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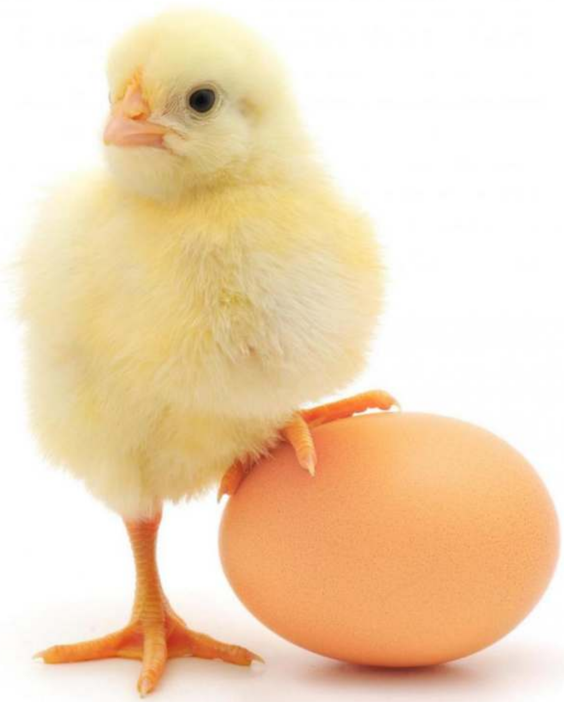

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Interactive Effects of Zinc, Copper and Manganese in Diets for Broilers[1]

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Abstract: An experiment was conducted to determine the interactive effects of Zn, Cu and Mn addition to diets of 0 to 14-d old broilers on growth performance, bone breaking strength, bone ash percentage and tissue mineral concentrations. Two levels of Zn (0 or 75 ppm, as Zn sulfate), 2 levels of Mn (0 or 100 ppm, as Mn sulfate) and 2 levels of Cu (0 or 7 ppm, as Cu sulfate) were supplemented to corn-soybean meal diets in a 2×2×2 factorial arrangement. Each treatment had 6 replications with 5 chicks each and the initial and final BW were 46 and 382 g. Daily gain, daily feed intake, gain:feed, bone breaking strength and bone and pancreatic Zn concentrations were increased ($p<0.05$) in chicks fed diets with supplemental Zn. Bone, liver and pancreatic Mn levels were increased ($p<0.01$) in chicks fed diets with supplemental Mn. Bone breaking strength was increased when Zn was added to the diets with or without Cu addition, but the increase was greater in chicks fed the diets without Cu addition (Zn×Cu, $p<0.05$). Bone ash percentage was increased in chicks fed the diets with Zn, Mn, or Cu addition but the increase was not additive (Zn×Mn×Cu, $p<0.03$). Liver Cu level was increased ($p<0.01$) in chicks fed diets with Cu addition. Bone and liver Cu levels were decreased ($p<0.05$) in chicks fed diets with Zn addition. Bone and pancreas Fe levels were decreased ($p<0.05$) in chicks fed diets with added Zn. Bone Zn level was decreased ($p<0.03$) in chicks fed diets with added Cu. Liver and pancreatic Mn levels were increased ($p<0.05$) in chicks fed diets with added Zn. This study indicates that Zn addition is necessary for normal growth and bone strength of chicks and that Zn addition had negative effects on tissue Cu and Fe levels and a positive effect on tissue Mn levels.

Key words: Chicks, growth, tissue, zinc, copper, manganese

Introduction

Previous research (Shelton *et al*., 2004b, 2005) in our lab indicated that removing the trace mineral premix (containing Zn, Cu, Mn, Fe, Se and I) from swine diets resulted in an increase in tissue Mn levels (most notably in bile and liver). Addition of phytase increased Zn availability and reduced the increase in tissue Mn levels. Therefore, there may be an interaction between Zn and Mn. Furthermore, because Zn and Cu interact with each other (Blakeborough and Salter, 1987; Frimpong and Magee, 1989), the effect we saw in tissue Mn may be due to Cu or a combination of all three minerals. Therefore, the objective of this experiment was to determine the interaction between Zn, Cu and Mn in 0- to 14-d old commercial broilers.

