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The Use of Copper Supplementation to Improve Growth Performance and Claw Health in Young Holstein Bulls

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ABSTRACT

This study was performed to determine the effect of copper on growth performance, blood metabolites and claw health in young Holstein bulls in a humid area in north of Iran. One hundred and two young Holstein bulls (Initial body weight= 377.3±17.1 kg) were randomly allocated to two treatments in a completely randomized design for 56 days. The treatment groups received: (1) the basal diet of no supplemental Cu (control; n= 50); (2) basal diet plus 30 mg of Cu kg^{-1} of Dry Matter (DM) as $CuSO_4$ (n= 52). Animals received fresh total mixed ration for ad libitum feeding allowing 10% refusals. The groups' Dry Matter Intake (DMI) was assessed daily. The Body Weights (BW) were recorded and jugular blood samples collected on days 0, 28 and 56. All claws of young bulls were examined every two weeks for an identification of claw lesions. Copper supplementation improved Average Daily Gain (ADG) and gain:feed (G:F; P < 0.05). Serum cholesterol decreased with Cu supplementation (P< 0.001). Serum Zn, Cu, and urea N were not affected by supplementation of Cu; however, plasma total protein (P< 0.001), and albumin (P< 0.001) were increased by a supplementation of Cu. The prevalence of lameness was 19.6% and control group had the highest Odds ratio (OR= 2.43). As a result, it is concluded that supplemental Cu might improve growth performance in the finishing bulls and decrease the prevalence of lameness.

Keywords: Blood metabolite, Claw health, Copper, Young bull.

INTRODUCTION

Copper (Cu) is required for cellular respiration, connective tissue development, and activity of such leukocytes as neutrophil (McDowell, 2003). Copper's role in the production of a healthy claw horn is related to the copper enzyme, thiol oxidase which forms the disulfide bonds between cysteine residues of adjoining keratin filaments (O'Dell, 1990). Some studies have indicated that ADG and G:F have been improved through Cu supplementation in feedlot cattle (Lee *et al.,* 2002; Hansen *et al.,* 2008). Copper, supplemented at physiological concentration levels, to beef cattle has been reported to be involved in cholesterol metabolism (Engle, 2010).

Copper deficiency has been reported to be linked to a variety of clinical signs, including anaemia, impaired reproductive performance, decreased resistance to infectious diseases, diarrhea and some other generalized ill-health signs (Tessman *et al.,* 2001), bringing about severe economic losses. Also, the increase in DNA damage found in hypocupremic cattle could be explained by higher oxidative stress suffered by some of these animals (Picco *et al.,* 2004). Enjalbert *et al.,* (2006) reported that herds with marginal or deficient plasma concentrations of either Zn or Cu were found to have suffered from increased risks

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of metritis, mastitis, as well as locomotion problems. Richeson and Kegley (2011) reported that administration of a trace mineral injection during initial processing of highly stressed, newly received heifers improved ADG, G:F, bovine respiratory disease morbidity as well as antibiotic treatment cost. Thus, proper trace mineral nutrition as well as overall nutrition are important components to optimizing animal health and performance. Therefore, it is hypothesized that such trace minerals as Cu levels should be increased in the diet when young bulls are kept under stressed conditions and in humid environments to control lameness and other probable diseases. Thus, the first objective of this research was to find out whether a supplementation of the diet with adequate levels of Cu would affect the performance and claw health of young bulls. The possible effects of Cu on the Cu concentration in the liver, and in the blood metabolites were also investigated.

