Journal of Animal and Veterinary Advances 12 (11): 1048-1054, 2013
ISSN: 1680-5593


# Effects of Chelated Zinc or Copper on Ruminal Fermentation Characteristics and Milk Production in Lactating Holstein Cows

[1]K.J. Jung, [1]Y.H. Ko, [1]G.S. Bae, [2]E.J. Kim, [3]S.S. Lee, [1]I.K. Paik,
[1]D.Y. Kil, [4]J.S. Chang, [1]C.H. Kim, [4]J.Y. Song and [1]M.B. Chang
[1]Department of Animal Science and Technology, School of Bioresource and Bioscience,
College of Natural Sciences, Chung-Ang University, 456-756 Anseong, Korea
[2]Department of Animal Science, Kyungpook National University, 742-711 Sangju, Republic of Korea
[3]Department of Animal Science, Sunchon National University, 540-742 Sunchon, Republic of Korea
[4]Department of Agricultural Science, Korea National Open University,
110-791 Seoul, Republic of Korea

**Abstract:** This study was conducted to examine the effects of supplemental Zn or Cu chelated with methionine or sulfate on ruminal fermentation, milk yield, milk composition and Immunoglobluin G (IgG) concentration in the blood of lactating Holstein cows. In this experiment, five Holstein dairy cows with permanent ruminal cannulae were subjected to five dietary treatments comprising either sulfate or methionine chelated Zn or Cu in a Total Mixed Ration (TMR) in a 5×5 Latin Square experimental design. The five dietary treatments were control, 40 ppm Dry Matter Intake (DMI)$^{-1}$ of Cu-Sulfate (CuS), 20 ppm DMI$^{-1}$ of Cu-Methionine (CuMet) and 200 ppm DMI$^{-1}$ of Zn-Sulfate (ZnS) and finally 100 ppm DMI$^{-1}$ of Zn-Methionine (ZnMet) chelate. Ruminal fermentation patterns including pH, ammonia-N and VFA post-feeding were significantly altered when animals were supplemented with chelated minerals relative to the control ($p<0.05$). In terms of animal performance, DMI (averaging 25.7 kg day$^{-1}$) did not differ among the dietary treatments whereas milk yield increased ($p<0.05$) upon supplementation of chelated trace minerals compared to control and was the highest in cows fed ZnMet. Somatic cell counts were much lower ($p<0.05$) in animals supplemented with chelated Zn than chelated Cu. The immune response as determined by the plasma IgG concentration of increased ($p<0.05$) in all cows supplemented with chelated Zn or Cu compared to control. The results of the study demonstrate that chelated trace mineral complexes are of use to improve animal performance and immune responses at lower or similar levels in comparison to non-chelated minerals although, further investigations are necessary to identify the modes of action and intestinal absorption rate.

**Key words:** Chelates, copper, zinc, dairy cow, rumen

## INTRODUCTION

Although, trace minerals are required in very small amounts they are an absolute necessity for farm animals. In many feeding practices, ruminants are supplemented with inorganic salts in several forms in order to improve meat and milk production (Spears, 1996). Of these trace minerals, Zn is essential for cow lactation as it is a component of bone, skin, hair, insulin as well as many metalloenzymes which are involved in protein and carbohydrate metabolism and functions to improve digestion. Zn also plays a role as a cofactor for many proteins and enzymes in the immune system, stimulating wound healing, reduction of body cell number in milk (Kellogg *et al*., 1989, 2004) and recovery from foot-rot. Cu is also an important component of metabolic enzymes for the formation of myelin of bone marrow, cell respiration, heart function, bon formation, connective tissue development, keratinization of tissues and pigmentation (McDowell, 1992). Cu is involved in Fe metabolism, hemoglobin synthesis (Kirchgessner and Grassmann, 1970) and it has been reported to affect growth stimulation (McDowell, 1992). The National Research Council (NRC) recommends optimum concentrations of Cu and Zn of 20 and 100 ppm in dairy cow, respectively (NRC, 2001). However, the different forms of the minerals often result in variable efficiency of availability to the animal.