Materials and Methods

The methods used in this experiment were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Two hundred and forty commercial broilers (120 female and 120 male Ross×Ross) were used for this study. The chicks were allotted to their respective treatment diets on d 0 posthatching and each treatment had 6 replications (3 pens of females and 3 pens of males) of 5 chicks per replicate pen. Initial and final weights were 46 and 382 g, respectively and the experimental period lasted 14 d. Two levels of Zn (0 and 75 ppm, as Zn sulfate), 2 levels of Mn (0 and 100 ppm, as Mn sulfate) and 2 levels of Cu (0 and 7 ppm, as Cu sulfate) were supplemented to corn-soybean meal based diets in a 2×2×2 factorial arrangement. Diets (Table 1) were formulated to meet or exceed the amino acid, mineral (except for Zn, Mn, or Cu where appropriate) and vitamin requirements of chicks 0 to 21 d posthatching (NRC, 1994). Analysis of the diets for Zn, Mn and Cu were close to anticipated values (Table 2).

Throughout the trial, chicks were housed in thermostatically controlled starter batteries with raised wire floors and continuous lighting. Feed (in mash form) and water were offered on an ad libitum basis. At the end of the experiment, the left tibia was removed from all chicks for determination of bone breaking strength, ash percentage and mineral concentration. Also, samples of the pancreas and liver were taken for determination of mineral concentration. Bone breaking strength was determined by using a HD 250 Texture Technology Machine fitted with a three point bend rig with a load cell capacity of 25 kg and a cross-head speed of 100 mm/min. After determination of bone breaking strength, fat was removed from the tibias by a 36-h Soxhlet extraction in ethyl alcohol followed by a 36-h extraction with diethyl ether and then dried in a forced air oven at 100°C for 24 h. Bone ash percentage was determined

Table 1: Basal diet composition

Ingredient	%
Corn	52.45
Soybean meal, 47.5% CP	37.96
Soy oil	5.33
Monocalcium phosphate	1.51
Limestone	1.65
Salt	0.50
Mineral premix[1]	0.25
Vitamin premix[2]	0.05
Choline chloride[3]	0.05
DL-methionine	0.20
Cellulose[4]	0.06
Calculated Composition[5]	
ME, kcal/kg	3,200
Crude protein, %	22.60
Lysine, %	1.26
Sulfur amino acids, %	0.91
Threonine, %	0.86
Ca, %	1.00
Available P, %	0.45
Zn, ppm	33.34
Mn, ppm	21.86
Cu, ppm	9.39

[1]Provided per kilogram of diet: iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate●$7H_2O$), 50 mg; selenium (sodium selenite), 0.15 mg; with calcium carbonate as the carrier. [2]Provided per kilogram of diet: vitamin A (retinyl palmitate), 1,366 IU; vitamin D_3 (cholecalciferol), 450 IU; vitamin E (DL-alpha-tocopheryl acetate), 50 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; d-pantothenic acid, 18.3 mg; pyridoxine (pyridoxine●HCL), 6.4 mg; riboflavin, 15 mg; thiamin (thiamin●HCL), 13.4 mg, [3]Contains 600,000 mg/kg of choline.
[4]Cellulose was replaced with zinc sulfate, manganese sulfate, or copper sulfate to provide Zn (75 ppm), Mn (100 ppm), or Cu (7 ppm), respectively, [5]Based on NRC (1994)

Table 2: Analyzed values of minerals in the diets (ppm)[1]

Diet[2]	Zn	Mn	Cu
Negative Control	28	18	6.2
+ Zn	117	20	6.1
+ Mn	29	109	6.0
+ Cu	30	18	12.2
+ Zn and Mn	129	125	5.2
+ Zn and Cu	136	20	13.1
+ Mn and Cu	27	128	13.0
+ Zn, Mn and Cu	113	127	12.9

