Effects of Supplementary Mineral Methionine Chelates (Zn, Cu, Mn) on the Performance and Eggshell Quality of Laying Hens

H. S. Lim and I. K. Paik*

Department of Animal Science, Chung-Ang University, Ansung-si, Kyonggi-do 456-756, Korea

ABSTRACT : A layer experiment was conducted to determine the effects of supplementary methionine chelates (Cu, Zn and Mn), individual or in combination, on laying performance, eggshell quality, gizzard erosion, and IgG level of serum for 8 weeks. Five hundred 96-wk-old force molted ISA Brown layers were assigned to five dietary treatments. Basal diet was formulated to meet or exceed the nutrients requirements listed in NRC (1994). Five experimental diets were control, Zn-methionine chelate (Zn-Met) supplemented, Cu-methionine chelate (Cu-Met) supplemented, Zn-Mn-methionine chelate (Zn-Mn-Met) supplemented and Zn-Mn-Cu-Met supplemented diet. Each treated diet was supplemented with respective mineral(s) at the level of 100 ppm in the form of methionine chelate. Egg production was increased by Cu-Met supplementation but decreased by Zn-Met supplementation. Egg weight was significantly (p<0.05) lower in Cu-Met treatment than those of the control and Zn-Met treatment. Specific gravity of eggs and eggshell strength were highest and soft egg production was lowest in Cu-Met treatment. Gizzard erosion index was significantly increased by supplementation of Cu-Met, Zn-Mn-Met or Zn-Mn-Cu-Met. Zinc content in liver significantly increased by Zn-Met, but not by Zn-Mn-Cu-Met treatment. In conclusion, 100 ppm Cu in Cu-Met chelate improved laying performance and eggshell quality but also increased gizzard erosion index. Supplementation of Zn-Met or its combination with other mineral chelates had no beneficial effects on laying performance and eggshell quality. *(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1804-1808)*

Key Words : Egg Production, Eggshell Quality, Chelates, Cu-methionine, Zn-methionine, Mn-methionine

INTRODUCTION

Copper, zinc and manganese are essential elements required by poultry. Copper is a component of various intracellular and extracellular enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998). Cu supplementation at the level of 125-250 ppm improved growth rate and feed conversion ratio in broilers (Baker et al., 1991; Paik, 2001) and pigs (Roof and Mahan, 1982; Cromwell et al., 1989; Paik, 2001).

Zinc is a component of metalloenzymes such as carbonic anhydrase, alcohol dehydrogenases, carboxy peptidase, alkaline phosphatase, thimidine kinase, RNA and DNA polymerase. It also involved in metabolism of protein and carbohydrate. Zn supplementation of 3,000-5,000 ppm from ZnO increased weight and feed intake in weanling pigs (Hahn and Baker, 1993; Carlson et al., 1995; Lemieux et al., 1995; Smith et al., 1995) and Harbaugh and Sanford (1970) reported hen-housed egg production was increased by optimal levels of zinc methionine supplemented to layer diet.

Manganese is an important activator of many enzymes and is also a component of arginase, pyruvate carboxylase, and manganese superoxide dismutase (Klasing, 1998). Lima et al. (2000) reported Mn supplementation of 40 ppm increased eggshell quality.

Recent information suggests that chelated or complexed

trace elements may improve bioavailability of minerals for animals. These metal-amino acid chelates or complexes furnish trace elements that are more efficiently absorbed from gut than those provided by inorganic salts (Wedekind et al., 1992; Aoyagi and Baker, 1993a). They also provide readily bioavailable amino acids (Aoyagi and Baker, 1993b).

Present experiment was conducted to determine effects of supplementary Cu-methionine (Cu-Met), Zn-methionine (Zn-Met), Mn-methionine (Mn-Met) chelates and their combination on laying performance and eggshell quality.

MATERIALS AND METHODS

Preparation of chelates

Cu methionine chelate : Eighty grams of D.L-methionine and 66.94 g CuSO$_4$·5H$_2$O were completely dissolved in 1 l and 500 ml of distilled water, respectively, at 65±5°C. These two solutions were mixed at a molar ratio of 2:1 for a reaction to occur to which 50% NaOH solution was gradually added to increase pH up to 7 for maximum precipitation. Precipitate was separated, dried in an oven at 50°C for 2 days and then made to powder which was subsequently tested to confirm approximately 16% Cu by analysis. The product was dissolved in distilled water and tested for chelation. Test with copper specific electrode (Model 720A, Orion Research Inc. 529 Main St., Boston, MA 02129 USA) showed app. 25% of Cu was ionized and the remaining undissociated Cu (app. 75%) was regarded as chelated (Kim et al., 1997).

