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The Impact of Copper and Zinc Supplementation on Serum Haptoglobin and Milk Production Performance on 20 Weeks of Lactation in Dairy Cows

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Abstract: The effect of copper and zinc supplementation on serum haptoglobin and milk production performance was tested in a 20 weeks feeding trial with 40 Holstein-Friesian lactating cows, devided in 2 equal groups (supplemented and control). At 10-20 days post calving cows began receiving either a diet containing supplemental Cu and Zn sulphate (19.1±0.8 ppm Cu and 61.7±6.8 ppm Zn) or a diet without mineral supplement (3.1±0.4 ppm Cu, 9.2±1.1 ppm Zn). Milk and blood samples were collected six times every 28 days. Ten subjects of each group were randomly assigned to skin biopsies of the periorbital area at the end and the beginning of the study. Feeding inorganic Cu and Zn increased ($p<0.001$) plasma Cu and Zn values and significant interactions ($p<0.001$) was found between time and supplementation on plasma Cu and Zn levels. Serum haptoglobin values were lower ($p = 0.24$) in cows from supplemented group (0.096±0.006 g L^{-1}) relative to the control group (0.099±0.009 g L^{-1}). There was no effect of time ($p = 0.21$) and no significant interaction ($p = 0.35$) between time and supplementation on serum haptoglobin levels. The Body Condition Score (BCS) was higher in supplemented group than in those that did not receive supplemental minerals (2.82 vs. 2.75, $p = 0.03$) but no significant interaction was found between time and supplementation on BCS ($p = 0.85$). Feeding inorganic Cu and Zn reduced periorbital hypopigmentation in cows. Supplementing Cu and Zn comparing to the control cows had higher yield of milk (23 vs. 22 kg day^{-1}, $p = 0.004$), milk crude protein (3.3 vs. 3.2%, $p = 0.14$), milk fat (4.16 vs. 4.09%, $p = 0.002$), milk lactose (4.82 vs. 4.79 g kg^{-1}, $p = 0.32$) and milk energy (0.74 vs. 0.75 Mcal kg^{-1}, $p = 0.27$) however, the response to supplementation tended to be inconsistent over time for production indicators as indicated by time x supplementation interactions ($p>0.15$). Supplemented group had lower SCC compared to the control cows (246,000 vs. 288,000 cells mL^{-1}, $p = 0.02$) but no significant interaction was found between time and supplementation on SCC ($p = 0.47$). Supplementing with 1.5 g $CuSO_4 \cdot 5H_2O$ and 5 g $ZnSO_4 \cdot 7H_2O$/cow/week for 1st 20 weeks of lactation can significant improve health and milk production performances in a deficient area.

Key words: Copper, dairy cows, haptoglobin, zinc, sulphate

INTRODUCTION

Enhancing of the cows performances here is an important way of increasing the profit of farmers, so that studying of the copper and zinc status in dairy cows might add value to it. Previous researches showed that adequate Cu and/or Zn input may be used as a strategy to optimize immune system function by the reduction of the metabolic stress (Cortinhas *et al*., 2010), oxidative stress (Tanaka *et al*., 2008) and milk production performance (Kellogg *et al*., 2004; Sharma and Joshi, 2005) by improving udder health (Andrieu, 2008). Clinically, Cu and Zn deficiency in ruminants results in alopecia associated with anorexia (Mecklenburg, 2009). Haptoglobin, an acute phase protein could improve the sanitary control of animals and the identification of pathologies even before clinical signs become apparent (Humblet and Godeau, 2005). The diagnosis of mineral deficiency is provided by a clinical or production response to supplements of the minerals or minerals thought to be lacking (Suttle, 1987). The objective of this study was to evaluate the influence of copper and zinc supplementation on serum haptoglobin levels and milk production performance of lactating dairy cows.

MATERIALS AND METHODS

Study area: The study was done in a large dairy farm located at 47°9'24"N and 27°38'50"E in an area where the climat is characterized by hot and dry summers and cold

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winters. Average annual rainfall totals about 550 mm characterized by a patchy distribution. The soil of this region have a low concentration of Cu and Zn, 10-20 ppm and <50 ppm, respectively (Andar *et al*., 2006). Drinking water in the region has an average Cu concentration of 0.022 mg L^{-1} and of 0.156 mg L^{-1} Zn, well below the maximum concentrations approved by the ECC (1998) and Diaconu (2009).