[1]Mineral composition of the diets was determined after digestion in nitric acid and hydrogen peroxide by inductively coupled plasma emission spectroscopy (AOAC, 1990), [2]The negative control indicates no added Zn, Mn, or Cu. The +Zn, +Mn and +Cu represent the addition of 75, 100 and 7 ppm, respectively, of each mineral as zinc sulfate, manganese sulfate, or copper sulfate

by placing the bones in a muffle furnace and ashing for 24 h at 550°C. Samples of liver and pancreas were dried in a forced air oven for 24 h at 100°C and mineral composition of the diets and tissues was determined after digestion in nitric acid and hydrogen peroxide by inductively coupled plasma emission spectroscopy (AOAC, 1990; Model Optima 3000, Perkin Elmer, Norwalk, CT 06859).

Data were analyzed by ANOVA procedures for a completely randomized design (Steel and Torrie, 1980) with treatment and sex in the model using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). There were no treatment by sex interactions; therefore, only the main effects of diet are presented. Orthogonal contrasts appropriate for a 2×2×2 factorial arrangement of treatments were used to determine treatment effects. The pen of chicks was the experimental unit for all data.

Results

Chicks fed the diets with added Zn had an increased ($p<0.01$) daily gain, daily feed intake, gain:feed and bone breaking strength relative to those not fed additional Zn (Table 3 and 4). Bone breaking strength was increased when Zn was added to the diets with or without Cu addition, but the increase was greater in chicks fed the diets without Cu addition (Table 5; Zn×Cu, $p<0.05$). Bone ash percentage was increased in chicks fed the diets with Zn, Mn, or Cu addition but the increase was not additive (Zn×Mn×Cu, $p<0.03$).

Tissue mineral concentrations are presented in Table 5, 6 and 7. Bone and pancreatic Zn levels were increased ($p<0.05$) in chicks fed diets with Zn addition. Also, liver and pancreatic Mn levels were increased ($p<0.05$), but bone and pancreas Fe levels and bone and liver Cu levels were decreased ($p<0.05$) in chicks fed diets with Zn addition. Bone, liver and pancreatic Mn levels were increased in chicks fed diets with Mn addition ($p<0.01$). Liver Cu levels were increased ($p<0.01$) in chicks fed the diets with Cu addition. Bone Zn and Fe levels were decreased ($p<0.01$) in chicks fed diets with Cu addition. Liver Cu levels were decreased when Mn was added to the diet with Cu addition but not affected when Mn was added to the diet without Cu addition (Mn×Cu, $p<0.03$). Liver Cu levels were decreased when Zn was added to the diet with Cu addition but not affected when Zn was added to the diet without Cu addition (Zn×Cu, $p<0.01$). Liver Cu levels were increased in chicks fed the diets with Zn, Mn, or Cu addition, but the increase was not as great as adding Cu alone (Zn×Mn×Cu, $p<0.01$).

Discussion

Previous research in our lab with swine has indicated that there may be a negative interaction between Zn and Mn. In several studies (Shelton *et al.*, 2004b, 2005), removing the trace mineral premix (containing Zn, Cu, Mn, Fe, Se and I) from swine diets resulted in an increased tissue Mn level (most notably in liver and bile). Increasing Zn availability by adding phytase reduced the increase in tissue Mn levels. This interaction could be a direct interaction between Zn and Mn, or possibly involving another trace mineral, such as Cu. However, results from this study indicated there was no negative interaction between Zn and Mn in poultry. These data

Table 3: Growth performance, bone breaking strength and bone ash percentage of chicks fed supplemental Zn, Mn, or Cu[1]

	Diet[2]								
Response	NC	+Zn	+Mn	+Cu	+Zn,Mn	+Zn,Cu	+Mn,Cu	+Zn,Mn,Cu	SEM
Daily gain,[3] g	22.57	26.51	21.38	23.20	25.46	25.60	21.57	25.63	0.76
Daily feed intake,[3] g	28.42	32.13	27.62	29.22	30.57	30.95	27.38	31.87	0.87
Gain:feed[3]	0.792	0.828	0.773	0.797	0.833	0.827	0.788	0.803	0.014
Bone strength,[3,4] kg	10.53	14.66	10.80	12.40	13.50	13.09	10.94	13.30	0.65
Bone ash,[5] %	52.80	54.74	54.58	54.05	54.27	54.18	53.85	54.36	0.40