Short peptides and amino acids can form chelate complexes which are the most efficient as mediators in living organisms (Kratzer and Vohra, 1986). Sufficient

**Corresponding Author:** Moon Baek Chang, Department of Animal Science and Technology, School of Bioresource and Bioscience, College of Natural Sciences, Chung-Ang University, 456-756 Anseong, Republic of Korea

chelation of Cu and Zn in animal feeds can reduce both inefficiency of nutrient absorption as well as soil pollution derived from Cu and Zn accumulation by using animal manure for a fertilizer (Paik, 2001). Cu and Zn based chelated forms of methionine (CuMet and ZnMet, respectively) and sulfate (CuS, ZnS) can be considered to reduce the total input of minerals in feeds. Therefore, the objective of this study was to examine the effects of chelated minerals either ZnMet or CuMet as well as additional CuS or ZnS on the characteristics of ruminal fermentation, milk yield and immune function in Holstein dairy cows.

## MATERIALS AND METHODS

The present experiment complied with the Korea Ministry for Health, Welfare and Family Affairs Law No. (August 3, 2008), Act No. 726, concerning the care of experimental animals.

**Preparation of mineral supplements (Cu-methionine chelate):** The 80 g of D, L-methionine and 66.94 g of $CuSO_4 \cdot 5H_2O$ were completely dissolved in 1 and 0.5 L of distilled water, respectively at 65±5°C. These two solutions were mixed at a molar ratio of 2:1 for a reaction to which 50% NaOH solution was gradually added in order to increase the pH up to 7 for maximum precipitation. The precipitate was separated, dried in an oven at 50°C for 2 days and then made into a powder which was subsequently tested to confirm that it was approximately 16% Cu by analysis. The product was dissolved in distilled water and tested for chelation. Tests with Cu-specific electrodes (Model 720A, Orion Research Inc. USA) showed that approximately 25% of the Cu was ionized, whereas the remaining undissociated Cu (approx. 75%) was regarded as chelated (Lim and Paik, 2003).

**Zn-methionine chelate:** The 80 g of D, L-methionine and 77.08 g of $ZnSO_4 \cdot 5H_2O$ were completely dissolved in 1 L of distilled water at 65±5°C. These two compounds were mixed at a molar ratio of 2:1 for a reaction to which a 50% NaOH solution was gradually added to increase the pH up to 10 for maximum precipitation. The precipitate was treated by the same procedure as above. The product contained approximately 17% Zn (Lim and Paik, 2003). CuS and ZnS were commercially available chemicals (www.dslab.co.kr) and were supplemented to the diet of experimental animals at appropriate levels.

**Experimental animal, design and sampling procedure:** Five clinically healthy, lactating (24±2 kg milk yield $day^{-1}$) Holstein cows (593±24 kg body weight) were used in a 5×5 Latin Square design experiment. The cows were surgically fitted with ruminal cannulae (#1C, Bar Diamond Parma, ID, USA) and were offered one of five dietary treatments: control (20 ppm Cu and 100 ppm Zn); CuS (control plus additional 20 ppm CuS); CuMet (same level as control for Cu chelated with methionine); ZnS (control plus additional 100 ppm ZnS and ZnMet (same level as control for Zn chelated with methionine).

Therefore, the cows supplemented with CuS and ZnS consumed double the amount of each mineral relative to the control whereas the chelated minerals were supplemented at the same levels in comparison with the control. Experimental minerals were top dressed on Total Mixed Ration (TMR) *ad libitum* and the feed compositions of the basal diets including the control are presented in Table 1. The experimental period comprised 15 days adaptation followed by 3 days for sampling.

Table 1: Ingredients and chemical composition of Total Mixed Ration (TMR) fed to lactating Holstein cows throughout the experimental period (% of total unless otherwise stated)

