthy animals.

The first group (group I) consisted of 17 cows, and included animals with no clinical symptoms during the course of the study, and for which the herdsman indicated periodic problems with appetite, particularly following morning milking and a decreased amount of milk produced as compared to the previous lactation (up to 15%). In laboratory tests, a significantly reduced Cu content in the blood in these animals but no changes in the total bilirubin and GGTP activity in the serum were found (Table 1).

The second group (group II) consisted of 14 cows with hair discoloration in the form of so-called "copper glasses" and minor scalp discoloration. Reduced appetite was reported as well as periodic difficulties rising up and an evident decrease in milk yield (in some individuals it reached 20%). The clinical trial showed an enlarged field of liver dullness (down and forward) with no pain in this organ. A biochemical examination revealed a

Table 1

Results of biochemical tests in cows

Specification	Bovine reference intervals according to Winnicka (2008)	Group I		Group II		Group III		Control group	
		X	SD	X	SD	X	SD	X	SD
Cu (μmol l^{-1})	the mean 18.84	10.37^{A}	1.00	9.49^{A}	0.29	8.10^{A}	0.90	12.45^{B}	0.84
Fe (μmol l^{-1})	21.5 - 35.8	30.05	4.11	16.03^{A}	3.09	25.50	1.98	29.40^{B}	5.10
Zn (μmol l^{-1})	the mean 15.3	43.73^{A}	3.18	20.50	4.34	42.30^{A}	3.68	27.13^{B}	9.58
AST (U l^{-1})	58 - 100	66.14	9.92	123.50^{A}	27.05	59.43	9.16	64.00^{B}	11.79
TB (μmol l^{-1})	1.9 - 7.0	3.59	1.54	8.60^{A}	4.96	2.22	3.25	2.91^{B}	2.05
GGTP (U l^{-1})	10 -30	19.21	6.76	47.50^{A}	8.93	36.86^{a}	16.50	$19.00^{B,b}$	5.44
Glucose (mmol l^{-1})	2.2 - 4.5	3.72	0.55	3.00^{a}	0.31	3.38	0.28	3.47^{b}	0.57
TP (g l^{-1})	51 - 71	67.1^{A}	3.8	67.9^{a}	4.6	67.7^{A}	3.6	$72.6^{B,b}$	3.10
Urea (mmol l^{-1})	1.66 - 7.47	3.72	1.39	6.57^{A}	0.78	4.55	3.78	4.43^{B}	1.82
CaAS (mmol l^{-1})	2.25 – 3.03	2.22	0.1	2.2	0.16	2.31	0.12	2.21	0.11
Mg (mmol l^{-1})	0.78 – 1.23	0.96	0.16	0.90	0.07	1.07	0.20	0.93	0.17

A,B – the significance of differences between mean values in the groups vs control group $p < 0.01$;
a, b – the significance of differences between mean values in the groups vs control group at $p < 0.05$; *X* – the mean; SD – standard deviation; TB – total bilirubin; TP – total protein.

significantly reduced copper content and an increase in the total bilirubin and GGTP activity in the serum (Table 1).

The third group (group III) consisted of 19 cows with hair discoloration around the eyes (“copper glasses”), on the head, neck, wither and the upper part of the front limbs. In some individuals (7 cows) inflammations occurred near the limbus of cloven hooves and dermatitis interdigitalis, most commonly in the pelvic limbs. An interview with the owner of the animals concluded that the cows suffered from reduced appetite (less intake of concentrated feed) and a significant decrease in milk yield by up to 20%. Laboratory tests showed a significant reduction in the serum Cu concentration, an increase in the GGTP activity in serum and no changes in the total bilirubin content (Table 1).

The fourth, control, group consisted of 30 animals, in which no pathological changes were found on the basis of an interview with the owner of the animals, clinical trial and blood biochemistry and no metabolic or infectious diseases occurred in the last year.

Blood was sampled after morning milking from the external jugular vein into 2 ml K_3EDTA tubes with a clotting agent from the Medlab company. In the morphological study the following was determined: the number of erythrocytes, hemoglobin concentration (Hb), hematocrit (Hct), mean red cell volume (MCV), mean erythrocyte hemoglobin (MCH), mean hemoglobin in the blood (MCHC), number of leucocytes, neutrophils (G), eosinophils (E) and basophils (B), lymphocytes (L) and monocytes (M) using a Horiba scil Vet abc Plus automatic analyzer; the preparations were stained using the May-Grünwald-Giemza method in a MYTHIC TS hematological smear staining apparatus. In the serum, the following were determined: the concentration of total calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), total bilirubin (TB), total protein (TP) and urea, the activity of gamma--glutamyl transpeptidase (GGTP), aspartate aminotransferase (AST), using a MINDRAY BS-130 Chemistry Analyzer. The concentrations of copper (Cu), zinc (Zn) and iron (Fe) were determined with the use of atomic absorption spectrometry (ASA) with excitation in an acetylene-air flame.

