# **ERRATUM**

# Erratum to: Effects of Short-Term Over-supplementation of Copper in Milk on Hematology, Serum Proteins, Weight Gain, and Health in Dairy Calves

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The original version of this article unfortunately contained a mistake. The name of Gholamreza Mohammadi is added. The correct full name of authors is shown above.

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# Effects of Short-Term Over-supplementation of Copper in Milk on Hematology, Serum Proteins, Weight Gain, and Health in Dairy Calves

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Abstract Thirty-six calves were used in the present study. The animals were divided equally into three groups (control, test 1, and test 2). The three groups of calves were homogeneous for parity of dams, sex, and month of birth. From 14 days of age, in the test 1 group copper as copper sulfate (Merck Co, Germany) was added to each meal of milk at a rate of 10 mg/kg of milk for 14 days and in test 2 group copper as copper sulfate was added to each meal of milk at a rate of 20 mg/kg of milk for 14 days. Blood samples were taken by jugular venipuncture using disposable syringes at 14 (before Cu supplementation), 30, 60, and 80 days of age. Anticoagulated blood was used for CBC determination. Plane tubes were used for harvesting of serum and the amounts of total serum protein, albumin, iron, and copper were measured. Calves were weighted at birth and at the end of trial (day 80) and total gain and mean daily gain were calculated. Days of treatment for ill calves were also recorded during experiment. Group (treatment) had no significant effect on the amounts of measured parameters except MCH values (p < 0.05) which were significantly lower in test 1 group than other trial groups. Age (sampling time) had significant effects on the values of most measured parameters (p < 0.05) except WBC, lymphocyte, total protein, and fibrinogen. Significant interactions between sampling time and group were not seen for any of measured parameters. No significant differences were seen for total weight gain and mean daily gain between trial groups. Chi-square test revealed no significant difference for the days of treatment between trials groups.

**Keywords** Calves · Copper · Health · Hematology · Weight gain

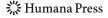
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## Introduction

Copper deficiency has been linked to a variety of clinical signs, including anemia, pale coat, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, and decreased resistance to infectious disease [1]. Copper has an important role in the metabolism and transition of iron in the body. Microcytic hypochromic anemia is the one of the outcomes of copper deficiency.

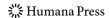
Enjalbert et al. [2] suggested inadequate copper status was not associated with adult disorders, but was an important risk factor for poor calf performance or health. Calves of dams fed deficient diets may show signs at 2–3 months of age. As a rule, the signs are severe in calves and yearling than adults. The occurrence of the disease and its failure to appear after weaning point to the importance of fetal store of copper and inadequacy of milk as a source of copper. Milk is always a poor source of copper and when it is the sole source of nourishment the intake of copper will be low [1]. Since copper absorption reduces with rumen activity [3] and its availability may be significantly reduced by some antagonistic elements in the diet (especially calf starter), including zinc, molybdenum, sulfur, and iron [4, 5], thus, during neonatal period of life, the deficiency of copper (and impaired iron utilization together) could exist and result to anemia and other related disorders. The aim of the present study was to investigate the effects of short-term over-supplementation of copper in milk for compensating lower copper availability during this critical period.

### **Materials and Methods**

The study was conducted in a dairy herd with approximately 600 calves per year at Mashhad suburb (Northeast of Iran). This herd consisted of pure bred animals of Holstein breed. The herd was totally confined in open-shed housing without access to pasture. Dry cows were fed with straw (26.19%), corn silage (57.04%), and concentrate (16.67%) which include barley, cotton seed, bran, beet root, and vitamin and mineral supplement (0.1% contain/kg: Vit A 500,000 IU, Vit D<sub>3</sub> 100,000 IU, Vit E 0.1 g, Ca 180 g, P 90 g, Mg 190 g, Na 60 g, Fe 3 g, Mn 2 g, Cu 0.3 g, Zn 3 g, Co 0.1 g, Iodine 0.1 g, Se 0.001 g, and antioxidant 3 g). The ration was balanced according to recommendations [3].

