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Interactive Effects of Dietary Copper, Water Copper, and *Eimeria* spp. Infection on Growth, Water Intake, and Plasma and Liver Copper Concentrations of Poults[1]

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ABSTRACT The effects of dietary Cu, water Cu, and coccidial infection on poult growth performance and selected tissue mineral concentrations were investigated in a 10-d experiment using 200 5-d-old Nicholas toms (five replicates of 5 poults each; initial weight = 85 g). Uninfected and coccidiosis-infected (*Eimeria meleagrimitis, Eimeria dispersa, Eimeria adenoeides*, and *Eimeria gallopavonis*; cocci) poults were assigned to two levels of dietary Cu [Basal (B) and B + 204 mg Cu/kg diet on Days 1 to 10] and two levels of water Cu (0 and 103 mg Cu/kg water on Days 6 to 10). Dietary Cu and water Cu (main effects) did not affect ($P > .10$) gain, feed intake, gain:feed, water intake, hemoglobin, hematocrit, or liver Fe and Zn concentrations. Dietary Cu and water Cu increased ($P < .03$) liver and plasma Cu concentrations. The combination of dietary Cu and water Cu increased plasma Cu more than the sum of the Cu additions (dietary Cu by water Cu, $P < .08$). Coccidial infection reduced ($P < .07$) gain, feed intake, gain:feed, water intake, and hemoglobin, and increased ($P < .02$) liver Zn. Water Cu reduced water intake in uninfected poults but increased water intake in coccidiosis-infected poults (water Cu by cocci, $P < .07$). Water Cu increased hemoglobin in uninfected poults but decreased hemoglobin in coccidiosis-infected poults (water Cu by cocci, $P < .07$). Water Cu increased plasma Cu and liver Cu more in coccidiosis-infected poults than in uninfected poults (water Cu by cocci, $P < .02$). Simultaneous supplementation of feed and water with Cu increases plasma and liver Cu concentrations in coccidiosis-infected poults.

(*Key words*: turkey, growth performance, copper, coccidiosis, liver copper)

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INTRODUCTION

Swine and poultry diets have been supplemented with dietary Cu in excess of the nutritional requirement for many years, purportedly for growth promotion. In addition to supplementing the diet with Cu, it is common practice to add Cu to the drinking water of poults for a short time period (approximately 5 d) during the growing phase. Recently, we reported that simultaneous supplementation of both the diet and water of poults with excess Cu resulted in decreased growth and increased liver Cu accumulation (Ward *et al.*, 1994).

Coccidial infections have been associated with perturbation of mineral absorption and utilization in poultry (Turk, 1986a,b; Ward *et al.*, 1990, 1993).

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Specifically, *Eimeria acervulina* infection has been shown to dramatically increase liver Cu accumulation of chicks fed excess dietary Cu (Southern and Baker, 1983a,b; Fox *et al.*, 1987). It is likely that coccidial infection also would exacerbate the tissue Cu accumulation of poults given excess Cu. Given the prevalence of coccidia in practical growing conditions, the known use of both dietary Cu and water Cu additions in the turkey industry, and that no research previously has evaluated the interactive effects of Cu intake and coccidiosis in poults, we felt it necessary to pursue this investigation. Therefore, this investigation was conducted to determine the interactive effects of dietary Cu, water Cu, and coccidial infection on growth performance, water intake, whole-blood hemoglobin (Hb), and plasma and liver Cu concentrations.

MATERIALS AND METHODS

An experiment was conducted using 200 Nicholas Large White toms[4] from 5 to 15 d of age. From hatching to 4 d posthatching, all poults were fed a basal diet formulated to meet the nutrient requirements of the growing poult (NRC, 1994; Table 1). The poults were deprived of feed for 16 h (but had continuous access to tap water), and then were weighed and randomly assigned to treatments. The average initial weight was 85 g. Poults were provided continuous fluorescent lighting and caged in heated, thermostatically controlled (mean temperature was 35 C) starter batteries with raised wire floors. Tap water was provided for *ad libitum* consumption from plastic water containers. The water containers were filled with a known weight of water and weighed daily (0700 h) to determine water disappearance. Evaporative loss (50.7 g water/d) was determined using an additional water container placed in the same room as the batteries. Pen feed intake was measured daily and pen poult weights were determined on Day 5. Individual poult weights were determined on Day 10. Due to the occurrence of mortality in the coccidiosis-infected poults, average daily gain was calculated using the sum of the final body weights of all poults minus the sum of the initial body weights, and this quantity was divided by the sum of the number of days each poult was on the experiment. Average daily feed intake was calculated by dividing the total feed intake by the sum of the number of days each poult was on the experiment. Gain:feed was calculated by dividing average daily gain by average daily feed intake.