MATERIALS AND METHODS

Animal Care and Experimental Design

This experiment was conducted on a beef cattle farm in Mazandaran Province (a humid area) in north Iran. One hundred and two young bulls (Initial body weight= 377.3±17.1 kg) were randomly allotted to two treatments in a completely randomized design for 56 days. The treatment groups received: (1) the basal diet with no supplemental Cu (control); (2) basal diet plus 30 mg of Cu kg^{-1} of DM as $CuSO_4$. Animals were randomly grouped in six pens (16 to 19 animals per pen). The copper supplemented diet provided copper in excess of the requirement for young bulls (NRC, 2000). However, copper content of the basal diet was less than adequate to meet the requirement in young bulls. The chemical composition and nutritive values of the diet components are shown in Table 1. The control diet contained 4.7 mg of Cu per kg of DM and 30.2 mg of Fe per kg of DM. Animals received fresh total mixed ration at 9.00, 12.00 and 15.00 (as feeding times) for ad libitum feeding allowing 10% as refusal. Water was available at free choice. The basal diet was dispensed to young bulls via a computerized feeder. Copper sulfate in a wheat bran carrier was fed following the morning meal as a top-dress.

Table 1. Composition of basal diet.

Ingredient	Total Mixed Ration
	% of DM
Corn silage	7.5
Wheat straw	2
Barley (Milled)	54
Potato	12
Cottonseed meal	3
Sunflower meal	4.78
Wheat bran	4
Rice bran	6
Beet pulp	2
Molasses	1.5
Zeolite	1.5
Urea	0.28
Calcium carbonate	0.56
Salt	0.28
Vitamin premix [a]	0.3
Mineral premix [b]	0.3
Chemical Composition	% of DM
CP	13.25
NDF	23.25
ADF	12.06
Ether extract	3.04
Ca	0.63
P	0.24
Mg	0.31
S	0.14
	mg kg^{-1} DM
Cu	4.7
Zn	44.8
Mn	59
Fe	30.2
Mo	1.2

[a] Contained per kilogram of premix: 500,000 IU of vitamin A; 100,000 IU of vitamin D, and 0.1 g of vitamin E.

[b] Contained per kilogram of premix: 180 g Ca; 90 g P; 20 g Mg; 60 g Na; 2 g Mn; 3 g Fe; 0.3 g Cu; 3 g Zn; 0.1 g Co; 0.1 g Se; 0.1 g I, 3 g antioxidant.

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Sampling

Diets were sampled weekly and stored for subsequent chemical analysis. For serum harvesting, blood samples were collected from each animal through venipuncture without any anticoagulant on days 0, 28, and 56 (2 hours following morning meal) and were stored at -20°C, until being analyzed. On days 0 and 28, serum samples were analyzed for Cu and Zn concentrations while on day 56 analyzed for urea N, cholesterol, and the concentrations of Zn and Cu. Whole blood was centrifuged at 1,760×g for 15 minutes at 4°C. For plasma harvesting, blood samples were collected on day 56 (2 hours following morning meal) *via* jugular venipuncture in EDTA tubes and then plasma harvested (by being centrifuged as similar to serum). At slaughter (on day 59 of the study), samples of liver were taken from a subgroup of animals (9 animals per treatment) to determine Cu concentration. The whole liver samples were cut into pieces with titanium coated scissors. Liver pieces were minced with a cutter before being deep–frozen.

Assessments

Daily feed intake was recorded throughout the trial, taking into account feed refusals. The body weights were taken on days 0, 28, and 56. Diets were analyzed for DM, Crude Protein (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Ether Extract (EE), calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), molybdenum (Mo), ferrous (Fe), copper (Cu), zinc (Zn) and manganese (Mn). Standard procedures of AOAC (2000) were employed in the analysis of samples of feeds and of refusals. The DM content of diets was obtained after being dried at 105°C for 24 hours. Kjeldahl' method following acid hydrolysis, was employed for N content measurement and then the percentage of crude protein (CP= N×6.25) calculated. Soxhlet method was employed to obtain EE percentage following an extraction with petroleum ether. A method of Van Soest *et al.* (1991) was utilized for NDF analysis, where feed samples were refluxed for 1 hour with neutral detergent solution without sodium sulfite and the use of an alpha-amylase. The residue was dried and reported as NDF. A method of AOAC (1990) was utilized for determining acid detergent fiber content. Total-tract apparent digestibility of DM was determined using acid-insoluble ash as an internal digestibility marker (Van Keulen and Young, 1977). Serum samples were analyzed for urea N, cholesterol, concentrations of Zn and Cu. Plasma samples were analyzed for total protein and albumin. Urea N, total protein, and albumin contents were determined using a COBAS MIRA plus Analyzer. Liver pieces were dried at 100°C for 24 hours and digested using a wet-ashing procedure in which 12 ml of a 4:1 mixture of HNO_3 and $HClO_4$ were added to 500 mg of liver tissue. Ash was filtered into 100 ml glass bottles containing warm distilled water. The solution was analyzed for Cu by atomic absorption spectrophotometry (Model: Perkin-Elmer, 1982). Concentrations of Ca, Zn, Cu, Fe, Mo, Mn, and Mg in the diets were determined through atomic absorption spectrophotometry, using a procedure similar to that employed in the determination of liver Cu content. Phosphorus content in the diets was determined through colorimetry (Fiske and Subbarow, 1925).