Zn and Mn methionine chelates : Eighty grams of D.L-

Table 1. Formula and composition of basal diet

Formula and composition	Amount
Ingredients	-------------- % ------------------
Corn	65.07
Soybean meal (44% CP)	20.00
Limestone	9.10
Wheat bran	2.89
Animal fat	1.50
Tricalcium phosphate (18%)	0.75
Salt	0.32
Layer premix[1]	0.17
DL-Methionine (98%)	0.12
Lysine-HCl (78%)	0.08
Total	100.00
Calculated composition	
ME, kcal/kg	2,800.0
Crude protein, %	15.0
Arginine, %	0.82
Lysine, %	0.70
Methionine, %	0.37
Met & Cystine, %	0.55
Calcium, %	3.80
Non-Phytate P, %	0.25
Total P, %	0.49
Salt, %	0.36
Copper, ppm	20.50

[1] Provides per kg: vitamin A, 10,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 15,000 IU; vitamin K_3, 2,000 mg; vitamin B_1, 1,500 mg; vitamin B_2, 4,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 3,000 μmg; pantothenic acid, 8,000 mg; niacin, 25,000 mg; folic acid, 500 mg; Zn, 52.5 g; Mn, 52.5 g; Fe, 52.5 g; Cu, 5.25 g; I, 1.155 g; Co, 0.315 g; Se, 0.315 g.

methionine and 77.08 g $ZnSO_4·5H_2O$ were completely dissolved in 1 l of distilled water at $65\pm5°C$. These two compounds were mixed at a molar ratio of 2:1 for a reaction to occur to which 50% NaOH solution was gradually added to increase pH up to 10 for maximum precipitation. Precipitate was treated with same procedure as above. The product contained approximately 17% Zn.

For Mn-Met preparation, 80g of D.L-methionine and 64.64g of $MnSO_4·5H_2O$ were dissolved in 1 liter of distilled water at $65\pm5°C$. The rest of the procedure is same as that of Zn-Met preparation. The product contained approximately 15% Mn.

Experimental diet

A basal diet was formulated to meet or exceed the nutrient requirements listed in NRC (1994) (Table 1). Five experimental diets were control, Zn-methionine chelate (Zn-Met) supplemented, Cu-methionine chelate (Cu-Met) supplemented, Zn-Met+Mn-methionine chelate (Zn-Mn-Met) supplemented, and Zn-Mn-Cu-Met chelate supplemented diet. Each treated diet was supplemented with respective mineral(s) at the level of 100 ppm in the form of methionine chelate.

Feeding regimen

Five hundred force molted 96-wk-old ISA brown layers were assigned to five dietary treatments. Each treatment consisted of 5 replications of 10 cages (2 birds per cage). Diets were presented in mash form, and feed and water were given *ad libitum* during the experimental period. The house for birds was provided with programmed lighting, which was 16:00 h per day.

Parameters of production performance and egg quality

The number of total eggs, broken and soft shell eggs and egg weight were determined on a daily basis. Feed consumption was measured weekly. On 6th day of each week, all eggs, except soft and broken eggs, were collected to measure egg specific gravity. Specific gravity of eggs was determined by using salt solutions made of incremental concentration of 0.005 in the range from 1.070 to 1.110. After measuring specific gravity, eggshell strength, eggshell thickness and Haugh unit (HU) were measured. Eggshell strength was measured by using Compression Test Cell in Texture Test Systems (Model T2100C, Food Technology Corp., Rockville, MD). Shell thickness was a mean value of measurements of the shell at three locations (air cell, equator and sharp end) of the egg measured by dial pipe gauge (Model 7360, Mitutoyo Corp., Kawasaki, Japan). Haugh unit was calculated using the HU formula (Eisen et al., 1962) based on the height of albumen determined by a micrometer (Model S-8400, AMES, Waltham, MA) and egg weight.