[4]Janssen Farms, Zeeland, MI 49464.

TABLE 1. Composition of the basal diet

Ingredients and composition	Percentage
Soybean meal, 44% CP	52.81
Corn	35.62
Corn oil	5.00
Fish meal, menhaden	2.50
Defluorinated rock phosphate	2.34
Vitamin-mineral premix[1]	.50
Limestone	.43
Salt	.40
DL-methionine	.20
Selenium premix[2]	.20
Total	100.00
Calculated composition	
CP	28.00
Calcium	1.20
Total phosphorus	.94
Available phosphorus	.60
Total sulfur amino acids	1.09
Lysine	1.75
Metabolizable energy, kcal/kg	2,881
Analyzed composition	
Copper,[3] mg/kg	18.40
Zinc, mg/kg	105.90
Iron, mg/kg	399.70

[1]Supplied the following per kilogram of diet: vitamin A, 22,000 IU (retinyl acetate); cholecalciferol, 3,300 IU (vitamin D-activated animal sterol); vitamin E, 16 IU (dl-α-tocopheryl acetate); menadione sodium bisulfite complex, 4.41 mg; thiamine, 2 mg; riboflavin, 8.8 mg; niacin, 66 mg; d-pantothenic acid, 16.2 mg; folic acid, .66 mg; d-biotin, .11 mg; vitamin B_{12}, .022 mg; choline chloride, 881 mg; manganese, 120 mg; zinc, 88 mg; iron, 40 mg; copper, 4 mg; iodine, 2.35 mg; cobalt, .4 mg.

[2]Supplied .2 mg Se/kg of diet as Na_2SeO_3.

[3]Diets supplemented with Cu were analyzed and found to contain 180.6 mg Cu/kg diet.

Experimental Treatments

Coccidial infections (cocci) were established by crop intubation of a 1-mL solution

containing 1×10^5 *Eimeria meleagrimitis*, 1×10^5 *Eimeria dispersa*, 5×10^4 *Eimeria adenoeides*, and 3×10^4 *Eimeria gallopavonis* oocysts on the day of experimental initiation. Uninfected poults were intubated with tap water.

A $2 \times 2 \times 2$ factorial arrangement of treatments was used. Poults were assigned to two levels of infection (uninfected and coccidiosis-infected), two levels of excess supplemental dietary Cu (0 and 204 mg Cu/kg diet, provided by .08% $CuSO_4 \cdot 5H_2O$), and two levels of water Cu (tap water and tap water plus 103 mg Cu/kg water). Each treatment was replicated five times with five poults each. Water Cu treatments resulted from adding acidified $CuSO_4 \cdot 5H_2O$ (21.97% Cu)[5] to tap water on a mass basis (i.e., milligrams of Cu per kilogram of water). The water Cu levels of 0 or 103 mg Cu/kg water represent 0 and 1 times the recommended use level.[5] Atomic absorption spectrophotometric analysis[6] (Anonymous, 1982) determined water Cu concentrations of .02 and 83.9 mg Cu/kg in tap water and Cu-supplemented water, respectively. Dietary Cu analysis revealed that the basal diet and Cu-supplemented diet contained 18.4 and 180.6 mg Cu/kg diet, respectively. However, except for calculation of Cu intake, dietary and water Cu levels will be referred to as 0 and 204, and 0 and 103 mg supplemental Cu/kg, respectively.