Claw Health

Clinical examinations were performed by one professional claw health expert, trained in the diagnosis of claw disorders through discussions with one of the authors of the paper Hadi Fagari-Nobijari and as well with a professional veterinarian during claw evaluations. Lameness prevalence was defined as the proportion of diagnosed animals with a given disorder during claw evaluations (every two weeks). Throughout

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this examination, animals were restrained, all the feet thoroughly washed and trimmed for a complete exposure of the lesions. At the time of visit, all cattle of the farm were evaluated for lameness using a 5-point lameness score as described by Sprecher *et al.* (1997). During claw evaluations both front and hind claws were examined for the presence of claw disorder, as described by Bielfeldt *et al.* (2005).

Statistical Analysis

Data analysis was performed through SAS 9.1 (SAS Inst., Inc., Cary, NC) using the General Linear Model Procedure (GLM) of SAS. Least square means with a significant *F*-test (P< 0.05) were compared using PDIFF of SAS. Animals were randomly grouped in six pens (16 to 19 animals per pen). Pen was employed as the experimental unit with three replications (pens) per treatment. Animals within treatments were used as random error terms. Serum Cu and Zn data were analyzed as repeated measures using the mixed procedure of SAS as described by Littell *et al.* (1998).

Claw disorder complexes and lameness were analyzed as binary traits; "non-lame" animals were distinguished from "severe lame" animals with score > 3. Animals were tested for the presence of lameness and of each claw disorder through Logistic Regression Analysis (PROC GENMOD of SAS). The model was run stepwise forward with probability for entry in the model *P*< 0.05. Independent variables (P> 0.10) were removed from the final model by stepwise backward elimination. The significance level was set at *P*< 0.05.

RESULTS

Performance and DM digestibility

Dry matter intake did not differ among treatments (Table 2). Average Daily Gain (ADG) was not affected by dietary Cu supplementation during days 0 to 28 period. During the days 28 to 56 period, ADG was affected by dietary Cu level (P< 0.05). During the entire treatment period, Cu supplementation increased ADG (P< 0.05) and Gain:Feed (G:F) (P< 0.001). Gain:feed was not affected by Cu supplementation during days 0 to 28 period, but, during the d 28 to 56 period, Cu supplemented group (P< 0.05) benefited from a greater G:F than the control. Apparent dry matter digestibility

Table 2. Least square means and standard errors of growth performance and DM digestibility for young bulls fed different diets*.

Variable	0 Cu	30	SE	*P*-Value
DMI [a] (kg d^{-1})				
d 0 to 28	9.92	9.83	0.16	0.49
d 28 to 56	10.94	10.58	0.13	0.29
d 0 to 56	10.43	10.20	0.11	0.2
ADG [b] (kg)				
d 0 to 28	1.46	1.47	0.04	0.2
d 28 to 56	1.29	1.35	0.01	<0.05
d 0 to 56	1.37	1.41	0.01	<0.05
G:F [c]				
d 0 to 28	0.14	0.15	0.006	0. 15
d 28 to 56	0.11	0.12	0.005	< 0.05
d 0 to 56	0.13	0.14	0.005	< 0.001
DM digestibility[d] (%)	63.75	72.25	2.1	< 0.001

* Diets included: (1) The basal diet (control), (2) Basal diet plus 30 mg of Cu kg^{-1} of DM as $CuSO_4$.
[a] Dry Matter Intake; [b] Average Daily Gain; [c] Gain:Feed, [d] Dry Matter digestibility.