Cows were dried 2 months before expected time of parturition and transferred to a separate stall. As the time of parturition approached, the cows were moved to straw bedded maternity pen. Prompt assistance was given to cows with dystocia. Following parturition, the umbilicus of each calf was treated with pavidone iodine and the calf was weighed and transferred to individual pen. Within first 6 h of life 2.5 kg of dam's colostrum was fed by nipple bottle and colostrum feeding was continued every 12 h for 48 h. Then, herd milk was replaced for feeding twice daily (2 kg every 12 h) until 30 days of life. After this time, calves were fed milk replacer (Table 1) twice daily (2 kg every 12 h) until 90 days of life. Calf starter (Table 2, started from 48 h of life) include concentrate (90% DM) and high quality alfalfa (10% DM) and also water offered free choice after transferring to individual pen. The calves were weaned at 90 days of life. The heifer calves were mainly used as herd replacements.

Thirty-six calves were used in the present study. The animals were divided equally into three groups (control, test 1, and test 2). The three groups of calves were homogeneous for parity of dams, sex, and month of birth. From 14 days of age, in the test 1 group, copper as copper sulfate (Merck Co, Germany) was added to each meal of milk at a rate of 10 mg/kg of milk for 14 days and in test 2 group, copper as copper sulfate was added to each meal of milk at a rate of 20 mg/kg of milk for 14 days. All other aspects of the diet were identical



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Table 1 Ingredient Composition of Milk Replacer Fed to Calves (DM%)

Ingredients	
Milk product	80%
Vegetable fat	15%
Premix of vitamins, minerals, etc. <sup>a</sup>	5%

<sup>&</sup>lt;sup>a</sup> Each kilogram of milk replacer contains: Vitamin A (55,000 IU), Vitamin D3 (4,500 IU), Vitamin E (80 mg), Vitamin C (120 mg), Vitamin B1 (16 mg), Vitamin B2 (10 mg), Vitamin B6 (8 mg), Vitamin B12 (40 mcg), Vitamin K (6 mg), Niacin (50 mg), Panthothenic acid (25 mg), Choline chloride (1,900 mg), Probiotic (1.4 × 10<sup>6</sup> CFU), Iron (110 mg), Copper (17 mg), Manganese (26 mg), Zinc (150 mg), Cobalt (2 mg), Iodine (0.6 mg), and Selenium (0.3 mg)

for all groups including the controls. Blood samples were taken by jugular venipuncture using disposable syringes at 14 (before Cu supplementation), 30, 60, and 80 days of age. Blood anticoagulated with disodium-EDTA (2.5 ml) and 7.5 ml transferred to plane tube for serum separation. All tubes were placed immediately on ice and were transferred to the laboratory. Blood anticoagulated was used for CBC determination using automated veterinary hematology analyzer (Nihon Kohden, Cell Tac α, MEK 6108, Tokyo, Japan). Differential leukocyte count was performed on Giemsa-stained blood film using cross-sectional method [6]. Plane tubes were centrifuged at 1,800 g for 10 min followed by removal of serum. Serum was stored at −20°C until analysis. The amounts of total serum protein (tp, Biuret method), albumin (alb, Bromcresol green method), iron (Fe, Ferene S method), and copper (Cu, 3,5-Di-Br-PAESA method) were measured by commercial kits (Pars Azmoon, Tehran, Iran and Randox, Antrim, UK for copper) using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). The concentration of globulin (glo) calculated as the difference between total serum protein and albumin. Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy.

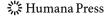
Calves were weighted at birth and at the end of trial (day 80) and total gain and mean daily gain were calculated. The health of calves was checked by a technician twice a day and any sign of illness, treatment (if needed), and duration of illness was recorded.