[1]Data are means of 6 replications (3 pens of females and 3 pens of males) of 5 birds each with an initial and final BW of 46 and 382 g, respectively. Bone strength was determined using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 25 kg and cross-head speed of 100 mm/min, [2]The Negative Control (NC) indicates no added Zn, Mn, or Cu. The +Zn, +Mn and +Cu represent the addition of 75, 100 and 7 ppm, respectively, of each mineral, [3]Zn effect, $p<0.01$, [4]Zn×Cu effect, $p<0.05$, [5]Zn×Mn×Cu effect, $p<0.03$

Table 4: Main effect of Zn, Mn, or Cu on growth performance, bone breaking strength and bone ash percentage of chicks[1]

	Zn		Mn		Cu		
	-	+	-	+	-	+	SEM
Daily gain,[2]g	22.18	25.80	24.47	23.51	23.98	24.00	0.38
Daily feed intake,[2]g	28.16	31.38	30.18	29.36	29.69	29.86	0.42
Gain:feed[2]	0.788	0.823	0.811	0.799	0.807	0.804	0.007
Bone strength,[2]kg	11.17	13.64	12.67	12.14	12.37	12.43	0.33
Bone ash, %	53.82	54.39	53.94	54.27	54.10	54.11	0.20

[1]Data are means of 24 replications (12 pens of females and 12 pens of males) of 5 birds each with an initial and final BW of 46 and 382 g, respectively. Bone strength was determined using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 25 kg and cross-head speed of 100 mm/min. The +Zn, +Mn and +Cu represent the addition of 75, 100 and 7 ppm, respectively, of each mineral, [2]Zn effect, $p<0.01$

Table 5: Trace mineral interaction on growth performance, bone breaking strength, bone ash percentage and tissue mineral levels of chicks fed supplemental Zn, Mn, or Cu[1]

Response	Mineral addition				
	-Zn, -Cu	+Zn, -Cu	-Zn, +Cu	+Zn, +Cu	SEM
Bone strength[2], kg	10.67	14.08	11.67	13.20	0.46
	Zn, -Cu	+Zn, -Cu	-Zn +Cu	+Zn, +Cu	SEM
Liver Cu[3], ppm	10.33	11.32	16.72	11.54	0.47
	Mn, -Cu	+Mn, -Cu	-Mn, +Cu	+Mn, +Cu	SEM
Liver Cu[4], ppm	10.77	10.91	14.77	13.97	0.48

[1]Data are means of 12 replications (6 pens of females and 6 pens of males) of 5 birds each with an initial and final BW of 46 and 382 g, respectively. Bone strength was determined using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 25 kg and cross-head speed of 100 mm/min. The +Zn, +Mn and +Cu represent the addition of 75, 100 and 7 ppm, respectively, of each mineral, [2]Zn×Cu effect, $p<0.05$, [3]Zn×Cu effect, $p<0.01$, [4]Mn×Cu effect, $p<0.03$

Table 6: Tissue mineral concentrations of chicks fed supplemental Zn, Mn, or Cu[1]