IgG level in serum and mineral content in liver

After 8 wk on experimental diets, fifteen birds per treatment (three hens from each replication) were killed by cervical dislocation, and blood samples were collected by heart puncture. After blood sampling, liver and gizzard were removed for further test. The gizzards were opened and the internal contents were removed. Samples were thoroughly cleaned in running tap water. Gizzard erosion indices were determined on the basis of a scoring system, 0 for normal, 1 for mild erosion, 2 for moderate erosion and 3 for severe erosion. Collected livers were dried, and mineral contents were measured using ICP (inductively coupled plasma) emission spectrometer (Model JY-24, Jobin Yvon, Longjumeau, Cedex, France).

Blood sample were centrifuged at 3,000 rpm for 15 minutes. Serum was removed and stored at -20°C until analysis. IgG of serum was measured by single radial immuno-diffusion test (RID test) method (Mancini et al., 1965).

Statistical analysis

Data were subjected to analysis of variance using

Table 2. Laying performance of hens fed mineral-methionine chelate diets for 8 weeks (96-103 wk of age)

	Treatments*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met+Mn-Met	Zn-Met+Mn-Met +Cu-Met	
Hen-day egg production, %	69.97[ab]	67.13[c]	72.57[a]	68.37[bc]	71.67[a]	0.9552
Hen-housed egg production, %	68.80[bc]	65.61[d]	72.57[a]	67.48[cd]	70.82[ab]	0.9974
Egg weight, g	70.27[ab]	70.77[a]	69.33[c]	69.72[bc]	70.04[abc]	0.2534
Feed intake, g/hen/day	127.94[ab]	127.31[ab]	129.18[ab]	126.87[b]	130.44[a]	1.0483
Feed conversion ratio, g/100 g egg mass	2.62[ab]	2.71[a]	2.58[b]	2.68[ab]	2.60[ab]	0.0364

* Each treated diet was supplemented with respective mineral at the level of 100 ppm in the form of methionine chelate.
[a-d] Means within each row with no common superscripts differ significantly (p<0.05).

Table 3. Egg quality of hens fed mineral-methionine chelate diets for 8 weeks (96-103 wk of age)

	Treatments*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met+Mn-Met	Zn-Met+Mn-Met +Cu-Met	
Albumin height, mm	6.305	6.259	6.375	6.296	6.382	0.0607
Haugh unit	74.96	74.55	75.46	74.63	75.77	0.5010
Specific gravity	1.0845[b]	1.0851[b]	1.0864[a]	1.0847[b]	1.0851[b]	0.0004
Eggshell strength, kg/egg	3.49[c]	3.52[bc]	3.70[a]	3.63[ab]	3.63[ab]	0.0400
Eggshell thickness, mm	0.366	0.375	0.374	0.376	0.369	0.0034
Soft egg production, %	0.594[ab]	0.778[ab]	0.311[b]	1.072[a]	0.598[ab]	0.1751
Broken egg production, %	3.808[c]	4.763[bc]	4.714[bc]	6.899[a]	5.593[ab]	0.5178

*Each treated diet was supplemented with respective mineral at the level of 100 ppm in the form of methionine chelate.
[a-c] Means within each row with no common superscripts differ significantly (p<0.05).

Table 4. Serum IgG, gizzard erosion index and mineral content in liver of hens fed mineral-methionine chelate diets for 8 weeks (96-103 wk of age)

	Treatments*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met+Mn-Met	Zn-Met+Mn-Met +Cu-Met	
IgG, mg/ml	24.3	25.5	24.5	26.2	24.0	1.7730
Gizzard erosion index	0.30[c]	0.50[bc]	1.25[a]	0.90[ab]	1.35[a]	0.1767
Mineral content in liver	------- ppm -------					
Zinc	99.50[ab]	104.53[a]	96.41[ab]	95.06[ab]	82.80[b]	6.3255
Copper	12.57	12.32	14.86	12.75	14.11	2.1827
Manganese	10.25	10.11	10.29	10.32	11.29	0.7185

* Each treated diet was supplemented with respective mineral at the level of 100ppm in the form of methionine chelate.
[a-c] Means within each row with no common superscripts differ significantly (p<0.05).

general linear model procedure (SAS Institute. 2000). Significant differences among treatment means were measured by Duncan's multiple range test at p<0.05 (Steel and Torrie. 1980).