Tissues

On Day 10 of the experiment, all poults were bled via cardiac puncture. Two milliliters of blood per poult were pooled by replicate into tubes[7] containing 286 USP units of sodium heparin. Hematocrit values were determined on each poult using heparinized hematocrit tubes,[8] which were centrifuged for 5 min.[9] Blood samples were kept on ice until Hb analysis[10] was completed (within 6 h). Blood was centrifuged (1,600 × g for 20 min at 4 C) and plasma was harvested and frozen for later analysis of Cu by atomic absorption spectrophotometry.[6] After bleeding, the poults were killed by cervical dislocation and liver samples were collected and pooled by replicate. Liver samples were dried in a forced-air oven at 100 C for 12 h, wet-ashed with HNO_3 and H_2O_2, and analyzed for Cu by atomic absorption spectrophotometry.[6]

Statistical Analyses

Growth performance data for Day 1 to 5 of the experiment were analyzed as a 2 × 2 factorial arrangement of treatments in a completely random design (Steel and Torrie, 1980). The water Cu treatments were not initiated until Day 6; therefore, the Day 1 to 5 data include only dietary Cu and coccidial infection effects with 10 replicates per dietary treatment. Growth performance data for Days 6 to 10 were analyzed as a 2 × 2 × 2 factorial arrangement of treatments in a completely random design with Day 5 BW as a covariate. The tissue data were analyzed as a factorial arrangement (2 × 2 × 2) of treatments without covariance analysis. Due to heterogeneity of variance, determined by Bartlett's test (Snedecor and Cochran, 1967), liver Cu data were log-transformed [ln (y + 1)] for statistical analysis. Day 6 to 10 water intake was analyzed as a split-plot in time with replicate within treatment as the error term for the treatment effects. Mortality data were transformed (arc sine of the square root of the percentage mortality within each pen) prior to statistical analysis (Snedecor and Cochran, 1967). Due to the occurrence of mortality only in the coccidiosis-infected poults, the effects of dietary Cu and water Cu were evaluated within the coccidiosis-infected treatments only. The pen was the experimental unit for all data.

RESULTS AND DISCUSSION

No interactions were evident for gain, feed intake, gain:feed, or water intake for Days 1 to 5 of the experiment; therefore

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[5]I. D. Russell Co. Laboratories, Longmont, CO 80501.

[6]Model 3030B, Perkin-Elmer Corp., Norwalk, CT 06856.

[7]Catalog No. 6489, Becton Dickinson Co., Rutherford, NJ 07070.

[8]Catalog No. 260-950, Curtin Matheson Scientific, Inc., Houston, TX 77038.

[9]Adams Autocrit Centrifuge, Clay Adams Co., Parsippany, NJ 07054.

[10]Sigma Technical Bulletin Number 525, Sigma Chemical Co., St. Louis, MO 63178-9916.

only the main effect of coccidial infection is presented (Table 2). Gain, feed intake, gain:feed, and water intake were decreased ($P < .01$) in coccidiosis-infected poults from Days 1 to 5 of the experiment. Although the literature is replete with examples of reduced body weight gain of turkeys due to coccidial infection, no data are available on feed and water intakes for coccidiosis-infected turkeys. However, Reid and Pitois (1965) reported that six different species of coccidia reduced both feed and water intake of growing chickens. Reduced weight gain of poultry by coccidial infection is well known and is due to many factors in addition to reduced feed and water intakes. However, it is possible that the reduction in water intake of cocci birds is more important, or at least as important, than the reduction in feed intake. Certainly these two factors are interrelated, but the multiple functions of water *in vivo* necessitates the knowledge of the effects of cocci as well as other factors on water intake. Much of the literature has neglected this important factor.

Dietary Cu supplementation did not affect ($P > .10$) gain, feed intake, gain:feed, or water intake from Days 1 to 5 of the experiment. Previously, we have reported that dietary Cu supplementation decreased gain of poults during the first 5 d of treatment (Ward *et al.*, 1994); however, the Cu-supplemented diet in the current experiment contained 57 ppm less Cu than in the previous investigation. Other researchers also have reported equivocal results regarding the benefit of Cu supplementation of turkey diets (Waibel *et al.*, 1964; Weeks and Sullivan, 1972; Guenthner *et al.*, 1978; Christmas and Harms, 1979; Kashani and Carlson, 1980; Kashani *et al.*, 1986). These investigations were conducted under a myriad of conditions (i.e., dietary Cu levels, length of feeding, housing conditions, and environments).