Response	NC	+Zn	+Mn	+Cu	+Zn,Mn	+Zn,Cu	+Mn,Cu	+Zn,Mn,Cu	SEM
Bone (Ash basis)									
Zn[2,3]	152.0	313.1	134.4	123.0	284.5	284.7	111.1	291.2	9.5
Mn[4]	1.36	1.96	3.96	1.17	4.42	0.96	3.60	3.91	0.57
Cu[2]	2.28	2.09	2.20	1.93	1.82	1.85	2.13	1.74	0.18
Fe[2,3]	298.2	229.3	279.8	242.3	216.8	211.9	268.8	199.5	15.3
Liver (DM basis)									
Zn	112.7	116.6	99.1	115.9	104.4	104.8	107.7	119.8	11.46
Mn[2,4]	6.65	7.17	7.55	6.52	9.69	8.03	7.57	9.21	0.52
Cu[2,4,5,6,7]	10.17	11.37	10.56	18.29	11.26	11.25	15.15	11.82	0.67
Fe	528.2	677.6	623.4	537.4	718.4	676.5	607.8	515.1	64.0
Pancreas (DM basis)									
Zn[2]	109.9	171.4	110.0	100.1	186.9	178.6	119.5	183.5	8.7
Mn[2,4]	2.56	4.56	4.52	2.68	7.69	5.19	4.47	6.99	0.37
Cu	4.20	4.30	4.38	4.05	4.12	4.55	4.38	4.56	0.25
Fe[2]	186.5	99.9	162.9	135.5	95.3	105.6	145.9	96.3	20.9

[1]Data are means of 6 replications (3 pens of females and 3 pens of males) of 5 birds each. Bone mineral concentration is based on an ash basis and liver and pancreas mineral concentrations are based on a DM basis. The negative control (NC) indicates no added Zn, Mn, or Cu. The +Zn, +Mn and +Cu represent the addition of 75, 100 and 7 ppm, respectively, of each mineral, [2]Zn effect, $p<0.05$, [3]Cu effect, $p<0.03$, [4]Mn effect, $p<0.01$, [5]Zn×Cu effect, $p<0.01$, [6]Mn×Cu effect, $p<0.03$, [7]Zn×Mn×Cu effect, $p<0.01$

Table 7: Main effect of Zn, Mn, or Cu on tissue mineral levels of chicks from 0 to 14-d posthatching[1]

	Zn		Mn		Cu		
Item, ppm	-	+	-	+	-	+	SEM
Bone Zn[2,3]	130.1	293.4	218.2	205.3	221.0	202.5	2.6
Bone Mn[4]	2.52	2.81	1.36	3.97	2.92	2.41	0.16
Bone Cu[2]	2.14	1.88	2.04	1.97	2.10	1.91	0.05
Bone Fe[2,3]	272.3	214.4	245.4	241.2	256.0	230.6	4.0
Liver Zn	108.9	111.4	112.5	107.8	108.2	112.1	5.7
Liver Mn[2,4]	7.07	8.53	7.09	8.50	7.77	7.83	0.25
Liver Cu[2,3]	13.54	11.43	12.77	12.20	10.84	14.13	0.34
Liver Fe	574.2	646.9	604.9	616.2	636.9	584.2	32.0
Pancreas Zn[2]	109.9	180.1	140.0	150.0	144.6	145.4	4.3
Pancreas Mn[2,4]	3.56	6.11	3.75	5.92	4.83	4.83	0.18
Pancreas Cu	4.25	4.38	4.28	4.36	4.25	4.39	0.12
Pancreas Fe[2]	157.7	99.3	131.9	125.1	136.2	120.8	10.7

[1]Data are means of 24 replications (12 pens of females and 12 pens of males) of 5 birds each. Bone mineral concentration is on an ash basis and liver and pancreas mineral concentrations are on a DM basis, [2]Zn effect, $p<0.05$, [3]Cu effect, $p<0.03$, [4]Mn effect, $p<0.01$

also indicate that Zn addition is necessary for normal growth and bone strength in young broilers which agrees with Shelton *et al.* (2004a) who indicated that Zn must be added in the diet for the first 6 d posthatching for normal growth and bone strength. The classic Cu×Zn interaction (Blakeborough and Salter, 1987; Frimpong and Magee, 1989) was seen in the bone and liver data in that adding Zn to the diet decreased bone and liver Cu levels and adding Cu decreased bone Zn levels. This study indicated that Zn is necessary for normal growth performance and bone response variables in broilers from 0 to 14-d of age. Furthermore, there were no negative interactions between Zn and Mn in young chicks.

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