Experimental design and diets: The present 20 weeks study was conducted on a large dairy farm according to the veterinary legal regulations for the farm animal protection (Council Directive, 1998) and involved 40 postpartum lactating (10-20 days of lactation) nonpregnant Holstein-Friesian cows, in good health condition.

The experimental design was completely randomized with one treatments given weekly for 20 weeks and repeated measures during the experimental period. All animals received the same basal diet including 29-31 kg maize silage, 7-8 kg alfalfa hay and 300 g concentrate mixed feed/L milk which provided 17.2±1.1 kg dry matter, 1563±50.5 g protein, 91.1±3.2 g Ca, 59±1.8 g P, 24.1±0.9 g Mg, 28±8.4 g Na, 711.3±39.2 ppm Mn, 3.1±0.4 ppm Cu, 9.2±1.1 ppm Zn and 78000±1800 UI vitamine A, for each subject. The concentrations of Cu in the drinking water for cows were 0.007±0.001 mg L^{-1} regarding Zn, the concentrations of water were below the detectable limits.

Cows were housed in individual pens with thin bed (2 kg sawdust/cow/day) and randomly assigned to one of the two groups: supplemented and control. The supplemented group was given 1.5 g $CuSO_4 \cdot 5H_2O$ and 5 g $ZnSO_4 \cdot 7H_2O$/cow/week for 20 weeks. The Cu and Zn substrates were dissolved in water before being orally administered to each subject. The control group received no mineral supplement. Both cupric sulphate ($CuSO_4 \cdot 5H_2O$) and zinc sulphate ($ZnSO_4 \cdot 7H_2O$) are accepted as feed aditives by the European Union (EC, 2003). Maximum content of the element in the total diet of cows is 35 mg kg^{-1} for copper and 150 mg kg^{-1} for zinc (EC, 2003). Standardized clinical examinations (Jackson and Cockcroft, 2002) were performed on each cow every week by a unique operator. Once a month, the body condition of each subject was scored as described by Edmonson *et al*. (1989) whose method is based on a 5-point scale (1 = emaciated; 5 = over-fat) and it is used as a preventive approach procedure of the production diseases.

Blood samples were collected from the coccidian vein into lithium-heparin coated tubes (Sarsted®) for further plasma copper and zinc quantitative and into no additive tubes (BD Vacutainer®) for serum haptoglobin evaluations. Samples were drawn before minerals were administered on the 1st day (t_0), on days 28 (t_1), 56 (t_2), 84 (t_3), 112 (t_4) and 140 (t_5). The tubes were centrifuged at 2500×g for 20 min and plasma was removed and frozen at -20°C. Plasma, after thawing was deproteinized with 0.6 M solution of trichloroacetic acid and hydrochloric acid (1:1, v:v).

The mixture was placed on a water bath at 90°C for 15 min and then separated by centrifugation at 3000×g for 10 min (Mihele, 2006). Cu and Zn concentrations were determined by a Flame Atomic Absorption Spectrophotometry Method (Parsons and Barbosa, 2007). Serum haptoglobin concentration was analysed with an automatic biochemical analyzer Cormay Accent 200 using a kit Cormay Haptoglobin.

Ten subjects of each group were randomly assigned to skin biopsies by excision of the periorbital area at the end and the beginning of the study. Fragments of tissue (about 1 cm^2) were displayed on filter study and fixed in 10% buffered formalin solution for 12 h. The Staining Method of skin sections was Hematoxylin Eosin methylene blue (HEA).

Cows were milked twice daily and individual milk weights recorded and samples collected every 28 days. Milk was analysed for fat, crude protein and lactose content using a IR Spectrometric Method (FTS). Somatic Cell Counts (SCC) were determined using the Flow Cytometry Method (Bentley FTS/FCM). The Net Energy Required for Lactation (NEL) which is defined as the energy contained in the milk produced was calculated using the equation (NRC, 2001):

$$NE_L \text{ (Mcal/kg)} = 0.0929 \times \text{Fat\%} + 0.0563 \times \text{Crude protein\%} + 0.0395 \times \text{Lactose\%}$$

Statistical analysis: The results obtained for each cow during the experimental period were expressed in terms of mean±standard error of the mean. Analyses of variance of milk production indicators, body condition score, serum haptoglobin levels and plasma Cu and Zn levels were performed with supplementation as a fixed effect factor and sampling day (time) as a repeated measures factor and with the interaction sampling day (time) x supplementation included in the model using a General Linear (GLM) of SPSS 16.0 for Windows (SPSS Inc., Chicago, Il, USA). Differences between means were tested using Duncans new multiple range test. The criterion for statistical significance was $p<0.05$.