| | Treatments | | | | |
|---|---|---|---|---|---|
| Items | Control | CuS | CuMet | ZnS | ZnMet |
| **Ingredients** | | | | | |
| Alfalfa (hay bale) | 6.21 | 6.21 | 6.21 | 6.21 | 6.21 |
| Tall fescue (straw) | 9.31 | 9.31 | 9.31 | 9.31 | 9.31 |
| Klein grass (hay) | 6.21 | 6.21 | 6.21 | 6.21 | 6.21 |
| Oats (hay) | 7.76 | 7.76 | 7.76 | 7.76 | 7.76 |
| Beet pulp | 3.10 | 3.10 | 3.10 | 3.10 | 3.10 |
| Whole cotton seed | 3.10 | 3.10 | 3.10 | 3.10 | 3.10 |
| $CaCO_3$ | 6.55 | 6.55 | 6.55 | 6.55 | 6.55 |
| $NaHCO_3$ | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Corn (mash) | 5.90 | 5.90 | 5.90 | 5.90 | 5.90 |
| Corn silage | 8.45 | 8.45 | 8.45 | 8.45 | 8.45 |
| Concentrate mix[1] | 33.39 | 33.39 | 33.39 | 33.39 | 33.39 |
| Cu (ppm) | 20.00 | 20.00 | - | 20.00 | 20.00 |
| Zn (ppm) | 100.00 | 100.00 | 100.00 | 100.00 | - |
| Chelate form of Cu, Zn | | | | | |
| CuS (ppm) | - | 20.00 | - | - | - |
| CuMet (ppm) | | - | 20.00 | - | - |
| ZnS (ppm) | - | - | - | 100.00 | |
| ZnMet (ppm) | - | - | - | - | 100.00 |
| **Chemical composition** | | | | | |
| Dry matter | | | | | 76.81 |
| Crude Protein (CP) | | | | | 14.60 |
| Undegradable Protein (%, CP) | | | | | 35.70 |
| Degradable Protein (%, CP) | | | | | 64.30 |
| Soluble Protein (%, CP) | | | | | 30.30 |
| Ether extract | | | | | 4.21 |
| Crude fiber | | | | | 17.09 |
| Neutral-Detergent Fiber (NDF) | | | | | 40.98 |
| Acid-Detergent Fiber (ADF) | | | | | 25.88 |
| Effective NDF (%, NDF) | | | | | 73.30 |
| Total digestible nutrients | | | | | 70.00 |
| NEl (Mcal) | | | | | 37.96 |

[1]Concentrate mix contained, 11.5% ground corn, 10.2% dried distiller's grains with solubles, 8.8% corn gluten feed, 7.1% corn germ meal, 7.0% palm kernel meal, 6.2% wheat bran, 6.2% rapeseed meal, 6.2% wheat flour, 5.3% wheat, 3.8% soybean meal (44% CP), 3.7% coconut meal, 2.3% full fat soya, 2.7% perilla meal, 0.4% bypass protein, 7.3% vitamin and mineral mixture

The cows were kept in an individual pen made of steel throughout the experimental period. Ruminal contents were sampled from cannulae at the time points 0, 2, 4, 6, 8, 10 and 12 h post-feeding for 3 days and then pooled for analysis. Feed consumption was recorded daily during the experimental period in which cows were fed a diet of TMR for 15 kg of Dry Matter (DM) twice daily at 08:00 and 18:00. Feed refusals were measured the next morning. Cows had free access to fresh water. Milk yield was measured using a milk meter (TRU-TEST, USA) twice a day at 05:00 am and 05:00 pm for 3 days. On the last day of sampling, blood was taken from the jugular vein into a vacutainer tube without heparin.

**Chemical analysis:** Total feed DM was determined by oven-drying at 60°C for 72 h to a constant weight by the AOAC (1990) Method. Neutral-Detergent Fiber (NDF) and Acid-Detergent Fiber (ADF) were determined by using the Fibertech System (FOSS 2010, FOSS, Denmark) according to Georing and Van Soest (1970). Crude protein was determined by the Kjeltec System (FOSS 2300, FOSS, Denmark) by the AOAC (1990) Method. Ether extract was determined by Soxtec (FOSS 2050, FOSS, Denmark) by the AOAC (1990) Method. Ruminal contents at different sampling time points were strained through cheesecloth into a flask. The pH of the rumen fluid was measured and immediately centrifuged at 3,000×g for 15 min at 4°C to remove the feed particles, after which the supernatant was kept for further analysis at -60°C. The ammonia-nitrogen ($NH_3$-N) concentration of the supernatant was measured according to the method of Chaney and Marbach (1962). Microbial protein synthesis was determined by the method of Lowry *et al.* (1951). The concentration of Volatile Fatty Acids (VFA) was measured by gas chromatography (Chrompack CP9002, Netherlands) according to the method of Erwin *et al.* (1961). In terms of milk analysis, 50 mL of milk samples was used to determine the milk composition by Milko-Scan (FOSS-4000, Foss Electric, Denmark). Serum was separated to determine the concentrations of IgG and proteins by using the Radial Immunodiffusion test (Mancini *et al.*, 1965).