RESULTS

All the laying performance parameters were significantly (p<0.05) different among treatments (Table 2). Cu-Met treatment showed highest hen-day and hen-housed egg production (72.57% in both) while Zn-Met treatments significantly decreased egg production (67.13% for hen-day and 65.61% for hen-housed). Compared with hen-day (69.97%) and hen-housed (68.80%) production of the control, Cu-Met treatment increased hen-day and hen-housed egg production by 2.60% (or 3.72% improvement) and 3.77% (or 5.48% improvement), respectively. Zn-Mn-Met treatment did not significantly affect egg production

but tended to decrease egg production. Combination of Cu-Met with Zn-Met and Mn-Met did not show any additive or synergistic effect. Egg weight was heaviest in Zn-Met treatment while lightest in Cu-Met treatment. Feed intake was highest in Zn-Mn-Cu-Met treatment and lowest in Zn-Mn-Met treatment. Feed conversion ratio (FCR) was best in Cu-Met treatment (2.58) and worst in Zn-Met treatment (2.71). Compared with that of the control (2.62), Cu-Met treatment improved FCR by 1.53%.

Among the egg quality parameters (Table 3). specific gravity. eggshell strength. soft egg production and broken egg production were significantly affected by treatments. Specific gravity of eggs laid in Cu-Met treatment was significantly higher than other treatments. Eggshell strength was strongest in Cu-Met treatment and weakest in the control. Soft shelled egg production was lowest in Cu-Met treatment while broken egg production was lowest in the control. Zn-Mn-Met treatment was highest in both soft

shelled egg production and broken egg production. Albumin height, Haugh unit and eggshell thickness were not significantly affected by treatments.

Table 4 shows serum IgG level, gizzard erosion index and mineral contents in the liver. Serum IgG level was not significantly affected by treatments. Gizzard erosion index was significantly higher in both Cu-Met treatment and Zn-Mn-Cu-Met treatment than those of the control and Zn-Met treatment. Zinc content in the liver was significantly affected by treatments. Zn-Met treatment showed the highest while Zn-Mn-Cu-Met treatment showed the lowest Zn content in the liver. Copper content in the liver was higher in Cu-Met treatment and Zn-Mn-Cu-Met treatment than those of others but the differences were not significant. Manganese content in the liver was not significantly different among treatments.

DISCUSSION

In the present experiment, Cu-Met chelate supplementation increased egg production by 2.60% in hen-day egg production and by 3.77% in hen-housed egg production compared with those fed control diet. Pesti and Bakalli (1998) reported the results of two layer experiments in which egg production were linearly increased as the supplementary Cu from Cu sulfate pentahydrate increased from 0 to 125 and 250 mg/kg diet. Jackson (1977) also reported that 256 mg/kg Cu supplementation increased egg production. On the other hand, two other studies reported that Cu supplementation decreased egg production in the same copper level (Pearce et al., 1983; Stevenson et al., 1983). Pesti and Bakalli (1998) explained that contrasting two results were caused by different methionine level in control diet. The best response to pharmacological levels of Cu feeding was observed with high levels of methionine supplementation (Wang et al., 1987). In the present experiment, the control diet contained 0.37% methionine including 0.122% of supplementary D.L-methionine, which is sufficient considering NRC(1994) requirement. Some extra methionine may have been supplied from chelate up to 0.02% at maximum. Thus Cu-Met supplementation could have been more effective if the above theory is applied. Some other researchers reported that egg production was depressed when copper was supplemented over 400 ppm in the form of copper sulfate (Chiou et al., 1997; Chiou et al., 1998). Effects of Zn-Mn-Met supplementation on the laying performance agree with those of Lima et al. (2000) who reported Zn and Mn methionine chelate supplementation, single or in combination, did not affect laying performance. Combination of Cu-Met with other mineral chelates tended to decrease egg production compared with Cu-Met alone. It may be due to interactions of other minerals, especially with zinc. Zinc has a strong antagonism with copper

(Ammerman et al., 1995). Other evidence of interaction between Zn and Cu was observed in mineral content of liver. Zinc level in the Zn-Mn-Cu-Met treatment was significantly lower than that of Zn-Met treatment. O'Dell et al. (1976) documented zinc-deficiency occurred when plasma, muscle and testis copper concentration were elevated above normal level in rats.