RESULTS

Health indicators: The nutrient composition of the basal diet including maize silage, alfalfa hay and concentrate mixed feed was consistent with values reported by NRC (2001) except copper and zinc content. Supplemented cows had the daily quantity of Cu and Zn intake higher (303.1±7 mg Cu/cow and 1065±34 mg Zn/cow) in comparison to the control group (48.5±5.6 mg Cu/cow and 154.8±24.6 mg Zn/cow). At t_0, plasma Cu level (Fig. 1) was deficient in both groups supplemented and control (0.64±0.11 and 0.68±0.09 mg L^{-1}). After 8 weeks of supplementation (t_2) there was a significant ($p = 0.001$) improvement in plasma Cu values in supplemented group which the plasma Cu levels reached (0.94±0.14 mg L^{-1}), while no significant ($p>0.05$) improvement was observed in control group. On the 84th day (t_3) plasma Cu values of supplemented group approached standard values (1.16±0.09 mg L^{-1}) as described by Radostits *et al.* (2007) while values in control group showed no significant changes remaining deficient till the end of the study (0.83±0.04 mg L^{-1}). Plasma Cu value showed significant ($p<0.001$) increase in supplemented group on the 140th day (1.20±0.05 mg L^{-1}) as compared to the 1st day of study (t_0). Plasma Cu concentrations of cows were affected by time ($p<0.001$) and minerals supplementation ($p<0.001$); significant interaction ($p<0.001$) was found between time and supplementation on plasma Cu concentrations. At t_0 plasma Zn level (Fig. 2) was found to be deficient in both groups supplemented and control (0.70±0.08 and 0.68±0.08 mg L^{-1}). The plasma Zn value showed significant ($p<0.001$) increase in supplemented group on the 140th day of study (1.05±0.09 mg L^{-1}) as compared to t_0 whereas no significant ($p>0.05$) improvement was observed in control group throughout the duration of study. After 8 weeks of supplementation (t_2) there was a significant ($p = 0.01$) improvement in plasma Zn values (0.86±0.14 mg L^{-1}) in supplemented group approaching standard values as described by Suttle (2004) while values in control group showed no significant changes remaining deficient till the end of the study (0.78±0.04 mg L^{-1}). Plasma Zn concentrations of cows were affected by time ($p<0.001$) and minerals supplementation ($p<0.001$); significant interaction ($p<0.001$) was found between time and supplementation on plasma Zn concentrations. In the experiment, the Cu:Zn ratio in the plasma ranged between 0.67 and 1.25.

The Body Condition Score (BCS) was higher in supplemented group than in those that did not receive supplemental minerals (2.82 versus 2.75) (Table 1). In this study, it was observed a positive effect ($p = 0.03$) of minerals supplementation on BCS. However, no effect of time ($p = 0.09$) and no significant interaction was found between time and supplementation on BCS in cows ($p = 0.85$). During the study serum haptoglobin values (Table 2) were lower in cows from supplemented group (0.096±0.006 g L^{-1}) relative to the control group (0.099±0.009 g L^{-1}) but the differences were not

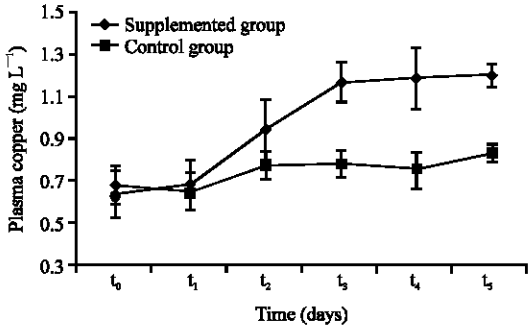

Fig. 1: Variation of plasma copper values during the 20 weeks of experiment in cows; t_0 = 1st day of study; t_1 = 28th day of study; t_2 = 56th day of study; t_3 = 84th day of study; t_4 = 112th day of study; t_5 = 140th day of study