**Statistical analysis:** Results of ruminal fermentation characteristics, milk composition and immune function indices were subjected to Analysis of Variance (ANOVA) using PROC GLM procedures, SAS Program Package (SAS, 2000) of a 5×5 Latin Square design. Results of the rumen fermentation characteristics were analyzed using PROC Mixed to account for repeated measures. Further comparisons between the means were verified at the level of 5% by Duncan's multiple range test.

## RESULTS AND DISCUSSION

**Rumen fermentation characteristics:** Neither abnormality nor toxicity of Cu and Zn was observed in cows throughout the experiment. Ruminal fermentation characteristics of lactating cows supplemented with chelated forms of Cu (20 or 40 ppm) and Zn (100 or 200 ppm) with sulfate or methionine in the TMR are presented in Fig. 1 and 2 as well as in Table 2.

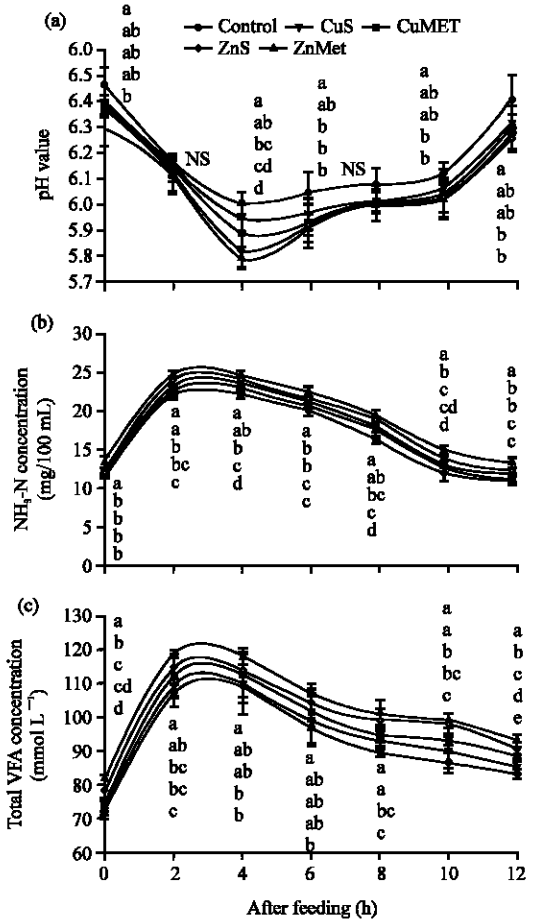


Fig. 1: Effects of supplementary CuS, CuMet, ZnS and ZnMet on ruminal pH, $NH_3$-N and total VFA concentrations of lactating Holstein cows (Control = 20 ppm Cu and 100 ppm Zn; CuS = Control plus additional 20 ppm CuS; CuMet = Same level as control for Cu chelated with methionine; ZnS = Control plus additional 100 ppm ZnS; ZnMet = Same level as the control for Zn chelated with methionine). Lowercase letters above or below the symbols indicate statistical significance and mean values with different letters are significantly different ($p<0.05$)

Table 2: Effects of chelated CuS, CuMet, ZnS and ZnMet on feed intake, milk yield and milk composition of lactating Holstein cows

