

## Effect of copper and zinc on blood and milk parameters and performance of dairy cows

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### ABSTRACT

Thirty five Holstein cows were chosen to study the blood biochemical indicators with a single-factor test design in three copper levels (21.5, 31.5 and 41.5 mg/kg) and three zinc levels (50.15, 90.15 and 130.15 mg/kg). The results showed that the supplement of 20 mg of Cu/kg DM and above 40 mg of Cu/kg DM may increase Cu-Zn-superoxide dismutase (Cu-Zn-SOD) in milk ( $P<0.05$ ), but did not increase milk yield and improve chemical composition ( $P>0.05$ ). Zinc concentration in milk could be higher as supplemented 80 mg of Zn/kg DM ( $P<0.05$ ). The concentration of copper, serum ceruloplasmin, haemoglobin and the activity of Cu-Zn-SOD in blood may be increased ( $P<0.01$ ) by 37, 60, 5.1 and 5.9%, respectively, as the supplement of dietary copper was above 31.5 mg/kg.

KEY WORDS: copper, zinc, performance, blood, milk, cows

### INTRODUCTION

Zinc is essential for the activities of more than 100 enzymes in the body. It is an essential component of the antioxidant enzyme, Cu-Zn superoxide dismutase (Bitman et al., 1991). Copper is contained in a number of enzymes and proteins, and its role has been defined for many physiological functions related mostly to a catalytic agent in the active sites of cuproenzymes (McDowell, 1992). Recent studies have also shown that cows supplemented with copper at 20 ppm in feed/day in addition to basal diet had more uninfected quarters compared to nonsupplemented cows (Singh and Bansal, 2001). The purpose of our research was to study the contribution of high level copper and zinc in dietary to the performance and biochemical parameters of dairy cows.

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## MATERIAL AND METHODS

### *Animals and diets*

Thirty five multiparous Holstein cows were housed individually. Based on individual feed intake data, a 2-week pre-trial period was sufficient for cows to adjust to the new diet. Before the initiation of the experiment, groups with five cows similar in parity, dry matter intake (DMI) and milk yield were assigned randomly to one of the following seven treatments: 1. control (no supplemental Cu, Zn, the levels of copper and zinc were 21.5 and 50.15 mg/kg in diet respectively), 2. 10 mg of Cu/kg DM from Cu sulphate (the levels of copper and zinc were 31.5 and 50.15 mg/kg in diet, respectively), 3. 20 mg of Cu/kg DM from Cu sulphate (the levels of copper and zinc were 41.5 and 50.15 mg/kg in diet, respectively), 4. 40 mg of Zn/kg DM from Zn sulphate (the levels of copper and zinc were 21.5 and 90.15 mg/kg in diet, respectively), and 5. 80 mg of Zn/kg DM from Zn sulphate (the levels of copper and zinc were 21.5 and 130.15 mg/kg in diet, respectively). The diets were formulated containing, %: CP 13.25, Ca 0.50, P 0.30, and other nutrients meeting or exceeding NRC (2001) recommendations with the exception of Cu and Zn, using maize silage (13.7%), peanut stalk (17.0%), sweet potato vine (17.0%) and supplement (52.3%).

### *Sample collection and analysis*

Blood samples were taken at the end of the experiment. Feed was removed for 1 h before the initiation of blood collection. A 15 ml sample of blood was collected by coccygeal venipuncture into heparinized evacuated tubes. Samples were placed with ice and then transported to the laboratory. Plasma was separated by centrifugation (3000 g) and then stored at the temperature -20°C before analysed.

The cows were milked twice daily at 07.00 and 16.00. Milk yield was recorded, and milk samples were collected at the end of the experiment and analysed the fat, protein, lactose (UL20AC, Ultrasun Technologies CO., Ltd.), and milk copper zinc superoxide dismutase (Cu-Zn-SOD) activity. Plasma Cu and Zn concentrations were measured after the samples were thawed at room temperature for 3 to 4 h. For Cu analysis, 1 ml of a 10% (w/v) trichloroacetic acid solution was added to 1 ml of plasma or standard and then vortexed vigorously for 20 sec. To aid in precipitation, the sample was placed in a -20°C freezer for 30 min, and then centrifuged at 1,200 g for 10 min at room temperature. The supernatant was removed and diluted in deionized water to the concentrations that fit within a linear range of a standard curve generated by linear regression of known concentrations. Plasma Cu concentrations were read at 324.7 nm using a flame atomic absorption

spectrophotometer (HITACHI 180-80). Plasma Zn concentrations were analysed in a similar manner as described above; they were determined at 213.9 nm.