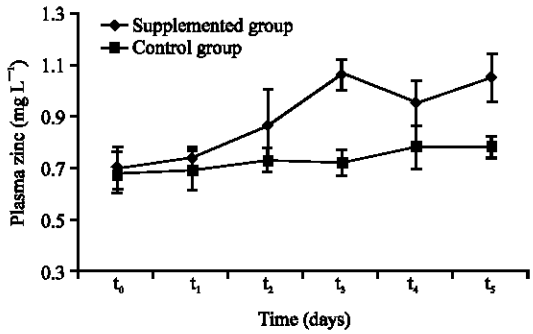

Fig. 2: Variation of plasma zinc values during the 20 weeks of experiment in cows; t_0 = 1st day of study; t_1 = 28th day of study; t_2 = 56th day of study; t_3 = 84th day of study; t_4 = 112th day of study; t_5 = 140th day of study

Table 1: Variation of the body condition score of lactating dairy cows

	Groups			
Time (days)	Supplemented	Control	SEM	p-values
t_0	3.13	3.11	0.05	0.59
t_1	2.72	2.61	0.13	0.42
t_2	2.63	2.52	0.06	0.10
t_3	2.77	2.63	0.09	0.17
t_4	2.80	2.77	0.06	0.68
t_5	2.88	2.86	0.06	0.68

t_0 = 1st day of study; t_1 = 28th day of study; t_2 = 56th day of study; t_3 = 84th day of study; t_4 = 112th day of study; t_5 = 140th day of study; SEM = Standard Error of the Mean

Table 2: Variation of serum haptoglobin concentrations (g L^{-1}) of lactating dairy cows

	Groups			
Time (days)	Supplemented	Control	SEM	p-values
t_0	0.105	0.097	0.006	0.181
t_1	0.098	0.117	0.008	0.049
t_2	0.099	0.097	0.001	0.137
t_3	0.094	0.089	0.005	0.171
t_4	0.086	0.097	0.003	0.009
t_5	0.094	0.098	0.003	0.157

t_0 = 1st day of study; t_1 = 28th day of study; t_2 = 56th day of study; t_3 = 84th day of study; t_4 = 112th day of study; t_5 = 140th day of study; SEM = Standard Error of the Mean

statistically significant (p = 0.24). In the cows that received Cu and Zn supplementation, serum haptoglobin levels tended (p = 0.09) to be higher on the 1st day of study than on day 140. Control group had higher on 28th (p = 0.049) and 112th (p = 0.009) day of study serum haptoglobin concentrations than supplemented cows however, no effect of time was observed on serum haptoglobin levels (p = 0.21). Response to treatment was inconsistent over time for serum haptoglobin values as indicated by time x supplementation interactions (p = 0.35).

In the study, at t_0 all the cows with histological features of the skin had different degrees of periorbital hypopigmentation (leukoderma and leukotrichia). After 20 weeks feeding trial, only the cows from control group had different degrees of periorbital hypopigmentation. The cows from supplemented group had no obvious periorbital hypopigmentation at t_5. Histological features of the skin in cows are shown in the Fig. 3 and 4. In the cases with periorbital hypopigmentation, epidermic discoloration with few melanocytes in the epidermis, agglutination of melanocytes in the dermis, hair follicle degeneration and poorly pigmented melanocytes in the hair follicle sheaths were found.

Production indicators: The production indicators of the cows during the experiment are summarized in Table 3. Milk yield tended to be higher (23 vs. 22 kg day^{-1}, p = 0.004) in supplemented group than in those that did not receive supplemental minerals. When analyzed by week, milk production was significantly higher at t_2 (p = 0.007) and t_5 (p = 0.01) in supplemented group comparing with control group. Overall supplementing cows had higher milk yield than control group from day 28 through day 140, nevertheless, no significant interaction (p = 0.23) was found between time and supplementation on milk yield. Supplementing cows with Cu and Zn results in 4.5 and 3% increase in production of milk and milk crude protein, respectively. The milk crude protein tended to be lower (3.2 vs. 3.3%, p = 0.14) in cows that did not receive Cu and Zn than in those that had received