The data were analyzed by SPSS 13 statistical package (SPSS Inc, Chicago, IL, USA). All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The metabolites without normal distribution were converted using a natural logarithmic transformation to achieve a normal distribution. Because serum parameters measured over time, a repeated measures approach using

Table 2 Ingredient Composition of Concentrate Mix Fed to Calves (DM%)

Ingredients	Percent	Ingredients	Percent
Corn	50%	Molasses	5.5%
Barley	15%	D.C.P	0.2%
Soybean meal	22%	Limestone	0.9%
Beet pup	3%	Supplement <sup>a</sup>	0.4%
Wheat bran	3%		

<sup>&</sup>lt;sup>a</sup> Each kilogram of supplement contains: Vitamin A (50,000 IU), Vitamin D3 (10,000 IU), Vitamin E (0.1 g), Calcium (196 g), Phosphorus (96 g), Sodium (71 g), Magnesium (19 g), Iron (3 g), Copper (0.3 g), Manganese (2 g), Zinc (3 g), Cobalt (0.1 g), Iodine (0.1 g), and Selenium (0.001 g)



ANOVA was used (fixed effects of group and covariates, random effect of calf). ANOVA with P value adjusted Bonferroni multiple comparison test was also used for total weight gain and mean daily gain comparison between groups. *Chi-square* test was used for comparison of disease occurrence between groups. P<0.05 was considered as significant.

### Results

The results are summarized in Tables 3, 4, and 5. Group (treatment) had no significant effect on the amounts of measured parameters except MCH values (p<0.05) which were significantly lower in test 1 group than other trial groups. Age (sampling time) had significant effects on the values of most measured parameters (p<0.05) except WBC, lymphocyte, total protein, and fibrinogen. Significant interactions between sampling time and group were not seen for any of measured parameters (Tables 3 and 4).

No significant differences were seen for total weight gain and mean daily gain between trial groups. Most of the treatments were performed for pneumonia and calf diarrhea. *Chi-square* test revealed no significant difference for the percent of days of treatment between trials groups, although it was numerically lower in test groups than control (Table 5).

### Discussion

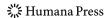
Animals are deficient in copper in many areas of the world and antagonistic minerals, such as molybdenum, sulfur, and iron can create a copper deficiency in ruminants even when

Table 3 Effects of Short-Term Copper Supplementation on Hematological Parameters Between Trial Groups

Parameters	Control	Test 1	Test 2	SE	Age	Group	Age × Group
HCT (1/1)	0.32	0.29	0.30	0.01	S	NS	NS
RBC (10 <sup>12</sup> /l)	9.37	8.84	8.83	0.36	S	NS	NS
Hb (g/l)	106.7	93.3	97.7	4.48	S	NS	NS
MCV (fl)	34.04	32.58	33.56	0.53	S	NS	NS
MCH (pg)	11.25 <sup>a</sup>	10.37 <sup>b</sup>	10.93 <sup>ab</sup>	0.22	S	S	NS
MCHC (%)	32.99	31.89	32.66	0.42	S	NS	NS
WBC (10 <sup>9</sup> /l)	10.51	10.70	11.15	0.94	NS	NS	NS
Neut (10 <sup>9</sup> /l)	3.50	3.95	4.12	0.58	S	NS	NS
Lymph (10 <sup>9</sup> /l)	9.02	6.48	6.78	1.07	NS	NS	NS
Mono (10 <sup>9</sup> /l)	0.20	0.27	0.24	0.04	S	NS	NS
Fib (g/l)	4.36	4.27	4.33	0.23	NS	NS	NS
Plt (10 <sup>9</sup> /l)	674	743	734	48.3	S	NS	NS
Fe (µmol/l)	20.12	20.15	21.28	1.20	S	NS	NS
Cu (µmol/l)	28.8	28.41	24.47	2.65	NS	NS	NS

Means within rows lacking a common superscript differ (P < 0.05)

S significant effect (P<0.05), NS non-significant effect, HCT hematocrit, RBC red cell count, Hb hemoglobin, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, WBC white blood cell count, Neut neutrophil, Lymph lymphocyte, Mono monocyte, Fib fibrinogen; Plt platelet; Fe iron, Cu copper



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Parameters	Control	Test 1	Test 2	SE	Age	Group	Age × Group
TP (g/l)	56.9	58.2	57.0	1.43	NS	NS	NS
Alb (g/l)	28.1	28.5	28.8	0.57	S	NS	NS
Glo (g/l)	28.8	29.6	28.2	0.13	S	NS	NS
A:G ratio	1.02	1.01	0.96	0.05	S	NS	NS

Table 4 Effects of Short-Term Copper Supplementation on Serum Protein Profile Between Trial Groups

S significant effect (P<0.05), NS non-significant effect, TP total protein, Alb albumin, Glo globulin

dietary copper is adequate [7]. Copper deficiency causes various disorders in animals such as anemia, retarded growth, immunological dysfunctions, reproductive disorders, and alter cardiovascular, nervous, and skeletal systems [1].