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Items | Control | CuS | CuMet | ZnS | ZnMet | SEM[1] |
| Dry matter intake (kg day$^{-1}$) | 25.40 | 25.90 | 25.70 | 25.90 | 25.70 | 0.580 |
| Crud protein intake (kg day$^{-1}$) | 3.70 | 3.78 | 3.75 | 3.79 | 3.76 | 0.084 |
| Milk yield (kg day$^{-1}$) | 27.20$^{d}$ | 28.00$^{c}$ | 28.50$^{bc}$ | 28.80$^{ab}$ | 29.20$^{a}$ | 0.390 |
| Milk fat (%) | 4.10 | 4.11 | 4.12 | 4.11 | 4.13 | 0.017 |
| 4% fat corrected milk (kg day$^{-1}$) | 27.90$^{d}$ | 28.50$^{c}$ | 28.90$^{b}$ | 29.20$^{b}$ | 29.80$^{a}$ | 0.489 |
| Milk protein (%) | 3.31$^{c}$ | 3.35$^{b}$ | 3.38$^{ab}$ | 3.40$^{a}$ | 3.40$^{a}$ | 0.019 |
| Somatic cell count (×10$^{3}$) | 122.00$^{a}$ | 121.00$^{a}$ | 122.00$^{a}$ | 119.00$^{b}$ | 118.00$^{b}$ | 1.300 |

[1]Standard error of the mean. Control = 20 ppm Cu and 100 ppm Zn; CuS = control plus additional 20 ppm CuS; CuMet = Same level as control for Cu chelated with methionine; ZnS = Control plus additional 100 ppm ZnS; ZnMet = Same level as control for Zn chelated with methionine; ,mean values with different letters are significantly different ($p<0.05$)

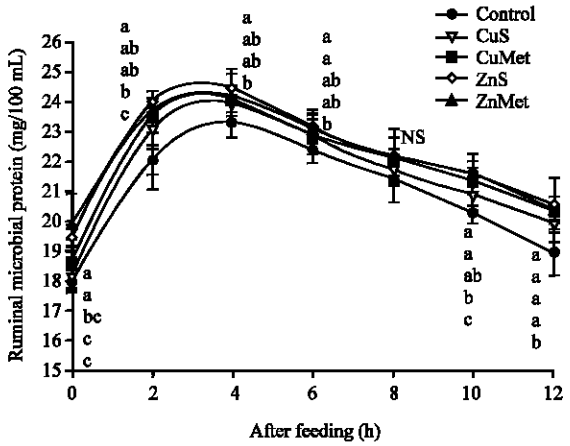


Fig. 2: Effects of supplementary CuS, CuMet, ZnS and ZnMet on ruminal microbial protein concentration of lactating Holstein cows (Control = 20 ppm Cu and 100 ppm Zn; CuS = Control plus additional 20 ppm CuS; CuMet = Same level as control for Cu chelated with methionine; ZnS = Control plus additional 100 ppm ZnS; ZnMet = Same level as control for Zn chelated with methionine). Lowercase letters above or below the symbols indicate statistical significance and mean values with different letters are significantly different ($p<0.05$)

During the adaptation period and sampling period, the pH value was maintained in the range from 5.8-6.5 for ruminal fermentation. The pH value rapidly decreased upon all treatments at 4 h post-feeding; specifically the pH value of cows supplemented with chelated minerals was significantly lower ($p<0.05$) than that of the control. The $NH_3$-N concentration increased upon all treatments at 2 h post-feeding; especially, the concentration of $NH_3$-N in cows with chelated minerals was consistently higher ($p<0.05$) than that of the control. These results are in agreement with previous reports, suggesting that supplementation of diets with additional or perhaps chelated Zn or Cu inhibited ureolysis which in turn reduces the accumulation of $NH_3$-N in the rumen (Rodriguez *et al*., 1995; Arelovich, 1998). Interestingly these two previous studies did not use chelated minerals while the present study provided minerals in chelated form. The results of the present study may indicate improved efficiency of nitrogen metabolism in the rumen of cows supplemented with chelated minerals.

The concentrations of total VFA increased, peaking at 3 h post-feeding and then gradually decreasing until the next feeding (Fig. 1). The diets supplemented with chelated minerals all showed higher ($p<0.05$) total VFA concentrations. The changes in the concentration of total VFA between the control and the diets with chelated minerals indicate that chelated Zn or Cu had marginal effects on ruminal VFA patterns. The effects of the chelated minerals on ruminal fermentation in relation to VFA metabolism have not been previously elucidated in the literature. However, it is speculated that Zn acts as an ionophore with antibiotic function that inhibits the growth of Gram-negative and hydrogen-producing microorganisms in the rumen (Van Nevel and Demeyer, 1988). Further Zn in the rumen is known to affect the transport of $K^+$ and $Na^+$ across the membranes of microbes in the rumen which consequently may change the ecology of the ruminal microflora and alter the VFA concentration (Froetschel *et al*., 1990; Arelovich *et al*., 2000).