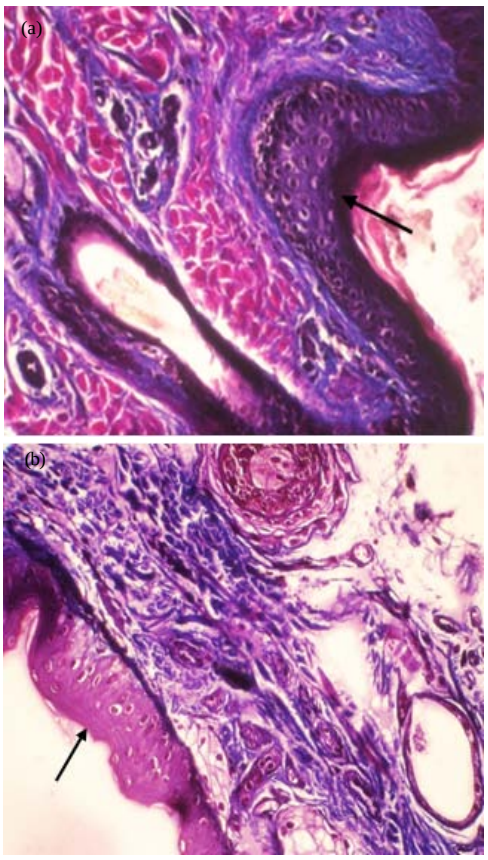

Fig. 3: Skin microscopic structure of 5 years old cows at the end of study-epidermis details; a) normal epidermis, HEA stain x400 (supplemeted group) and b) epidermic discoloration; few melanocytes in the epidermis, HEA stain x400 (control group)

supplemental minerals. When analyzed by week, milk crude protein was significantly higher at t_5 (p = 0.05) in supplemented group comparing with control group. No significant interaction was found between time and supplementation on milk crude protein (p = 0.16).

Control cows had lower milk fat than supplemented cows (4.09 vs. 4.16%, p = 0.002), however when analyzed by week, fat milk was significantly higher at t_3 (p = 0.03) and t_4 (p = 0.03) in supplemented group comparing with control group. Supplementing cows with Cu and Zn resulted in (p = 0.02) 2% increase in milk fat. However, response to supplementation was inconsistent over time for milk fat as indicated by time x supplementation interactions (p = 0.83). Milk lactose was higher (4.82 vs. 4.79 g kg^{-1}) in supplemented group than in those that did not receive supplemental minerals but the differences were not statistically significant (p = 0.42).

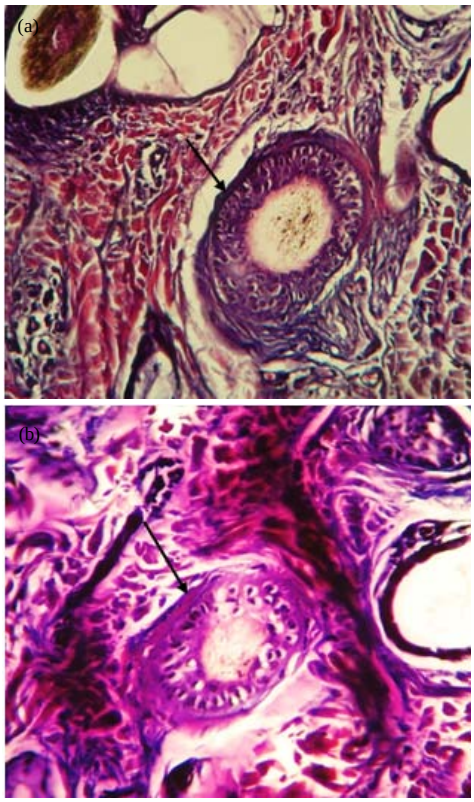

Fig. 4: Skin microscopic structure of 5 years old cows at the end of study-hair follicle details; a) normal hair follicle, HEA stain x400 (supplemeted group) and b) hair follicle degenerated, poorly pigmented melanocytes in hair follicle sheaths, HEA stain x400 (control group)

Table 3: Effect of Cu and Zn supplementation on yield of milk and milk components of lactating dairy cows