### Statistical analysis

Datas were analysed by ANOVA using the statistical program SAS.

## RESULTS

Dry matter intake averaged 26.6 kg across all treatments and was not affected ( $P>0.05$ ) by the inclusion of supplemental copper and zinc (Table 1).

Table 1. Effect of supplemental different levels of copper and zinc on production, chemical composition and copper-zinc-superoxide dismutase activity of milk in Holstein cows

Item	Supplemental Cu mg Cu/kg DM			SEM	Supplemental Zn mg Zn/kg DM			SEM
	0	10	20		0	40	80	
DMI, kg/d	26.55	26.73	26.60	0.21	26.70	26.67	26.57	0.26
Milk yield, kg/d	19.03 <sup>a</sup>	19.93 <sup>a</sup>	19.60 <sup>a</sup>	1.88	19.03 <sup>a</sup>	19.92 <sup>a</sup>	19.87 <sup>a</sup>	2.77
<i>Milk composition</i>								
fat, %	3.02 <sup>a</sup>	3.05 <sup>a</sup>	2.98 <sup>a</sup>	0.09	3.02 <sup>a</sup>	3.01 <sup>a</sup>	3.07 <sup>a</sup>	0.70
protein, %	3.04 <sup>a</sup>	3.01 <sup>a</sup>	3.06 <sup>a</sup>	0.06	3.04 <sup>a</sup>	3.12 <sup>a</sup>	3.05 <sup>a</sup>	0.21
lactose, %	4.77 <sup>a</sup>	4.72 <sup>a</sup>	4.75 <sup>a</sup>	0.12	4.77 <sup>a</sup>	4.87 <sup>a</sup>	4.82 <sup>a</sup>	0.13
copper, mg/l	0.25 <sup>a</sup>	0.30 <sup>a</sup>	0.33 <sup>a</sup>	0.06	0.25 <sup>a</sup>	0.24 <sup>a</sup>	0.26 <sup>a</sup>	0.02
zinc, mg/l	5.66 <sup>a</sup>	5.65 <sup>a</sup>	5.63 <sup>a</sup>	0.81	5.66 <sup>a</sup>	5.91 <sup>a</sup>	6.12 <sup>b</sup>	0.84
iron, mg/l	4.30 <sup>a</sup>	4.82 <sup>a</sup>	4.42 <sup>a</sup>	0.19	4.30 <sup>a</sup>	4.32 <sup>a</sup>	4.23 <sup>a</sup>	0.19
Cu-Zn-SOD, U/ml	47.31 <sup>a</sup>	48.25 <sup>a</sup>	54.11 <sup>b</sup>	4.24	47.31 <sup>a</sup>	66.52 <sup>b</sup>	84.14 <sup>c</sup>	4.58

Supplemental copper and zinc have no effect on average daily milk yield and milk composition. But Cu-Zn-SOD in supplemental 20 mg of Cu/kg DM treatment was higher than that of other two treatments ( $P<0.05$ ), and was greater from cows supplemented with high relative to low dietary zinc levels ( $P<0.05$ ). Milk zinc concentration in supplemental 80 mg of Zn/kg DM treatment was higher than that of other two treatments ( $P<0.05$ ).

The concentration of copper, serum ceruloplasmin, and the activity of Cu-Zn-SOD in blood were raised up ( $P<0.01$ ) by 37, 60 and 5.9%, respectively (Table 2), as the copper level was raised from 21.5 to 31.5 mg/kg, but the concentration of zinc in blood was not affected ( $P>0.05$ ) by the inclusion of supplemental copper and zinc. In the same certain level of zinc there was a significant increase (by 5.1%) in the concentration of haemoglobin in blood ( $P<0.01$ ) when the copper level in diet was raised from 21.5 to 41.5 mg/kg. In the same certain level of copper, there was no

effect ( $P>0.05$ ) on concentration of copper, zinc, serum ceruloplasmin, haemoglobin and the activity of Cu-Zn-SOD in blood as the zinc level was raised up.