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was increased by Cu supplementation (P< 0.001).

Metabolic Profile

Serum cholesterol decreased with Cu supplementation (P< 0.001; Table 3). Serum Zn, Cu and urea N concentrations were not affected by Cu supplementation. However, plasma total protein (P< 0.001), and albumin (P< 0.001) were increased by Cu supplementation. Supplemental Cu (P< 0.001) increased Cu concentration in the liver. The serum concentrations of Cu (P< 0.05) and Zn (P< 0.001) were affected by time (Table 4). Effect of treatment across time on serum Zn and Cu was recorded as consistent.

Claw Health

The prevalence of lameness was 19.6%. Lameness was most frequently observed in control group (OR = 2.43; Table 5). The prevalence of skin and Interdigital space Disorders (ID) was the most common cause of lameness (70% of lameness) and was higher in the control animals than that in the copper supplemented animals. The prevalence of Sole Disorders (SD), White Line Disorders (WD), and Heel Erosions (HE) did not differ by treatment (data not shown).

DISCUSSION

Performance and Dry Matter Digestibility

The findings indicated that addition of 30 mg of Cu kg^{-1} of DM to the control diet (control diet contained 4.7 mg of Cu kg^{-1} of DM) improved ADG, G:F and DM digestibility. However, treatment did not affect DMI. In agreement with the present study, finishing steers supplemented with 10 or 40 mg Cu kg^{-1} DM had higher ADG and average daily feed intake than controls as found out by Engle *et al.* (2000b). Previous research also reported higher ADG and G:F in finishing steers supplemented with 5 mg Cu kg^{-1} DM (basal diet contained 3 mg Cu kg^{-1} DM) relative to unsupplemented steers (Ward and Spears, 1997). In contrast, Essig *et al.* (1972) reported that ADG and G:F were not affected by the level of Cu sulfate (5.73 g 100 kg^{-1} DM diet) fed to steers. Similarly, Engle and Spears (2001) reported that Cu supplementation in Simmental steers had no effect on performance while Ivan *et al.* (1975) reported that the addition of 20 mg Cu kg^{-1} DM (basal diet containing 6 mg Cu kg^{-1} DM) to diets of Holstein calves had no effect on DM digestibility. Wagner *et al.* (2008) reported that certain environmental, genetic, and dietary factors may have influenced the animal performance response to trace mineral supplementation. The effect of Cu supplementation on DM digestibility may be related to enhancement of ruminal

Table4. Probabilities of main effects on serum Zn and Cu of young Holstein bulls.

Variables	Cu	Time	Cu× Time
Serum Zn	0.63	< 0.05	0.60
Serum Cu	0.88	< 0.001	0.88

Table 5. Associations between treatments and the presence of claw disorders in young Holstein bulls fed different diets.

	n	OR[a]	CL_L, CL_U[b]	*P*–value
Control	14/50	2.43	0.91-6.84	< 0.05
Cu	6/52	1		

[a] Odds Ratio; [b] Lower, Upper Confidence Limit, *P*= 0.05.

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fermentation and as well to other factors.

Metabolic Profile

In the present study, Cu supplementation decreased serum cholesterol while increasing Cu concentrations in liver (Table 3). These results are consistent with those of the previous researches in which serum cholesterol was lower in steers supplemented with Cu by day 84 of the finishing period (Engle *et al.,* 2000a). Adding 10 or 40 mg Cu kg^{-1} DM of Cu sulfate increased plasma and liver Cu concentrations in finishing steers, but did not affect serum cholesterol concentration (Engle and Spears, 2001). Dorton *et al.* (2003) reported that steers receiving 20 mg Cu kg^{-1} DM had higher liver Cu concentrations than steers receiving 10 mg Cu kg^{-1} DM or no supplemental Cu.

