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ORIGINAL PAPER

COPPER AND IRON DEFICIENCY IN DAIRY CATTLE*

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Abstract

Copper deficiency is a common homeostatic disorder in dairy cattle farms in Poland. A simultaneous decrease in blood copper and iron concentrations is very rarely diagnosed and described in dairy cows. Copper is necessary for the mobilization of iron from the liver and its transport to bone marrow, where it is used for erythropoiesis. The aim of the study was to observe changes in haematological parameters in cows, resulting from copper deficiency or simultaneous deficiency of copper and iron, in comparison with animals from the same herds in which no deficiencies of these micronutrients were found. The study was conducted on 56 cows of the HF breed, aged from 3 to 6 years, from 3 dairy farms. The animals selected for the study were divided into three groups, two deficiency groups and one control group – without deficiency. Group I comprised animals with reduced concentration of copper in the serum, while group II comprised animals with low copper and iron concentrations. Group III comprised healthy cows without clinical symptoms and mineral deficiency. In both deficiency groups, the one characterized by a low concentration of copper, as well as the one with low concentrations of copper and iron, a reduction in red blood cell parameters, which was statistically significant versus the control group, was observed. In the examined animals, normocytic normochromic anaemia was found in group I, while normocytic hypochromic anaemia was diagnosed in group II. Cows with simultaneous Cu and Fe deficiency have worse milk yield results and they are more often culled from the herd. It is not clear why only some animals in a herd develop simultaneous deficiency of these micronutrients.

Keywords: haematology, copper and iron deficiency, dairy cows.

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INTRODUCTION

Copper (Cu) and iron (Fe) are the most abundant trace elements in living organisms. The most common deficiency in cows is copper deficiency accompanied by elevated iron content in the serum. Deficiency of iron in cows' blood is rare due to the high content of this element in water and feed.

Cu deficiency (hypocupraemia) occurs in either primary or secondary form. Primary deficiency occurs in areas where copper is less abundant in the soil and feed. Secondary deficiency results from the presence of Cu antagonists, namely high levels of molybdenum, sulphur, iron and zinc in the diet reduce the absorption of copper from the gastrointestinal tract (Kurek et al. 2017, Menzir, Dessie 2017). Iron deficiency, which is often manifested in calves before weaning (Joerling, Doll 2019), is rare in adult animals. Iron antagonists in a diet (e.g. phosphates, manganese, nickel, selenium) interfere with the absorption of Fe in the proximal small intestine (Radwińska, Żarczyńska 2014).

Cu deficiency evoke haemoglobin production, which is inefficient despite normal Fe concentration in the serum. The mechanism of anaemia in copper deficiency cases has not yet been clarified (Collins et al. 2010). Cu is necessary to maintain Fe homeostasis because it is a component of proteins involved in hematopoiesis, such as ceruloplasmin and hephaestin. Ceruloplasmin catalyses the oxidation of Fe^{2+} to Fe^{3+}, which allows the binding of iron to transferrin and transport in the blood plasma. Copper is necessary for the mobilization of iron from the liver and its transport to bone marrow, where it is used for erythropoiesis. In the case of copper deficiency, iron is stored in the liver and its bioavailability is reduced. Hephaestin acts as a factor that facilitates the transportation of iron from enterocytes to the blood. Deficiency of this protein may lead to hypochromic microcytic anaemia and the accumulation of iron in the intestinal epithelium (Wu et al. 2006, Wierzbicka, Gromadzka 2014, D'Angelo 2016).

There is no information concerning the simultaneous deficiency of copper and iron in cows. In experimental studies on rats (Collins et al. 2010), it was observed that copper (Cu) deficiency led to iron deficiency by disrupting its utilization in organism. Collins et al. (2010) also stated that in the case of anaemia caused by Cu deficiency, after the administration of iron preparations alone, Fe and haemoglobin (Hgb) concentrations returned to the normal range. The unexplained phenomenon of iron utilization in bone marrow, which is dependent on the presence of copper, has also been observed (Collins et al. 2010).

The aim of the study was to follow changes in haematological parameters in cows resulting from copper deficiency or simultaneous deficiency of copper and iron in comparison with animals from the same herds in which no deficiencies of these micronutrients were found.

MATERIALS AND METHODS

The study was conducted on cows of the HF (Holstein Friesians Cows) breed, from 3 dairy farms located in the same region, aged from 3 to 6 years with an average BCS (Body Condition Score) of approximately 3.5/5, with Fe and Cu deficiencies. The cows were privately owned and were presented to the Department and Clinic of Animal Internal Diseases. The Local Ethics Board in Lublin considered all procedures routine veterinary ones and did not require institutional approval.

The average milk yield was within 9-10 thousand kg of milk per lactation. The nutrition provided to the cattle in the study was based on the TMR (Total Mixed Ration) method. The feed ration was designed based on the milk yield, current physiological period, and body weight of the cows. The feed ration included maize silage (41%), haylage (23%), hay (6.49%), straw (4%), soybean meal (2.70%), on-farm produced cereal (4%), brewer's spent grain (5.39%), rapeseed meal (4%), fodder chalk (0.40%), sodium chloride (0.08%), molasses (0.94%), pressed sugar-beet pulp (8%), feed additives with a protein content from 18 to 24%, compound feed as well as mineral and vitamin supplements.