Production indicator	Time (days)	Groups: Supplemented	Groups: Control	SEM	p-values
Milk yield (kg day^{-1})	t_0	22.73	22.82	0.460	0.850
	t_1	23.15	22.85	0.940	0.750
	t_2	24.81	23.52	0.350	0.007
	t_3	23.21	22.11	0.690	0.150
	t_4	21.86	20.96	0.600	0.170
	t_5	22.55	20.93	0.530	0.010
Milk crude protein (%)	t_0	3.39	3.33	0.040	0.090
	t_1	3.33	3.21	0.060	0.060
	t_2	3.24	3.21	0.030	0.290
	t_3	3.22	3.18	0.090	0.330
	t_4	3.23	3.19	0.040	0.230
	t_5	3.24	3.17	0.040	0.050
Milk fat (%)	t_0	4.35	4.28	0.050	0.200
	t_1	4.20	4.18	0.050	0.720
	t_2	4.06	4.02	0.040	0.410
	t_3	4.10	4.03	0.030	0.030
	t_4	4.11	4.01	0.040	0.030
	t_5	4.15	4.05	0.060	0.070
Milk lactose (g kg^{-1})	t_0	4.76	4.80	0.020	0.280
	t_1	4.87	4.76	0.100	0.380
	t_2	4.83	4.84	0.110	0.910
	t_3	4.73	4.80	0.110	0.570
	t_4	4.87	4.74	0.100	0.320
	t_5	4.90	4.85	0.040	0.420
Milk energy (Mcal kg^{-1})	t_0	0.78	0.77	0.003	0.420
	t_1	0.77	0.76	0.003	0.420
	t_2	0.75	0.74	0.003	0.180
	t_3	0.74	0.74	0.006	0.660
	t_4	0.75	0.73	0.003	0.060
	t_5	0.76	0.75	0.003	0.180
Somatic cell count ($\times 10^3$ mL^{-1})	t_0	321.00	308.00	26.340	0.320
	t_1	259.00	372.00	62.140	0.050
	t_2	262.00	308.00	61.730	0.240
	t_3	254.00	206.00	47.210	0.160
	t_4	184.00	245.00	21.680	0.010
	t_5	197.00	293.00	55.310	0.030

t_0 = 1st day of study; t_1 = 28th day of study; t_2 = 56th day of study; t_3 = 84th day of study; t_4 = 112th day of study; t_5 = 140th day of study; SEM = Standard Error of the Mean

Control cows had lower milk energy than supplemented cows (0.74 vs. 0.75 Mcal kg^{-1}) during the study but differences were not statistically significant ($p = 0.27$) when analyzed by week, milk energy tended to be higher ($p = 0.06$) at t_4 in supplemented group comparing with control group. Supplementing cows with Cu and Zn resulted in 1% improvement in milk lactose and 3% improvement in milk energy. However, no significant interaction was found between time and supplementation on lactose ($p = 0.53$) and milk energy ($p = 0.67$). There was no effect of time ($p>0.05$) on milk yield, crude protein, fat, lactose and milk energy.

In the study cows supplemented with Cu and Zn had lower SCC compared to the control cows (246,000 vs. 288,000 cells mL^{-1}, $p = 0.01$). The 40 cows had higher total number of somatic cells in their milk more than the highest value for the milk from a healthy mammary gland that is 200,000 cells mL^{-1} milk (Burvenich *et al*., 2004). When analyzed by week, SCC was significantly lower at t_1 ($p = 0.05$), t_4 ($p = 0.01$) and t_5 ($p = 0.03$) in supplemented group comparing with control group however, no effect of time was observed on SCC ($p = 0.33$). Response to treatment was inconsistent over time for SCC as indicated by time x supplementation interactions ($p = 0.47$).

DISCUSSION

In the present experiment carried on in a region known for imbalances of the soil trace elements, diet supplementation with Cu and Zn was necessary. The chemical analysis of the dairy cow basal diet showed values of copper and zinc well below the recommended concentrations. Subjects belonging to supplemented group had a daily intake of 19.1±0.8 ppm Cu and 61.7±6.8 ppm Zn in dry matter which meet the diet requirements for lactating dairy cows 10-20 ppm Cu and 43-73 ppm Zn (NRC, 2001).