Mean values of Microbial Protein Synthesis (MPS) in the rumens of cows fed control, CuS, CuMet, ZnS and ZnMet supplemented diets were 20.88, 21.49, 21.82, 21.97 and 21.52 mg 100 mL$^{-1}$, respectively (Fig. 2). Throughout the day, the MPS of animals treated with chelated minerals did not differ whereas animals fed control diet showed reduced ($p<0.05$) MPS than the rest of the dietary treatments. This is somewhat different from a previous study in which MPS in the rumen decreased when ZnS and Zn-salts were fed to steers (Froetschel *et al*., 1990). Nevertheless with other rumen parameters obtained, it seems logical to conclude that the chelated forms of Zn and Cu had improved fermentation characteristics in the rumen.

**Animal performance:** Dry Matter Intake (DMI) of cows fed control, CuS, CuMet, ZnS and ZnMet supplemented

diets did not differ among the dietary treatments, averaging 25.7 kg day$^{-1}$ (Table 2). Milk yield of lactating dairy cows treated with chelated minerals significantly increased ($p<0.05$) relative to that of the control and the diet containing ZnMet showed the highest milk yield among the dietary treatments (29.2 kg day$^{-1}$, $p<0.05$). The 4% Fat-Corrected Milk (FCM) production in animals fed diets with chelated minerals was higher ($p<0.05$) than that of the control and the diet containing ZnMet produced about 1.9 kg day$^{-1}$ more milk than the control ($p<0.05$). Milk protein content was significantly higher ($p<0.05$) in animals supplemented with chelated minerals than that of the control.

The results on animal performance in relation to milk production were in agreement with previous studies that similarly reported increased milk yield of lactating dairy cows supplemented with chelated Cu or Zn (Kincaid *et al*., 1976; Kellogg *et al*., 1989; Spears, 1996). With regards to materials for chelation, essential amino acids such as methionine are often used so that degradation during ruminal fermentation does not occur (Kincaid and Cronrath, 1993) which increases the absorption of Zn and/or Cu in the small intestine. In another study, supplementing diets of dairy cows with chelated Zn-methionine increased milk yield (Kellogg *et al*., 2004). Additionally, methionine is known to be a limiting amino acid for milk production and results using ZnMet showed reduced degradation during ruminal fermentation which improves the methionine available for absorption in the small intestine (Casper and Schingoethe, 1988; Pruekvimolphan and Grummer, 2001). In this study, improved availability of a limiting amino acid using chelated Cu or Zn appeared to improve milk yield as well as milk protein composition relative to the control. However, milk fat was not significantly affected by the chelated minerals which supports the results of a previous study (Kellogg *et al*., 2004). In addition, there were little differences between sulfate and chelated materials in terms of milk production and composition (Table 2) which indicate that both sulfate with additional Cu or Zn and methionine were effective.

The number of somatic cells in the milk from cows fed ZnS and ZnMet-supplemented diets were significantly decreased ($p<0.05$) whereas cows fed diets with Cu-chelates were not affected compared with the control (Table 2). Zn and Cu complexes containing either amino acids or polysaccharide can improve disease resistance as improved absorption of Cu and Zn stimulate T cell development and immune responses (Kukral *et al*., 1988; Chirase *et al*., 1991; Prasad and Kundu, 1995; Tran *et al*., 2004). Kincaid *et al*. (1976) reported that supplementation of Zn-methionine complexes to the diet of lactating dairy cows has beneficial effects in terms of milk production and SCC compared to oxidized or sulfate supplements. Cu chelates also promote Cu absorption into the blood, resulting in stimulation of cerulplamin, an immune-reinforcing element and reduction of SCC (Engle *et al*., 2001; Middleton *et al*., 2004). However, the Cu and Zn-chelates showed different results in this study.