Day 6 to 10 gain, feed intake, gain:feed, and water intake were not affected ($P > .10$) by dietary Cu or water Cu supplementation (Table 3). Gain, feed intake, gain:feed, and water intake were reduced ($P < .07$) by the coccidial infection. Water intake was reduced by water Cu supplementation in uninfected poults but was increased by water Cu supplementation in coccidiosis-infected poults (water Cu by cocci, $P < .07$).

Hematocrit and Hb were not affected ($P > .10$) by dietary Cu or water Cu supplementation (Table 4). Dietary Cu supplementation of swine diets has been reported to reduce hematocrit and Hb levels (Ritchie *et al.*, 1963; Kline *et al.*, 1972; Gipp *et al.*, 1973, 1974). However, we previously have reported no affect of dietary Cu or water Cu on Hb concentration of poults (Ward *et al.*, 1994). The reduction in Hb due to excess Cu has been reported to occur only in combination with Fe deficiency (Waibel *et al.*, 1964;

TABLE 2. Growth performance and water intake of uninfected and coccidiosis-infected poults fed 0 or 204 ppm supplemental dietary Cu from Day 1 to 5 of the experiment[1]

Treatment[2]	Average daily gain	Average daily feed intake	Gain: feed	Average daily water intake
	(g/poult/d)		(g:g)	(g/poult/d)
Cocci				
–	20.0	22.4	.895	76.0
+	7.6	12.6	.541	46.9
SEM	1.0	.7	.078	3.0
	Probability > F			
Cocci	.01	.01	.01	.01

[1]Data are means of 10 replicates per treatment. Average initial weight was 85 g. No main effect or interactive effect of dietary Cu level was evident ($P > .10$).

[2]Cocci = uninfected (–) or coccidiosis-infected (+) poults.

TABLE 3. Growth performance and water and Cu intake of uninfected and coccidiosis-infected poults fed 0 or 204 ppm supplemental dietary Cu with 0 or 103 ppm water Cu from Day 6 to 10 of the experiment[1]

Cu							
Diet	Water	Cocci[2]	Calculated Cu intake[3]	Average daily gain	Average daily feed intake	Gain: feed	Average daily water intake
(ppm)			(mg/poult/5 d)	(g/poult/d)		(g:g)	(g/poult/d)
0	0	−	3.20	30.6	34.9	.886	134.3
0	103	−	51.65	30.4	33.3	.914	115.8
204	0	−	31.70	32.3	35.1	.923	124.6
204	103	−	78.55	29.8	32.9	.908	116.4
0	0	+	2.75	18.4	29.8	.602	111.6
0	103	+	52.10	19.9	31.3	.612	117.3
204	0	+	27.10	18.2	30.0	.586	108.1
204	103	+	72.85	18.5	29.5	.614	110.1
Main effects							
Dietary Cu							
0				24.8	32.3	.754	119.8
204				24.7	31.9	.758	114.8
Water Cu							
0				24.9	32.5	.749	119.7
103				24.7	31.8	.762	114.9
Cocci							
−				30.8	34.1	.908	122.8
+				18.8	30.2	.604	111.8
SEM				1.3	1.0	.031	6.3
				Probability > F[4]			
Day 5 BW[5]				NS	.01	.07	
Cocci				.01	.07	.01	.02
Water Cu by cocci				NS	NS	NS	.07

[1]Data are least squares means of five replicates per treatment.

[2]Cocci = uninfected (−) or coccidiosis-infected (+) poults.

[3]Copper intake was calculated using analyzed dietary Cu levels of 18.4 and 180.6 mg/kg for 0 and 204 ppm diets, respectively, and analyzed water Cu levels of .02 and 83.9 mg/kg for 0 and 103 ppm, respectively.

[4]Only sources of variation with at least one effect (P < .10) are presented.