All the cows were copper and zinc deficient at the beginning of experiment but those blood minerals increased significantly after 8-12 weeks of Cu and Zn supplementation when the blood levels of Cu and Zn were found within the normal limits of 0.97-1.57 mg Cu L^{-1} according to Radostits *et al*. (2007) and 0.8-1.2 mg Zn L^{-1} according to Suttle (2004). Many factors can influence concentrations of trace elements in blood. For example, inflammation stress and infection may increase plasma Cu and decrease plasma Zn (Herdt *et al*., 2000). These difficulties in interpreting the results can be ruled out by discarding results for samples with high Cu:Zn ratios (>3-4) (Suttle, 2004). In the experiment, the Cu:Zn ratio in the plasma was lower than 3. In the study, the response to supplementation was consistent over time for plasma Cu and Zn levels as indicated by time x supplementation interactions ($p<0.001$). Minerals supplementation had a positive effect on BCS, the cows from control group had lower values than supplemented group. Clinically, Cu and Zn deficiency in ruminants results also in anorexia (Mecklenburg, 2009). The loss of appetite is regarded as the earliest clinical sign of Zn deficiency (Underwood and Suttle, 1999). However, all the cows from the study had the BCS according to Braun *et al*. (1987), 2.5-3.0 (1 month postpartum), 3.0 (mid lactation) and 3.25-3.75 (end of lactation).

Serum haptoglobin values were higher in control group than in those that had received supplemental Cu and Zn. Increased serum haptoglobin has been found in association with for example pneumonia (Young *et al*., 1996), metritis (Smith *et al*., 1998), uterine torsions (Schonfelder *et al*., 2006), ketosis, fatty liver syndrome (Stengarde *et al*., 2008), stress (Lomborg *et al*., 2008) and claw disorders (Smith *et al*., 2009). Among acute phase proteins, haptoglobin is considered as an excellent marker and the most interesting in cows (Humblet and Godeau, 2005). According to Heegaard *et al*. (2000) and Grell *et al*. (2005) the increase of serum haptoglobin concentration was often correlated with severity of infection. In the study, the cows with the highest somatic cell count had the highest serum haptoglobin concentrations.

All cows from the both groups had serum haptoglobin concentrations in the physiological parameters of adult cows that 0-0.4 g L^{-1} (Radostits *et al*., 2007). Due to its early and marked increase in acute inflammation, it is useful for the diagnosis and prognosis of acute inflammation and for the evaluation of treatment (Humblet and Godeau, 2005). Cu and Zn supplementation had no significantly effect on serum haptoglobin concentrations. All the cows with Cu and Zn deficiency had different degrees of periorbital hypopigmentation. In the study, feeding inorganic Cu and Zn for 20 weeks reduced periorbital hypopigmentation in cows. Cutaneous hypopigmentation is the result of a decreased amount of melanin in the epidermis or dermis (or both) and may be congenital or acquired (leukoderma). Melanin a black-brown pigment produced from tyrosine in the presence of tyrosinase and copper is deposited on the protein matrix in the melanosomes (membrane-bound organelles produced by melanocytes) (Smith, 2009; Thiruvenkadan *et al*., 2008). In the study, histological features of the skin in all Cu and Zn deficient cows, revealed epidermic discoloration due to fewer melanocytes in the epidermis and agglutination of melanocytes in the dermis. Leukoderma may be caused by several factors including dietary imbalances, trauma, inflammation, hormonal influences and immunologic disorders and is usually accompanied by leukotrichie (Smith, 2009). Copper has an important influence on the skin pigmentation by stimulating the conversion of tyrosine into melanin by tyrosinase or other enzymes containing Cu (McDowell, 2003). Leukotrichia is the results of decreased amount of melanin in the hair shaft (Smith, 2009). In the study, the cows with Cu and Zn deficiency had hair follicle degeneration and poorly pigmented melanocytes in the hair follicle sheaths. Achromotrichia is the earliest clinical sign of copper deprivation in all species (Underwood and Suttle, 1999). Regarding the impact of Zn deficiency on skin, cows can present alopecia and parakeratotic hyperkeratosis. Masters *et al*. (1985) affirmed that alopecia in Zn deficiency is due to malformed hair fibres that become distorted within the follicular infundibulum. In the study, it was not observed alterations of histological features of the skin to support the Zn deficiency in cows. In the study, periorbital leukoderma and leukotrichia may be due to the reduced tyrosinase activity that inhibits the conversion of tyrosine into melanin, most probably because of Cu deficiency.