Table 2. Comparison of blood biochemistry indices with different diet copper and zinc levels

Item	Supplemental Cu mg Cu/kg DM			SEM	Supplemental Zn mg Zn /kg DM			SEM
	0	10	20		0	40	80	
	Copper in blood, ug/ml	0.97 <sup>a</sup>	1.33 <sup>b</sup>		1.53 <sup>b</sup>	0.15	0.97 <sup>a</sup>	
Zinc in blood, ug/ml	5.79 <sup>a</sup>	5.69 <sup>a</sup>	5.39 <sup>a</sup>	0.04	5.79 <sup>a</sup>	5.73 <sup>a</sup>	5.61 <sup>a</sup>	0.59
Cu-Zn-SOD, U/ml	119.95 <sup>a</sup>	127.05 <sup>b</sup>	128.87 <sup>b</sup>	2.88	119.95 <sup>a</sup>	114.75 <sup>a</sup>	108.46 <sup>a</sup>	6.52
Ceruloplasmin, U/l	9.67 <sup>a</sup>	15.48 <sup>b</sup>	16.19 <sup>b</sup>	0.1	9.67 <sup>a</sup>	9.36 <sup>a</sup>	9.28 <sup>a</sup>	1.33
Haemoglobin, g/l	97.5 <sup>a</sup>	100 <sup>ab</sup>	102.5 <sup>b</sup>	1.62	97.5 <sup>a</sup>	98 <sup>a</sup>	101.5 <sup>a</sup>	2.82

## DISCUSSION

The addition of 10 and 20 mg of Cu/kg DM, or 40 and 80 mg of Zn/kg DM to the control diet did not improve cows performance or change milk chemical composition. This suggests that the control diet was adequate in Cu and Zn to meet requirements of producing cows. Cu-Zn-SOD in supplemental 20 mg of Cu/kg DM and above 40 mg of Zn/kg DM was higher than that of other treatments ( $P<0.05$ ). This suggests that 41.5 mg of dietary copper and above 90.15 mg of dietary zinc could improve the activity of superoxide dismutase in milk. Holstein×Simmental calves fed a control diet (35 mg of Zn/kg) or the control supplemented with 10 mg of Zn/kg had similar Zn concentration in muscle, liver, bone, and hair at the end of a 284-day study (Kessler et al., 2003). With the exception of rib cartilage and rumen, tissue Zn concentration also were similar in lactating dairy cows fed diets containing 16.6 or 39.5 mg of Zn/kg for 42 days (Neathery et al., 1973). Tissue Zn concentration are well controlled by homeostatic changes in absorption and faecal endogenous excretion of Zn. Supplementation with high concentration (500 mg/kg) of Zn in the present study resulted in elevated concentration of Zn in plasma, liver, kidney, and bone shaft (Kincaid et al., 1997).

The concentration of copper, serum ceruloplasmin, haemoglobin and the activity of Cu-Zn-SOD in blood were raised up ( $P<0.01$ ), as the copper level was raised up, but there were no effects ( $P>0.05$ ), as the zinc level was raised up. The concentration of zinc in blood was not affected ( $P>0.05$ ) by the inclusion of supplemental copper or zinc. This indicates that high level of zinc in diet blocked the absorption of copper in diet. The concentration of copper in dairy cow blood was decreased as the zinc in the diet reached 2000 mg/kg. While the zinc level is 1000 mg/kg, there was no significant affect to the concentration of copper in cow blood (Miller et al., 1989). Copper and zinc are known components of SOD, and Cu seems to regulate the enzyme's activity (Paynter et al., 1979).

## CONCLUSIONS

Supplement of dietary copper and zinc may increase Cu-Zn-superoxide dismutase in milk, but did not increase milk yield and improve chemical compositions. The concentration of copper, serum ceruloplasmin, haemoglobin and the activity of Cu-Zn-SOD in blood may be increased by the supplement of dietary copper, not the dietary zinc.

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