In the present study, copper supplementation decreased serum cholesterol. In most mammals, the primary site of endogenous cholesterol synthesis is the liver (Siperstein *et al.,* 1970); but, in ruminants the primary site of cholesterol synthesis is the small intestine and adipose tissue, with a small proportion of the total endogenous cholesterol being produced by liver (Liepa *et al.,* 1978). Engle *et al*. (2001) reported that total serum cholesterol concentrations were higher in cows receiving supplemental Cu (10 or 40 ppm) when initial liver copper concentrations were high. When copper status was reported at a lower rate in cows, the effect of the element's supplementation on lipid metabolism varied (Engle *et al.,* 2001).

In the present study, the concentration of serum Cu and also, the concentration of serum Zn in young bulls were affected by time. The increase of serum Cu concentration might be related to the increase in liver Cu concentrations during the experimental period in animals supplemented with Cu (Table 4). Etzel *et al.* (1982) indicated that serum Cu values were increased by infection, possibly due to increased serum concentrations of the Cu containing acute phase protein, ceruloplasmin. Ceruloplasmin, binds up to 95% of circulating Cu, regulates iron availability, takes part in oxidation-reduction reactions, and may regulate immune function (Healy and Tipton, 2007). There was no indication in this study that bulls were fighting a clinical or subclinical infection. However, the effect of time on serum Cu and Zn concentrations remained unknown.

Claw Health

The percentages of animals affected by various claw lesions amounted to SD, 2.94%, WD, 2.94%, HE, 0%, and ID, 13.72%. In the present study, Cu supplementation reduced the incidence of ID. The higher incidence of the infectious ID lesions in control group might be due to impaired immune function as related to Cu deficiency. Copper has been shown to have an important role in immune function including neutrophil activity (McDowell, 2003).

Holzhauer *et al.* (2008) reported that digital dermatitis, which is an important cause of lameness in dairy cattle in many countries, increased when dairy cattle were fed a high energy diet during lactation. Therefore, consumption of high concentration diets by dairy and beef cattle may be an important risk factor for skin and interdigital space lesions. Other risk factors for high prevalence of ID in dairy herds included wet floors, purchase of replacement stock, restricted grazing time, low parity, early lactation and serious heel horn erosion (Wells *et al.*, 1999; Rodriguez-Lainz *et al.*, 1996; Somers *et al.*, 2005). Digital dermatitis might cause pain and lameness in infected dairy cows, thus negatively affecting animal well being (Almeida *et al.* 2007). It is debated whether or not digital dermatitis causes a reduction in milk production (Losinger, 2006).

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In the present study, wet floors may have predisposed cattle to increased incidence of ID. Copper supplementation of the basal diet with marginal Cu content decreased ID incidence reflecting the important role of Cu in reducing some types of lameness. In addition, Cu may reduce lameness by improving hoof texture due to its role in the activity of thiol oxidase which forms the disulfide bonds between keratin filaments. Torre *et al.* (1996) indicated that neutrophils isolated were less effective at killing ingested bacteria in heifers fed approximately 7 ppm Cu than neutrophils of heifers supplemented with 20 ppm Cu, although phagocytosis and superoxide production were not affected. Percival (1998) demonstrated that even in marginal deficiency, when common indexes of copper are not affected by the diet, the proliferative response and interleukin concentrations are reduced. The number of neutrophils in human peripheral blood is not reduced in cases of marginal copper deficiency, but their ability to generate superoxide anion and kill ingested microorganisms is reduced in both overt and marginal copper deficiency (Percival, 1998). Dorton *et al.* (2003) indicated that the immune response to an antigen varies depending on the type of antigen administered as well as the concentration of Cu supplemented when steers were supplemented with 10 and 20 mg Cu kg^{-1} DM.