The results were subjected to mathematical statistical analysis with a nonparametric *t*-Student test using a Statistica® 10.0 computer program from Statsoft.

RESULTS AND DISCUSSION

A decreased Cu concentration in the serum was observed in all groups (groups I, II, III) and the results were statistically significant ($p < 0.01$) as compared with the control group. A physical examination revealed increasing appetite disorders and disturbances in the reticulum, rumen and omasum. There was an enlargement of the hepatic suppression field and periodic rumen bloating only in the second group, where an increase in GGTP activity, AST and an increase in total bilirubin with a simultaneous decrease in iron concentration were detected as well (Table 1). The concentration of glucose and total protein content in blood serum were within normal values for all groups, but, at the same time, lower than the average values for the control group. The concentration of urea remained at normal levels, but this parameter reached statistically significant higher values in the second group than in the control group. Calcium levels were relatively low, which is normal for cows at this physiological stage. Concentrations of magnesium also remained within the norm and its average value was close to the values observed in the control group. In group I, no changes in hair (discolouring, loss) were reported. Changes in the form of inflammation in the area of limbus of cloven hooves and dermatitis interdigitalis were found in cows with the lowest Cu content in serum.

In the groups of animals studied, a decrease in the parameters of the red blood cell system was found in all the three groups as compared with the control group (Table 2). The number of erythrocytes in all the groups was within the reference range; however, it was statistically significant lower ($p < 0.01$) than in the control group. The Hb concentration, Hct and MCH values were below the reference range (Baumgartner 2005) and were statistically significantly lower ($p < 0.01$) than in the control group. Although the MCV parameter was within the reference range (Baumgartner 2005) in all the groups studied, it was statistically significantly lower ($p < 0.01$) than in the control group.

The number of leukocytes in groups I and II was within the reference range; however, it was statistically significantly lower ($p < 0.01$) than in the control group. In group III, leukopenia was reported and this result was significantly different from the control group. Neutrophilia was reported in groups II and III, while in group I, the percentage of neutrophils was lower than in the control group. All the studied groups were found to have lymphopenia and mild *eosinophilia* and basophilia, in addition, groups I and II were diagnosed with monocytosis. Platelet counts were within the reference range (Baumgartner 2005) in all the studied groups (Table 2)

Decreased copper concentration in cows is often found in herds around the world (Fazzio et al. 2010, Menzir, Dessie 2017). The causes of Cu deficiency in clinical cases are often multifactorial and may be difficult to diagnose, especially in the initial stages of the deficiency, when clinical symptoms are not observable (Menzir, Dessie 2017). In the case of Cu deficiency, hypochromic microcytic anemia occurs (Mohammed et al. 2014), while in humans normocytic or macrocytic hypochromic anemia is observed, and in individual cases microcytic anemia is reported as well (Wu et al. 2006, Lazarchick 2012).

In the studied groups of cows, a decrease in erythrocyte system parameters was observed, and normocytic hypochromic anemia was found, as compared with the reference range according to Baumgartner (2005). In turn,

Table 2

Hematological values means in cows

Specification	Bovine reference intervals according to Baumgartnera (2005)	Group I		Group II		Group III		Control group	
		X	SD	*X*	SD	*X*	SD	*X*	SD
RBC x 10^{12} l^{-1}	5 - 7	6.91^A	1.09	6.8^A	0.72	6.53^A	1.03	8.03^B	0.50
Hb (g l^{-1})	105 - 140	94.8^A	9.8	95.4^A	12.1	89.5^A	11.6	112.4^B	1.04
Hct (l l^{-1})	0.30 - 0.40	0.29^A	0.03	0.29^A	0.04	0.27^A	0.04	0.33^B	0.03
MCV (fl)	40 - 60	42.93^A	5.11	43.09^A	4.35	42.18^A	4.96	51.08^B	3.80
MCH (fmol)	0.9 - 1.5	0.86^A	0.11	0.87^A	0.09	0.86^A	0.09	1.08^B	0.07
MCHC (mmol l^{-1})	16 - 21	19.74	0.48	19.84	0.41	19.95^a	0.46	19.32^b	0.98
WBC x 10^9 l^{-1}	6.2 - 9.5	6.52^A	1.16	6.29^A	1.29	5.91^A	1.78	8.37^B	0.85
GP (%)	0 - 2	0.07^A	0.27	0.73^A	0.47	0.55^A	0.93	1.50^B	0.67
GS (%)	23 - 37	33.29^a	5.50	37.27	6.97	40.39^a	6.30	36.17^b	1.19
E (%)	1 - 7	7.79^A	4.81	7.09^A	3.88	7.18^A	4.05	2.75^B	1.48
B (%)	0 - 1	1.21^a	1.42	1.00^a	0.77	1.55^A	1.37	0.25	0.45
L (%)	53 - 67	52.21^A	7.98	47.73^A	7.82	46.64^A	6.98	58.75^B	1.14
M (%)	0 - 4	5.43^A	4.05	5.73^A	5.83	3.73^A	3.26	0.58^B	0.51
PLT x 10^9 l^{-1}	200 - 800	561.57	162.69	501.09	95.10	507.55	106.60	550	75.74