Cu-Met chelate treatments increased eggshell strength in the present experiment. It is postulated that lysyl oxidase, a copper containing enzyme which functions in stability and strength of collagen (Underwood, 1977), may have influenced the result. Collagen affects eggshell membrane and eggshell strength. Defective collagen in the shell membrane by copper-deficiency often instigates infertile and abnormal shell (Klasing, 1998). Zn is a component of carbonic anhydrase which catalyzes synthesis of bicarbonate. Solid portion of eggshell is $CaCO_3$ which is made of Ca ion (Ca^{--}) and bicarbonate (HCO_3^-). Manganese is essential for development of the organic matrix of the bone which is composed largely of mucopolysaccharides. Deficiency of manganese increases incidence of thin-shelled and shell less eggs (Scott et al., 1978). Therefore, improvement of eggshell quality was expected by supplementation of Zn-Met or combination of Zn-Met and Mn-Met (Zn-Mn-Met).

Lima et al. (2000) reported that supplementation of 40 ppm Zn-Met chelate in single or in combination with 40 ppm Mn-Met chelate increased specific gravity of egg, eggshell strength and eggshell thickness. In present experiment, Zn-Mn-Met treatment showed significantly higher eggshell strength than the control. However, eggshell strength, specific gravity of egg and eggshell thickness of Zn-Met treatment were not significantly different from the control although those parameters tended to increase in Zn-Met treatment.

Serum IgG tended to increase by supplementation of Zn-Met alone or in combination with Mn-Met. Zn is known to be involved in immune response. Ferket and Qureshi (1992) reported that combination of Zn-Met and Mn-Met chelate increased serum IgG level in male turkey. However, in the present experiment, the effect of Zn supplementation on serum IgG level was not significant.

Gizzard erosion index was increased by Cu-Met chelate supplementation. Fisher et al. (1973) reported that 600ppm Cu supplementation increased gizzard erosion in broiler. Zn-Mn-Met treatment also increased gizzard erosion, although the severity was less than Cu-Met treatment. The result may need more verification. Zn level in the liver of Zn-Met treatment tended to increase but that of Zn-Mn-Cu-Met significantly decreased indicating strong interactions among these elements especially between Cu and Zn. Cu-Met supplementation tended to increase Cu level in the liver but the effect was not statistically significant. This result

indicates that supplementation of 100 ppm Cu in the form of Cu-Met may not accumulate Cu in the liver to the extend that can cause adverse effects.

In conclusion. 100 ppm Cu in Cu-Met chelate improved laying performance and eggshell quality but also increased gizzard erosion index. Supplementation of Zn-Met or its combination with other mineral chelates had no beneficial effects on laying performance and eggshell quality.

ACKNOWLEDGMENTS

REFERENCES

Ammerman, C. B., D. A. Baker and A. J. Lewis. 1995. Bioavailabilty of nutrients for animals: amino acids, minerals, vitamins. Academic Press, INC. London.

Aoyagi, S. and D. H. Baker. 1993a. Protective effect of copper-amino acid complexes against inhibitory effects of L-cysteine and ascorbic acid. Poult. Sci. 72 (Suppl. 1):82(Abs.).

Aoyagi, S. and D. H. Baker. 1993b. Nutritional evaluation of copper-lysine and zinc-lysine complexs for chicks. Poult. Sci. 72:165-171.

Baker, D. H., J. Odle, M. A. Funk and T. M. Wieland. 1991. Bioavailability of copper in cupric oxide, cuprous, and in a copper-lysine complex. Poult. Sci. 70:177.

Carlson, M. S., G. M. Hill, J. E. Link, G. A. McCully, D. W. Rozeboom and R. L. Weavers. 1995. Impact of zinc oxide and copper sulfate supplementation on the newly weaned pig. J. Anim. Sci. 73 (Suppl. 1):72.

Chiou, P. W. S., K. L. Chen and B. Yu. 1997. Toxicity, tissue accumulation and residue in egg and excreta of copper in laying hens. Anim. Feed Sci. Technol. 67:49-60.

Chiou, P. W. S., K. L. Chen and B. Yu. 1998. Effect of dietary organic arsenicals and cupric sulfate on copper toxicity, liver accumulation and residue in eggs and excreta of laying hens. Anim. Feed Sci. Technol. 73:161-171.

Cromwell, G. L., T. S. Stahly and H. J. Mongue. 1989. Effect of source and level of copper on performance and liver copper stores in weanling pigs. J. Anim. Sci. 67:2996-3002.