Table 3: Effects of supplementary CuS, CuMet, ZnS and ZnMet on plasma IgG and protein concentration in the blood of lactating Holstein cows

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Items | Control | CuS | CuMet | ZnS | ZnMet | SEM[1] |
| Plasma IgG (mg mL$^{-1}$) | 23.42$^{c}$ | 25.10$^{b}$ | 25.17$^{b}$ | 27.10$^{ab}$ | 27.60$^{a}$ | 0.945 |
| Plasma protein (mg mL$^{-1}$) | 39.20$^{c}$ | 41.15$^{b}$ | 41.72$^{b}$ | 42.81$^{a}$ | 43.59$^{a}$ | 0.604 |

[1]Standard error of the mean. Control = 20 ppm Cu and 100 ppm Zn; CuS = Control plus additional 20 ppm CuS; CuMet = Same level as control for Cu chelated with methionine; ZnS = Control plus additional 100 ppm ZnS; ZnMet = Same level as control for Zn chelated with methionine; mean values with different letters are significantly different ($p<0.05$)

**Immune function response:** The immune response of cows fed either chelated or sulfate minerals as determined by plasma IgG levels in the blood appeared to be better ($p<0.05$) than that of the control (Table 3). In a study by Uchida *et al*. (2001) there were no changes in milk production of lactating dairy cows with Cu at 0, 10 and 40 mg kg$^{-1}$ DM as well as no differences in all measured parameters, except for the plasma IgG concentration and milk content. On the other hand, a study by Hempe and Cousins (1989) reported the adverse effect of Zn-methionine on Zn absorption rate in rat. Further research is needed to elucidate the mode of action in particular on the intestinal absorption rate of trace mineral complexes containing amino acids. Nevertheless, several reports showed an improved immune response or disease resistance in relation to trace mineral chelates in animals.

## CONCLUSION

Researchers suggested a simple method for amino acid chelates or sulfur complexes containing Cu or Zn that considers the effects on rumen fermentation characteristics and animal performance in lactating dairy cows. Diets containing chelated minerals provided animals with improved ruminal environments and produced more milk compared to cows fed the control diet, even at lower consumption of the same minerals. Further investigation of the absorption efficiency of the chelates in the intestine can provide economic insights into the optimum dose of these supplements.

## ACKNOWLEDGEMENT

Researchers are grateful to the farm staff for the care of the experimental animals. This study was supported by the Chung-Ang University Excellent Student Scholarship as well as by the Bio-Industry Technology Development Program (No. 20121011), Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

## REFERENCES

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Arelovich, H.M., 1998. Effects of zinc and manganese on digestion, ruminal and blood parameters of cattle fed prairie hay. Ph.D. Thesis, Oklahoma State University, Stillwater, OK., USA.

Arelovich, H.M., F.N. Owens, G.W. Horn and J.A. Vizcarra, 2000. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake and digestion by cattle fed prairie hay and urea. J. Anim. Sci., 78: 2972-2979.

Casper, D.P. and D.J. Schingoethe, 1988. Protected methionine supplementation to a barley-based diet for cows during early lactation. J. Dairy Sci., 71: 164-172.

Chaney, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. Clin. Chem., 8: 130-132.

Chirase, N.K., D.P. Hutcheson and G.B. Thompson, 1991. Feed intake, rectal temperature and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci., 69: 4137-4145.

Engle, T.E., V. Fellner and J.W. Spears, 2001. Copper status, serum cholesterol and milk fatty acid profile in holstein cows fed varying concentrations of copper. J. Dairy Sci., 84: 2308-2313.

Erwin, E.S., G.J. Marco and E.M. Emery, 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44: 1768-1771.

Froetschel, M.A., A.C. Martin, H.E. Amos and J.J. Evans, 1990. Effects of zinc sulfate concentration and feeding frequency on ruminal protozoal numbers, fermentation patterns and amino acid passage in steers. J. Anim. Sci., 68: 2874-2884.

Georing, H.K. and P.J. Van Soest, 1970. Forage Fiber Analysis, Agriculture Handbook No. 379. Agriculture Research Service, USDA, Washington, DC., USA.

Hempe, J.M. and R.J. Cousins, 1989. Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. J. Nutr., 119: 1179-1187.