[5]Day 5 body weight was used as a covariate for daily gain, daily feed intake, and gain:feed.

Kline *et al.*, 1972). The diets in the current experiment contained Fe in great excess of the nutritional requirement.

Hemoglobin was reduced (P < .01) by coccidial infection. Others also have reported decreased Hb by coccidial infection in chicks (Bafundo *et al.*, 1984; Turk, 1986a). Mathis *et al.* (1984) reported decreased Hb and increased methemoglobin in chicks infected with *Eimeria* spp., and in turkeys infected with *Histomonas meleagridis*. These authors concluded that the decreased Hb and increased methemoglobin were due to a common response to inflammation and not due to coccidial infection *per se*. In the current study, Hb was increased in uninfected poults given supplemental water Cu but was decreased in coccidiosis-infected poults given supplemental water Cu (water Cu by cocci, P < .07).

Plasma and liver Cu were increased (P < .03) by both dietary Cu and water Cu supplementation. Plasma Cu was increased more in poults given both supplemental dietary Cu and water Cu than the sum of the plasma Cu of poults given dietary Cu and water Cu individually (dietary Cu by water Cu, P < .08). Previous data from our laboratory showed that dietary Cu did not affect plasma Cu concentration of 9- to 19-d-old poults (Ward *et al.*, 1994). The reason for this discrepancy is not known. Vohra and associates reported increased liver Cu in turkeys fed dietary Cu at relatively low

TABLE 4. Hematocrit, hemoglobin, plasma Cu, and liver Cu, Fe, and Zn concentrations of uninfected and coccidiosis-infected poults fed 0 or 204 ppm supplemental dietary Cu with 0 or 103 ppm water Cu[1]

Cu							Liver		
Diet	Water	Cocci[2]	Calculated Cu intake[3]	Hemato-crit	Hemo-globin	Plasma Cu	Cu[4]	Fe	Zn
(ppm)			(mg/poult/10 d)	(%)	(g/dL)	(μg/mL)	(μg/g dry tissue)		
0	0	–	5.25	32.2	9.6	.19	26	255	95
0	103	–	53.70	32.8	9.8	.21	94	276	95
204	0	–	51.95	32.8	9.4	.20	64	327	90
204	103	–	98.80	33.2	9.7	.25	229	263	89
0	0	+	3.85	34.7	9.3	.12	18	249	121
0	103	+	53.20	31.8	9.1	.22	157	254	110
204	0	+	39.20	33.3	9.1	.14	35	268	108
204	103	+	84.95	34.2	8.6	.45	348	259	99
Main effects									
Dietary Cu, ppm									
0				32.9	9.5	.19	74	258	105
204				33.4	9.2	.26	169	279	96
Water Cu, ppm									
0				33.3	9.4	.16	36	275	103
103				33.0	9.3	.28	207	263	98
Cocci									
–				32.8	9.6	.21	104	280	92
+				33.5	9.0	.23	139	258	110
SEM				1.0	.2	.05	15	26	10
				Probability > F[5]					
Dietary Cu				NS	NS	.03	.01	NS	NS
Water Cu				NS	NS	.01	.01	NS	NS
Cocci				NS	.01	NS	NS	NS	.02
Dietary Cu by water Cu				NS	NS	.07	NS	NS	NS
Water Cu by cocci				NS	.07	.02	.01	NS	NS

[1]Data are means of five replicates per treatment.

[2]Cocci = uninfected (–) or coccidiosis-infected (+) poults.

[3]Copper intake was calculated using analyzed dietary Cu levels of 18.4 and 180.6 mg/kg for 0 and 204 ppm diets, respectively, and analyzed water Cu levels of .02 and 83.9 mg/kg for 0 and 103 ppm treatments, respectively. Feed and water intake values are from Table 2 (Days 1 to 5) and Table 3 (Days 6 to 10), respectively.

[4]Due to heterogeneity of variance, liver copper data were transformed (ln [y + 1]) for statistical analysis; transformed data SEM = .12.

[5]Only sources of variation with at least one effect ($P < .10$) are presented.