Eisen, E. J., B. B. Bohren and H. E. McKean. 1962. The Haugh unit as a measure of egg albumen quality. Poult. Sci. 41:1461-1468.

Ferket, P. R. and M. A. Qureshi. 1992. Effect of manganese on the immune function of turkey toms. Poult. Sci. 71 (Supp 1):60.

Fisher, C., A. P. Lauren-Jones, K. J. Hill and W. S. Hardy. 1973. The effect of copper sulfate on performance and the structure of gizzard in broilers. Br. Poult. Sci. 14:55-68.

Hahn, J. D. and K. H. Baker. 1993. Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. J. Anim. Sci. 71:3020.

Harbaugh, D. D. and P. E. Sanford. 1970. The effect of various levels of zinc-methionie supplement on flock performance, egg size and shell quality. Poult. Sci. 49:1393 (Abs.).

Jackson, N. 1977. The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels

of the mature, laying, domestic fowl. Br. J. Nutr. 38:93-100.

Kim, D. H., J. S. UM., S. H. Ahn and I. K. Paik. 1997. Studies on the development and utilization of copper chelate as a supplementation to the pig diet. Kor. J. Nutr. Feed. 21(5):429-438.

Klasing, C. K. 1998. Minerals. pages. 234-276 in Comparative Avian Nutrition. CAB International. New York. USA

LeMieux, F. M., L. V. Ellison, T. L. Ward, L. L. Southern and T. D. Bidner. 1995. Excess dietary zinc for pigs weaned at 28 days. J. Anim. Sci. 73 (Suppl. 1):72.

Lima, F. R., I. Mabe and R. Albuquerque. 2000. Zinc and manganese additional supplementations may benefit egg production and quality. Poult. Sci. 79:46 (Abs.).

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

National Research Council. 1994. Nutrient Requirements of Poultry. 9th. rev. ed. National Academy Press, Washington DC.

O'Dell, B. L., P. G. Reeves and R. F. Morgan. 1976. Interrelationships of tissue copper and zinc concentrations in rats nutritionally deficient in one or the other of those elements. In: "Trace Substances in Environmental Health" (Ed. D. D. Hemphill), University of Missouri, Columbia, MO. p. 411.

Paik, I. K. 2001. Application of chelated mineral in animal production. Asian-Aust. J. Animal. Sci. 14, Special Issue: 191-198.

Pearce, J., N. Jackson and M. H. Stevenson. 1983. The effects of dietary intake and of dietary concentration of copper sulfate on the laying domestic fowl: Effects of some aspects of lipid, carbohydrate and amino acid metabolism. Br. Poult. Sci. 24:337-348

Pesti, G. M. and R. I. Bakalli. 1998. Studies on the feeding cupric sulfate pentahydrate to laying hens on egg cholesterol content. Poultry Sci. 77:1540-1545.

Roof, M. D. and D. C. Mahan. 1982. Effect of carbadox and various dietary copper level for weanling swine. J. Anim. Sci. 55:1109.

SAS Institute. 2000. SAS system for window V 8.01. SAS Institute Inc., Cary, NC.

Scott, M. L., M. C. Nesheim and R. J. Young. 1982. Nutrition of the Chicken(Third edition),. Ithaca, New York. p. 321.

Smith, J. W. II., M. D. Tokach, R. D. Goodband, J. L. Nelssen, Jr. Nessmith, K. Q. Owen, and B. T. Richert. 1995. The effect of increasing zinc oxide supplementation on starter pig growth performance. J. Anim. Sci. 67:835.

Steel, R. G. O., J. H. Torrie. 1980. Principle and Procedures of Statistics. 2nd ed. McGraw-Hill Publishing Co., New York, NY

Stevenson, M. H., J. Pearce and N. Jackson. 1983. The effects of dietary intake and of dietary concentration of copper sulfate on the laying domestic fowl: Effects on laying performance and tissue mineral contents. Br. Poult. Sci. 24:327-335.

Underwood, J. E. 1977. Trace Elements in Human and Animal Nutrition. Academic Press, INC. London. p. 85.

Wang, J. S., S. R. Rogers and G. M. Pesti. 1987. Influence of copper and antagonism between methionine and copper supplements to chick diets. Poult. Sci. 66:1500-1507.

Wedekind, K. J., A. E. Hortin and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.