Zinc, Cu, and Mn are integral components in the antioxidant system due to their presence in Superoxide Dismutase (SOD) which reduces the free radical superoxide to hydrogen peroxide (Gressley, 2009). Spears and Weiss (2008) demonstrated that white blood cells are particularly sensitive to oxidative damage because their membranes contain high concentrations of unsaturated fatty acids. Oxidative damage to these membrane fatty acids reduces the ability of white blood cells to defend the cow against disease challenges. If trace mineral supplementation reduces oxidative damage to white blood cells, it may reduce disease susceptibility in oxidatively stressed animals. Gressley, (2009) reported that trace mineral supplementation (such as Zn, Cu and Mn) above predicted requirements during times of oxidative stress (calving, infection, and heat stress) may reduce oxidative damage to cells and metabolites and may finally, increase disease resistance. Some studies have indicated that stress in the form of an infection (IBRV), a metabolic disorder (ketosis), or deprivation of feed and (or) water can increase copper and zinc depletion in the body of the animal (Orr *et al.* 1990; Nockels *et al.* 1993). Therefore, impaired immunity due to inadequate Cu supplementation may increase lameness caused by infectious agents as observed through increased Interdigital space Disorders (ID) in the control group in this study. Despite the apparent involvement of certain trace minerals in animal production and disease resistance, deficiencies of trace minerals have not always been observed to increase the susceptibility of domesticated livestock species to natural or experimentally induced infections or having decreased the performance (Engle, 2010).

It was concluded that Cu supplementation may improve ADG, G:F, plus apparent DM digestibility and decrease lameness. Stress induced by lameness in the unsupplemented feed group of cattle may have contributed to the lower performance in the control animals. Therefore, adequate Cu supplementation of cattle in such stress conditions as high humidity environment may help to control infectious lesions that could lead to lameness. Future research should concentrate on the effects of Cu on immunity and the microbial resistance of the animal over extended periods of time.

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استفاده از مکمل مس برای بهبود عملکرد رشد و سلامت سم در گوساله های نر هلشتاین

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چکیده

هدف از انجام آزمایش بررسی اثر مس بر عملکرد رشد، فراسنجه های خون و سلامت سم گوساله‌های نر هلشتاین در یک منطقه مرطوب واقع در شمال ایران بود. ۱۰۲ گوساله نر هلشتاین (با وزن اولیه = ۱۷/۱± ۳۷۷/۳ کیلوگرم) به مدت ۵۶ روز در قالب یک طرح کاملا تصادفی به دو تیمار اختصاص داده شدند. تیمارها شامل: ۱) جیره شاهد (تعداد = ۵۰ ; بدون افزودن مس) ۲) جیره شاهد به علاوه ۳۰ میلی گرم مس به ازای هر کیلوگرم ماده خشک مصرفی (تعداد = ۵۲ ; به صورت سولفات مس) بود. گوساله‌های نر جیره پایه را به صورت کاملا مخلوط شده دریافت نمودند. میزان ماده خشک مصرفی گروهی به صورت روزانه اندازه گیری شد. اندازه گیری وزن بدن و خون گیری از سیاهرگ گردنی گوساله‌ها در روزهای آغاز، بیست و هشتم و پنجاه و پنجم انجام شد. برای تشخیص انواع لنگش، سم تمام گوساله‌ها هر دو هفته یک بار مورد بررسی قرار گرفت. افزودن مس میانگین افزایش وزن روزانه و بازده تولید را افزایش داد ($P < 0.05$). با افزودن مس میزان کلسترول سرم کاهش یافت ($P < 0.001$). مقادیر نیتروژن اوره‌ای، مس و روی سرم تحت تأثیر مکمل مس قرار نگرفت، اما میزان پروتئین تام ($P < 0.001$) و آلبومین پلاسما ($P < 0.001$) با افزودن مس افزایش یافتند. میزان وقوع لنگش در کل گله مورد آزمایش ۱۹/۶ درصد بود و بیشترین میزان وقوع لنگش در گروه شاهد مشاهده شد (نسبت وقوع لنگش = ۲/۴۳). در نتیجه، افزودن مس عملکرد رشد گوساله های نر پرواری را بهبود و میزان وقوع لنگش را کاهش داد.

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