(150 ppm; Vohra and Heil, 1969) and high (940 ppm, Vohra *et al.*, 1968) levels. Wiederanders (1968) reported that high levels of Cu injected in 13-wk-old turkeys increased plasma and liver Cu concentrations, as well as plasma ceruloplasmin.

Plasma and liver Cu concentrations were increased by water Cu supplementation to a greater extent in coccidiosis-infected poults than in uninfected poults (water Cu by cocci, $P < .02$) even though the cocci poults consumed less Cu. Coccidial infection also has been reported to increase liver Cu in chicks fed excess dietary Cu (Fox *et al.*, 1987; Southern and Baker, 1983a,b). Somewhat of a paradox exists regarding the effect of coccidial infection on plasma Cu concentrations. Copper absorption is greatest in the duodenum of chicks (Starcher, 1969) and probably turkeys. Therefore, the greatest effect on Cu absorption would be expected with the species of coccidia that infect the upper digestive tract (*E. dispersa* and *E. meleagrimitis*). However, Turk (1986b) reported that plasma Cu concentration was increased during the acute phase of infection by lower intestinal tract (*E.*

TABLE 5. Mortality of uninfected and coccidiosis-infected poults fed 0 or 204 ppm supplemental dietary Cu with 0 or 103 ppm water Cu[1]

Cu				Mortality			
Diet	Water	Cocci[2]	Total live poults	Days 1 to 5		Days 6 to 10	
(ppm)			(n)	(n)	(%)	(n)	(%)
0	0	–	25	0	0	0	0
0	103	–	25	0	0	0	0
204	0	–	25	0	0	0	0
204	103	–	25	0	0	0	0
0	0	+	25	3	12	2	8
0	103	+	25	12	48	0	0
204	0	+	25	4	16	1	4
204	103	+	25	3	12	1	4
				Probability > F^3			
SEM					.01		.05
Cocci					5		3

[1]Data are means of five replicates per treatment. Data were transformed (arcsin of the square root of the percentage mortality) prior to statistical analysis. Analysis of Day 1 to 5 data included only the treatment effects of dietary Cu and coccidial infection. Dietary Cu and water Cu effects were evaluated within the coccidiosis-infected treatments.

[2]Cocci = uninfected (–) or coccidiosis-infected (+) poults.

[3]Only sources of variation with at least one effect ($P < .10$) are presented.

brunetti, E. tenella) infections but not by upper tract (*E. acervulina, E. necatrix*) infections in chicks fed low Cu levels (less than 20 mg/kg). It was not possible to differentiate the effects of different species of coccidia in the current experiment. Turk (1986b) also reported that plasma Cu concentrations were not different during the recovery phase of the infections. However, Giraldo and Southern (1988) reported that *E. acervulina* infection induced liver Cu accumulation in chicks fed excess Cu during both the acute and recovery phases of infection. Southern and Stewart (1984) have reported that *Ascaris suum* infection tended to decrease (not significantly) tissue Cu accumulation in pigs fed excess dietary Cu. Thus, level of Cu supplementation, species of animal, species of coccidia (or other intestinal parasite), stage of infection, as well as other factors may affect the tissue response to Cu supplementation.

Liver Fe and Zn concentrations were not affected ($P > .10$) by dietary Cu or water Cu supplementation. Incongruent with work done in the chick (Southern and Baker, 1983a,b; Bafundo *et al.*, 1984; Ward *et al.*, 1993), liver Zn concentration was increased ($P < .02$) by coccidial infection in poults. Tissue Zn response also may be influenced by a variety of factors.

Mortality occurred ($P < .05$) only in the coccidiosis-infected poults and was most pronounced from Days 1 to 5 of the experiment (Table 5). There were no main effects or interactions ($P > .10$) of dietary Cu or water Cu level on mortality.

The growth response of 5- to 15-d-old poults to dietary Cu and water Cu supplementation was not positive. In addition, simultaneous supplementation of feed and water with Cu for coccidiosis-infected poults increases liver Cu accumulation and thus the potential for Cu toxicity. The likelihood of the concurrent occurrence of these factors in commercial practice accentuates the importance of this information for the turkey industry.

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