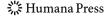
The effects of copper supplementation were reported in deficient dairy cows and calves using different levels and various form of copper with or without antagonists [8–12]. Similar studies were also performed in beef cattle [13–19]. However, limited information is available concerning to the effects of supplementation or over-supplementation of copper in non-deficient dairy calves in neonatal period along with decrease copper absorption due to rumen function.

In agree with our results, Heidarpour Bami et al. [20] revealed parenteral oversupplementation of copper at 14 days of life in dairy calves caused higher RBC parameters than control calves although the differences were not significantly different. Non-significant differences were also obtained for RBC indices and different types of leukocytes. In goats, over-supplementation with copper sulfate resulted in non-significant difference in PCV levels. WBC counts were not also affected when goats were supplemented with the amounts of 50, 150, and 300 mg/day/head of copper although positive significant correlation was seen between copper amount and WBC number [21]. In another study in goats, over-supplementation with the amounts of 100 and 200 mg/day/head of copper had no effect on the RBC parameters and MCV but MCH for the 100 mg copper group was lower than other groups. In our study, significant decrease in MCH amount was seen in calves of test 1 group when compared with control and test 2 group. The reason of this change is not clear. An increase in leukocyte count was reported as an increase in copper supplementation. Copper group (100 mg) had a higher percentage of neutrophils and a lower percentage of lymphocytes [22]. In copper depleted heifers copper supplementation as sulfate or lysine at a rate of 8 or 16 mg/kg revealed no difference between groups for hemoglobin concentrations [17]. In copper depleted calves, the effects of supplementation with molybdenum (5 mg/kg DM), copper (10 mg/kg DM), and copper plus molybdenum (5 mg/kg DM and 5 mg/kg DM, respectively) were compared with control calves. Hematocrit and total leukocyte counts were similar for all calves at all sampling times [10].

Table 5 Mean±SE of Weight Gain and Health Between Trial Groups

Parameters	Control	Test 1	Test 2	p value
Total weight gain (kg)	43.1±1.91	43.2±1.49	$43.04 \pm 0.94$	NS
Mean daily gain (kg)	$0.54 \pm 0.02$	$0.54 \pm 0.02$	$0.54 \pm 0.02$	NS
Days of treatment	4.5±1.9	$3.07 \pm 1.0$	$2.46 \pm 1.05$	NS

NS non-significant difference

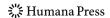


Xin et al. [9] studied the effects of molybdenum supplementation (copper depleting diet) in Holstein steers. In comparing with copper sufficient group, there was no evidence of blood hemoglobin difference between two groups. On the other hand, in a previous study, calves fed the molybdenum supplementation exhibited lower hematocrit values than the iron or copper-supplemented calves [13].

Anemia can result along with other abnormality in ruminants with copper deficiency. It is more likely to occur as primary form of deficiency due to eating of diet with low copper amounts, it is less common in secondary copper deficiency where copper absorption is impaired by high dietary antagonists such as iron, molybdenum, and sulfate [23]. However, in cattle it is unlikely that calves will be born with depleted liver copper reserves unless the dams were severely copper deficient during pregnancy. Priority is given to the fetus to ensure adequate liver copper reserves at birth. However, liver copper concentrations in calves decreased rapidly during early neonatal period [24]. With attention to the results of previous studies and present study, it seems the hemogram is not a sensitive indicator for copper deficiency/sufficiency conditions and the changes in RBC parameters only occur in clinical situations.