GP – band neutrophils, GS – segmented neutrophils, E – eosinophils, B – basophils, L – lymphocytes, M – monocytes;
A, *B* – the significance of differences between mean values in the groups vs control group $p < 0.01$;
a, *b* – the significance of differences between mean values in the groups vs control group at $p < 0.05$; *X* – the mean; SD – standard deviation.

Jones and Allison (2007), Roland et al. (2014), noted that in the case of Cu and Fe deficiency and in the course of chronic diseases, MCV is reduced, which indicates microcytic anemia. Our study showed that MCH is below the reference standards (Baumgartner 2005). The reduction in the mean hemoglobin mass of erythrocytes may be an early indicator of iron deficiency, as this index decreases faster than MCV and MCHC in microcytic anemia (Mazzullo et al., 2014). Our research showed that in the cow groups studied, MCV is within the reference standards (Baumgartner 2005). With regards to Baumgartner's (2005) norms, normocytic anemia occurred in all groups. Comparing the average blood cell size (MCV) in healthy animals with those recorded in the study groups, a significant difference in the size of erythrocytes was observed.

In groups I and III, it was observed that the iron concentration in serum was within the reference standards (Winnicka 2008) but, despite this, anemia occurred in the examined cows. Similar observations were made by Collins et al. (2010) in humans. They found (Collins et al. 2010) that in the course of copper deficiency, hemoglobin production is ineffective, despite a normal Fe concentration in serum.

A decrease in the number of leukocytes in all the three groups was found as compared with the control group, whereas in group I, the neutrophil count decreased relative to the control group, while a decrease in the serum copper concentration in groups II and III was concurrent with an increase in the number of neutrophils. In humans, leukopenia with neutropenia is observed as the earliest symptom of a decline in the Cu concentration in serum (Lazarchick 2012). Our findings also confirmed a decrease in the number of lymphocytes with a decrease in the copper concentration, which is opposite to a change observed in the number of neutrophils. Similar changes were noted by Karimbakas et al. (1998), where the lymphocyte population predominated in healthy individuals, whereas in Cu-deficient animals the number of lymphocytes decreased together with an increase in neutrophil counts. Cerone et al. (1998) did not observe changes in the number of neutrophils in cows suffering from copper deficiency. In sheep with hypocupremia, the number of monocytes increased with a decrease in neutrophils (Cerone et al. 1998). In all the three groups of cows studied in our research, an increase in the number of monocytes was observed as compared with the control group. It was also found that in the third group, with the lowest concentration of Cu in serum, the increase was the least notable. Based on our own research, a mild eosinophilia was diagnosed in animals with low Cu levels in the serum. There are reports concerning the relationship between the histamine metabolism enzymes and Cu concentration in the serum (DAO histaminase), which may lead to mild histaminaemia and furthermore, a minor change in the appearance of the eosinophiles may be observed. Thus, further studies are required to provide an explanation for such changes (Schuschke 1994, Maintz, Novak 2007, Robinson 2013) .

Based on our own research, the severity of clinical symptoms in cows in the course of hypocupremia may also be largely associated with hematological disorders. Only a temporary decrease in appetite occurred in group I, whereas in group III, where numerically the lowest Cu concertation occurred, the reduction of appetite was more severe. For some animals in group III, inflammations occurred in the area limbus of cloven hooves and dermatitis interdigitalis, with simultaneous neutrophilia and lymphopenia.

CONCLUSION

A hematological examination is of prognostic significance in the early diagnosis of Cu deficiency in cows, when clinical symptoms are not yet present or are atypical. Anemia and a decrease in the number of leukocytes in cows with the deficiency are observed. The decrease in Cu concentration in the serum deteriorates the erythrocyte system's parameters; the lower the Cu concentration in the serum is, the more advanced the hypochromic anemia becomes. In the course of Cu deficiency, the normal ratio of neutrophil and lymphocyte populations is disturbed. It will require further research to elucidate the pathomechanism of hematological changes in the course of hypocupremia in cows.

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