Cu and Zn supplementation as inorganic had a significant positive effect on milk yield ($p = 0.004$) and milk fat ($p = 0.002$) and no significant effect on milk crude protein ($p = 0.14$), lactose ($p = 0.42$) and milk energy ($p = 0.27$) however, the response to supplementation tended to be inconsistent over time ($p>0.15$) for milk yield, crude protein, fat, lactose and milk energy. This is in accordance with Sharma and Joshi (2005) who found that zinc sulphate supplementation is highly effective in alleviating Zn deficiency and improving the milk yield in cows.

In contrast, Formigoni *et al*. (1993) and Campbell *et al*. (1999) reported no increase in milk yield from feeding organic trace mineral complexes. The positive effect or Cu and Zn supplementation on fat milk

from the study is in accordance with Nocek *et al*. (2006) but not consistent with Uchida *et al*. (2001) who reported no effect on milk production, milk fat and protein content, somatic cell count or animal body condition score from feeding a combination of zinc amino acid, manganese amino acid and copper amino acid complexes and cobalt glucoheptonate to early lactation Holstein cows. De Frain *et al*. (2010) also reported that supplementing with minerals (zinc, manganese and copper from amino acid complexes, cobalt glucoheptonate) had no effect on lactational performance. Regarding milk crude protein, supplemented cows had an increase of 3% but the effect of minerals supplementation was not statistically significan. Griffiths *et al*. (2007) observed an increase in milk protein in cows fed a combination of Zn, Mn, Cu and Co but Cope *et al*. (2009) found that milk composition was unaffected by dietary Zn treatment. Milk energy also increased in supplemented cows from the study is in accordance with Ballantine *et al*. (2002) and Kellogg *et al*. (2003) who obtained similar results using Co glucoheptonate and specific amino acid complexes of Zn, Mn and Cu.

In the study, Cu and Zn supplementation had a positive effect on SCC. Weiss and Wyatt (2002) observed that adequate mineral nutrition may have a positive effect on the defense mechanisms of mammary gland against mastitis, Cu and Zn supplementation being associated with higher antioxidant capacity resulting in reduced SCC. Cope *et al*. (2009) also, concluded that supplementing Zn at the recommended level reduced SCC. Kinal *et al*. (2007) associated the reduction of SCC with quick formation of keratin in teat canal provided by the supplementation of Zn. Scaletti *et al*. (2003) found Cu sulfate suplemented cows had decreased severity of clinical mastitis cases. SCC is a primary indicator of mastitis and milk quality in dairy heard (Janosi and Baltay, 2004; Weiss, 2003). The high SCC values in this study suggested the high incidence of the incipient subclinical masititis.

Deficiencies of Cu and Zn have been associated with increased incidence and severity of intra-mammary infections, increased clinical mastitis cases and higher somatic cell counts in individual cows and bulk tank milk (Kellogg, 1990; Scaletti *et al*., 2003). The control of mastitis is based on two principles: reduction of the nipple extremity exposure to pathogens and maximizing the defense system of the milking cow. In this farm, the routine milking is done in the confining places where the bedding is thin and thus the exposure of nipples to pathogens is increased which can also explain a high somatic cell count in their milk of all 40 cows. The health and milk production performances responses to feeding Cu and Zn sulphate, in the study, may reflect the high mineral content of the diet in supplemented group, comparing with the minerals deficient diet of cows from control group.

CONCLUSION

Overall, this study suggests that copper and zinc deficiencies are risk factors for impared production and health in lactating dairy cows. Based on the results of this study it may be concluded that supplementing with 1.5 g $CuSO_4 \cdot 5H_2O$ and 5 g $ZnSO_4 \cdot 7H_2O$/cow/week for 1st 20 weeks of lactation can significant improve health and milk production performances in a deficient area. Nevertheless, the serious errors of management concerning housing of the animals probably masked a better benefit of the copper and/or zinc supplementation.

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