The animals were under constant veterinary care. Only animals without parasitic and infectious diseases, injuries or any kind of bleeding were included in the study. The animals which qualified for the study were divided into three groups.

Group I comprised animals with reduced concentration of copper in their serum, while group II comprised animals with low copper and iron concentrations. Group III comprised healthy cows without clinical symptoms and mineral deficiency.

Blood samples were collected in the same manner in all cases, i.e. after morning milking blood was drawn from the jugular vein into test tubes containing K_2EDTA (dipotassium ethylenediaminetetraacetic acid) and a clot activator. The following parameters were determined: erythrocyte count (RBC), haemoglobin concentration (Hgb), haematocrit index (Htc), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), quantitative leukocytes (WBC), neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L) and monocytes (M). These measurements were performed using a Horiba scil Vet abc Plus automatic analyser (Scil Animal Care Company, Altorf, France). The samples were stained with the May-Grünwald-Giemza method using a MYTHIC TS stainer for haematological smears (PZ Cormay S.A. Łomianki, Poland).

The concentrations of copper and iron in serum samples were determined using the method of flame atomic absorption spectrometry (FAAS) by Avanta PM, GBC, Australia. Serum samples were diluted appropriately with a matrix modifier containing 5g L^{-1} Triton-X-100 to fit the linear range

of the calibration curve. The methods were controlled by analysing a series of samples from a certified reference material called Seronorm (Nycomed Pharma AS, Oslo, Norway). Recoveries between 90% – 110% were accepted to validate the calibration for all elements.

The results were subjected to statistical analysis and the significance of differences between the groups was calculated using the Mann-Whitney *U* rank test and Fisher's exact test (Table 1). The calculations were made at a significance level of $p<0.05$.

Table 1

Mean values of haematological parameters with the corresponding Cu and Fe concentrations in cows

Parameters	Bovine reference intervals (Baumgartner 2005)	Group I (n=24)	Group II (n=12)	Group III (n=20)
RBC x10^{12} l^{-1}	5–7	6.04±0.72^{A,a}	5.87 ±0.44^{A,a}	7.96±0.55^{B,b}
Hgb (g l^{-1})	105–140	86.95±8.64^{A,a}	84.75±6.78^{A,b}	112.62±10.03^{B,c}
Hct (l l^{-1})	0.30–0.40	0.26±0.03^{A,a}	0.25±0.02^{A,a}	0.33±0.03^{B,b}
MCV (fl)	40–60	43.26±4.34^{A}	42.25±1.54^{A}	50.92±3.68^{B}
MCH (fmol)	0.9–1.5	0.9±0.08^{A,a}	0.89±0.03^{A,a}	1.08±0.07^{B,b}
MCHC (mmol l^{-1})	16–21	20.82±0.34	20.12±0.29	19.41±1.00
WBC x10^{9} l^{-1}	6.2–9.5	7.76±1.80	7.20±1.29	8.15±1.12
N (%)	23–37	44.89±15.84^{A}	45.75±5.19^{A}	36.00±1.29^{B}
E (%)	1–7	5.84±4.60^{A}	5.00±2.34^{A}	2.92±1.55^{B}
B (%)	0–1	2.00±1.18	1.67±1.00	1.00±0.00
L (%)	53–67	46.53±14.10^{A,a}	46.50±4.93^{A,a}	58.85±1.14^{B,b}
M (%)	0–4	2.25±1.14	1.67±1.00	1.00±0.00
PLT x10^{9} l^{-1}	200–800	493.05±147.89	544±118.32	532.23±96.77
Fe (µmol l^{-1})	21.5-38.5 (Dirksen 2002)	29.53±5.15^{A,a}	12.32±1.53^{B,b}	29.40±5.10^{A,a}
Cu (µmol l^{-1})	12-20 (Dirksen 2002)	6.49±1.81^{A,a}	6.57±0.37^{A,a}	12.45±0.84^{B,b}

A, B – the significance of differences between mean values in the groups vs control group at $p<0.001$ (using the Mann-Whitney *U* rank test), *a*, *b* – the significance of differences between mean values in the groups vs control group at $p\leq0.001$ (using the Fisher's exact test), X±SD – the mean± standard deviation.

RESULTS

The first study group (group I) comprised 24 cows which exhibited appetite disorders and produced a lower milk yield than the other animals in the herd. During clinical examinations, these animals showed pallor of the mucous membranes, dry matted coat and diarrhoea. Greying and the loss of the hair

coat around the eyes ("copper glasses") were found in only 5 cows. Decreased copper concentration in serum was noted.

The second group (group II) included 12 cows which also had diminished appetite and slow weight loss with a simultaneous decrease in milk yield. In the clinical examination, shallow and rapid breath, redness, swelling and painfulness in the hoof's coronary band, as well as interdigital dermatitis were detected. The pallor of the mucous membranes and dry matted coat were exhibited in only 3 cows. Discolouration (greying) of the hair around the head and neck was also observed in all animals in this group. A decrease in the iron and copper concentrations in the serum was detected.