In contrast with our results, Heidarpour Bami et al. [20] suggested parenteral supplementation of copper resulted in significantly better weekly gain, daily gain for each trial weeks, total weight gain, and total daily gain in dairy calves. Solaiman et al. [21] reported a 28% better in daily gain in Nubian doe goats were supplemented with copper at levels of 100-150 mg/day for 23 weeks. In another study, higher average daily gain was detected after 70 days of copper supplementation in the 100 mg/day group when compared to the other two groups [22]. In the study performed by Gengelbach and Spears [10] in copper depleted calves, calves that were fed the Cu and Mo diets gained weight more efficiently than those fed with the control and Cu + Mo diets during the 112-day study. In another study rate of gain did not differ among calves fed the control, Fe-supplemented (600 mg of FeCO<sub>3</sub>/kg) or copper-supplemented (10 mg CuSo<sub>4</sub>/kg) diets, whereas the molybdenumsupplemented (5 mg Na<sub>2</sub>MoO<sub>4</sub>/kg) calves gained significantly lower amount [13]. Rabiansky et al. [17] reported that body weight was not affected by the administration of copper in copper depleted heifers. Xin et al. [9] studied the weight gain between steers with copper sufficient diet (20 ppm copper sulfate/DM) and those received copper depleting diet (10 ppm of ammonium molybdate/DM). There was no significant difference between two groups. In pigs high dietary copper supplementation at a rate of 125 and 250 mg/kg were resulted to significant improve of average daily gain and average daily feed intake in comparing with control group (10 mg/kg). High dietary copper appears to stimulate appetite in pigs by upregulating neuropeptide Y concentration in hypothalamus, which in turn resulted to better gain [25]. Presence of neuropeptide Y in somatotrophs of cattle was reported [26]. According to the controversial results, it seems that route and levels of supplementation have significant effects on performance of studied animals. Parenteral supplementation was not affected by dietary antagonists but the results of dietary supplementation were at least partially dependent to the levels of antagonists in diet.

In vitro studies have revealed that copper deficiency can affect various cells functions in the immune system [9, 16, 19]. In goat kids, over-supplementation of copper resulted to better lymphocyte proliferation induced by concanavalin A and phytohemagglutinine A in comparing with control groups [22]. Gengelbach et al. [15] reported that the antimicrobial activity of neutrophils from copper deficient calves decreased compared with neutrophils from copper-supplemented calves. The activity of antioxidant enzyme cu,zn-superoxidedismutase in neutrophils from cattle and sheep and in peritoneal macrophages of rats decreased due to copper deficiency. Ward et al. [27] reported that control calves had higher secondary antibody



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response to pig erythrocytes than copper-, molybdenum-, and iron-supplemented calves. Heidarpour Bami et al. [20] reported that parenteral copper over-supplementation did not have any effect on the health of the dairy calves. In our study, no significant differences were detected in incidence of neonatal diseases and days of treatment due to administration of copper. The concentration of globulin and A:G ratio were not also affected by copper supplementation. Dorton et al. [19] suggested that nonspecific IgG and total IgM in serum were not different between steers supplemented with different form and amounts of copper with control steers whereas specific antibody responses were better than control group. It seems that immunological responses would be affected in clinical copper deficiency and in subclinical situations specific immune response affected more than other responses.

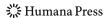
Solaiman et al. [22] believed that the discrepancy of response to copper supplementation in cattle may be related to three basic factors: dietary, animal, and animal×diet interactions. Dietary factors contain ingredient composition of the diet (mainly carbohydrate source), copper source, copper level in basal diet, and concentration of copper antagonists in the diet. Animal factors include breed, species, gender, and physiological stages of the animal. Interaction of the above two factors such as initial copper status of the animals or duration of copper supplementation may also affect the results. The results obtained by Heidarpour Bami et al. [20] are examples of these probable factors. Based on their results, parenteral supplementation of copper in calves revealed to better weight gain during the study but in the present study copper supplementation in diets did not have any significant effect on measured parameters.

In conclusion, short-term (14 days) over-supplementation of copper in milk did not induce any significant effect on the hematological, serum biochemical, health, and performance parameters in dairy calves. However, it could probably promote copper reserve of the liver for higher amounts of need in the productive phase of life. It must be considered that if the available copper amount in neonatal period is not sufficient for maximum performance, the calf proceeds to use copper reserve of liver (if sufficient) without any changes in serum or plasma copper concentrations and other clinical and laboratory findings. This may result to lower copper availability (and liver reserve) for future pregnancy and milk production and would result to lower performance. Thus, long-term monitoring of over-supplementing calves may be required for definitive results.

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