Kellogg, D.W., D.J. Tomlinson, M.T. Socha and A.B. Johnson, 2004. Review: Effects of zinc methionine complex on milk production and somatic cell count of dairy cows: Twelve-trial summary. Professional Anim. Sci., 20: 295-301.

Kellogg, D.W., J.M. Rakes and D.W. Gliedt, 1989. Effect of zinc methionine supplementation on performance and selected blood parameters of lactating dairy cows. Nutr. Rep. Int., 40: 1049-1057.

Kincaid, R.L. and J.D. Cronrath, 1993. Effects of added dietary fat and amino acids on performance of lactating cows. J. Dairy Sci., 76: 1601-1606.

Kincaid, R.L., W.J. Miller, P.R. Fowler, R.P. Gentry, D.L. Hampton and M.W. Neathery, 1976. Effect of high dietary zinc upon zinc metabolism and intracellular distribution in cows and calves. J. Dairy Sci., 59: 1580-1584.

Kirchgessner, M. and E. Grassmann, 1970. The Dynamics of Copper Absorption. In: Trace Element Metabolism in Animals: Proceedings of WAAP/IBP International Symposium, Mills, C.F. (Ed.). Vol. 1, Livingston, Edinburgh, pp: 277-287.

Kratzer, F.H. and P. Vohra, 1986. Chelates in Nutrition. CRC Press, Boca Raton, Florida, ISBN: 9780849365447, Pages: 169.

Kukral, H.W., M. Kirchgessner and H.P. Roth, 1988. [Effect of marginal zinc deficiency on humoral immunity in rats]. Z. Ernahrungswiss., 27: 126-136.

Lim, H.S. and I.K. Paik, 2003. Effects of supplementary mineral methionine chelates (Zn, Cu, Mn) on the performance and eggshell quality of laying hens. Asian-Aust. J. Anim. Sci., 16: 1804-1808.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mancini, G., A.O. Carbonara and J.F. Heremans, 1965. Immunochemical quantitation of antigens by single radialimmunodiffusion. Immunochemistry, 2: 235-254.

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press, San Diego, CA., USA.

Middleton, J.R., C.D. Luby, L. Viera, J.W. Tyler and S. Casteel, 2004. Short communication: Influence of *Staphylococcus aureus* intramammary infection on serum copper, zinc and iron concentrations. J. Dairy Sci., 87: 976-979.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academy Press, Washington, DC.

Paik, I.K., 2001. Management of excretion of phosphorus, nitrogen and pharmacological level minerals to reduce environmental pollution from animal production: Review. Asian-Australas. J. Anim. Sci., 14: 384-394.

Prasad, T. and M.S. Kundu, 1995. Serum IgG and IgM responses to sheep red blood cells (SRBC) in weaned calves fed milk supplemented with Zn and CU. Nutrition, 11: 712-715.

Pruekvimolphan, S. and R.R. Grummer, 2001. Lactation responses to sulfur-containing amino acids from feather meal or rumen-protected methionine. J. Dairy Sci., 84: 2515-2522.

Rodriguez, B.T., H.M. Arelovich, J.J. Villalba and H.E. Laborde, 1995. Dietary supplementation with zinc and manganese improves the efficiency of nitrogen utilization by lambs. J. Anim. Sci., 73: 277-277.

SAS, 2000. User's Guide: Statistics. 8th Edn., SAS Institute Inc., Carry, NC., USA.

Spears, J.W., 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol., 58: 151-163.

Tran, C.D., L.V. Miller, N.F. Krebs, S. Lei and K.M. Hambidge, 2004. Zinc absorption as a function of the dose of zinc sulfate in aqueous solution. Am. J. Clin. Nutr., 80: 1570-1573.

Uchida, K., P. Mandebvu, C.S. Ballard, C.J. Sniffen and M.P. Carter, 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Anim. Feed Sci. Technol., 93: 193-203.

Van Nevel, C.J. and D.I. Demeyer, 1988. Manipulation of Rumen Fermentation. In: The Rumen Microbial Ecosystem, Hobson, P.N. (Ed.). Elsevier Applied Science, New York, London, pp: 387-443.