The control group (gr III) comprised 20 clinically healthy cows, originating from farms in the same area.

In the examined animals, concentrations of the abovementioned microelements were determined, and simultaneous hypocupraemia and Fe deficiency were detected. We observed changes in haematological parameters in both deficiency groups. The mean values of the examined haematological parameters are presented in Table 1. The reported blood copper concentrations were statistically significantly different than in the healthy animals (group I vs. III, $p<0.000002$; group II vs. III, $p<0.0000002$). Similarly, in group II, the iron concentration was statistically significantly different than in the two other groups (group I vs. II, $p<0.00005$; group II vs. III, $p<0.00009$).

The results of the erythrocyte parameters (except the MCHC) in groups I and II were statistically significantly ($p<0.001$) different than in group III (Table 1).

A statistically significant increase ($P<0.001$) in the number of neutrophils in group I and II was observed. Also, a statistically significant decrease ($p<0.001$) in the lymphocyte count in groups I and II in comparison with healthy cows was noted.

DISCUSSION

Copper deficiency is a common homeostatic disorder in dairy cattle, especially in Poland (Kurek et al. 2017, Menzir, Dessie 2017). From our observations, it may be concluded that a simultaneous decrease in blood copper and iron concentration is very rarely diagnosed and described in dairy cows. In most cases, deficiencies of these micronutrients are referenced separately (Radwińska, Żarczyńska 2014). The cases of copper deficiency with a simultaneous excess of the serum iron concentration are common, and this condition has a negative effect of the absorption of Cu and is the cause of secondary hypocupraemia (Kendall, Bone 2006). On the other hand, a long-term copper deficiency in the body may lead to a low concentration of iron (D'Angelo

2016). In the case of copper deficiency in animals, Mohammmed et al. (2014) observed microcytic hypochromic anaemia. Based on our research, we found that hypocupraemia was accompanied by normocytic normochromic anaemia, which also occurs in humans (Lazarchick 2012). In the available literature (Joerling, Doll 2019), the most frequently described disorder that coexists with iron deficiency is microcytic hypochromic anaemia. In our research, this type of anaemia was also expected with the simultaneous deficiency of Cu and Fe, although hypochromic normocytic anaemia was observed. In both groups with mineral deficiencies, very similar clinical symptoms were observed, and the owners emphasized a decrease in milk yield and the occurrence of appetite disorders. Comparing groups I and II, it was noted that while clinical symptoms were very similar, changes in the red blood cell system progressed more in animals with simultaneous Cu and Fe deficiency.

In animals with copper deficiency, normocytic normochromic anaemia was found, while Roland et al. (2014) reported that anaemia which occurs with simultaneous Cu and Fe deficiency is one of the microcytic hypochromic type. As regards red blood cell parameters, it was found that the red blood cell indices in group II are lower than in group I. In the group of cows with Cu and Fe deficiency, hypochromic normocytic anaemia occurred. It is known that in the presence of Cu deficiency, bioavailability of iron decreases and this disrupts erythropoiesis (Wierzbicka, Gromadzka 2014). This is the reason why the authors expected microcytic hypochromic anaemia in group II. In human medicine, there are reports (Johnson-Wimbley, Graham 2011) that normocytic hypochromic anaemia may occur in the case of Fe deficiency. These observations are in agreement with the results of our study. Johnson--Wimbley and Graham (2011) stated that the MCV which is within the normal reference range does not exclude the presence of anaemia resulting from Fe deficiency. Mazzullo et al. (2014) mentioned that a decrease in the MCH in erythrocytes is an early indicator of iron deficiency, and decreases more rapidly than the MCV and MCHC in the case of microcytic anaemia.

The total white blood cells (WBC) count in the examined cows was within the normal reference range. In groups I and II, the number of neutrophils increased as compared to the control group. Leukopoenia with neutropoenia are the earliest symptoms of low serum Cu levels in humans (Lazarchick 2012). In our research, a decrease in the lymphocyte count was observed in both deficiency groups. Similar changes were noted by Karimbakas et al. (1998), who observed the lymphocyte population domination in healthy animals, whereas in the Cu deficiency group, the number of lymphocytes decreased with a simultaneous increase in the neutrophil count. Cerone et al. (1998) did not observe changes in the number of neutrophils in cows with copper deficiency.

Based on the results and our observations, it was found that more intense haematological changes were observed in animals with simultaneous Cu and Fe deficiency than in ones with Cu deficiency alone. It should

be noted that the simultaneous deficiency of Cu and Fe is more difficult to compensate for with Cu supplementation only. Cows with simultaneous Cu and Fe deficiency were more often eliminated from the herds and did not produce an adequate milk yield at the peak of lactation. Based on these observations, it is not clear why only some animals in the herd develop simultaneous deficiency of these micronutrients and whether it is possible to eliminate these disorders through the appropriate selection of animals for breeding.

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