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Ward, T., Watkins, K., & Southern, L. (1994). Interactive effects of dietary copper and water copper level on growth, water intake, and plasma and liver copper concentrations of poults.. *Poultry science, 73* (8), 1306-1311. https://doi.org/10.3382/ps.0731306

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Interactive Effects of Dietary Copper and Water Copper Level on Growth, Water Intake, and Plasma and Liver Copper Concentrations of Poults[1]

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ABSTRACT The interactive effects of dietary Cu and water Cu on poult growth, water intake, whole-blood hemoglobin (Hb), and plasma and liver Cu concentrations were investigated using 232 9-d-old toms. Poults were randomly assigned to two levels of dietary Cu [Basal (B) and B + 204 mg Cu/kg diet] and to four levels of water Cu (0, 51.5, 103, and 206 mg Cu/kg water) in a 2 × 4 factorial arrangement of treatments. Each treatment was replicated six times. All poults received tap water during Days 1 to 5 followed by the water Cu treatments during Days 6 to 10. Water intake was measured daily and gain and feed intake were determined on Days 5 and 10. Day 1 to 5 gain was reduced ($P < .02$) in poults fed supplemental dietary Cu. Day 1 to 5 feed intake and gain:feed and Day 6 to 10 gain, feed intake, gain:feed, and water intake were not affected ($P > .10$) by dietary Cu. Liver Cu concentration was increased ($P < .01$) in poults fed supplemental dietary Cu, but Hb and plasma Cu concentrations were not affected ($P > .10$) by dietary Cu level. Day 6 to 10 gain, feed intake, gain:feed, and daily water intake were decreased (quadratic effect, $P < .01$) in poults fed supplemental water Cu. Plasma Cu concentration was increased (linear effect, $P < .04$) in poults fed supplemental water Cu, but Hb concentration was not affected ($P > .10$) by water Cu. Liver Cu concentration was increased more in poults receiving both supplemental dietary Cu and water Cu than in poults receiving either Cu source alone (dietary Cu by water Cu quadratic, $P < .10$). Addition of 206 ppm supplemental Cu to the water decreased poult growth and water intake. Supplementation of feed and water with Cu increased liver Cu accumulation in poults, and the effect was greater when feed and water Cu were provided simultaneously.

(*Key words*: poult, growth performance, copper, water intake, liver copper)

1994 Poultry Science 73:1306–1311

INTRODUCTION

The addition of dietary Cu in excess of the nutritional requirement to swine and poultry diets has been common practice for many years. The excess supplemental Cu has been reported to have growth-promoting effects (Burnell *et al.*, 1988; Cromwell *et al.*, 1989; Dove, 1993), which have been attributed to the antibacterial activity of Cu (Johnson *et al.*, 1985; Varel *et al.*, 1987). The data regarding Cu supplementation of turkey diets have yielded variable results. Weeks and Sullivan (1972) reported positive growth responses of poults fed 500 and 1,000 ppm Cu in

Received for publication December 6, 1993.
Accepted for publication March 15, 1994.
[1]Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript number 93-11-7412.
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practical rearing conditions, but decreased growth in poults reared in battery cages. Guenthner *et al.* (1978) reported positive growth response at 120 ppm Cu, but Kashani *et al.* (1986) found decreased growth to 8 wk of age from 120 and 240 ppm Cu. Negative responses were observed for both 500 and 750 ppm Cu in studies conducted by Christmas and Harms (1979); however, Harms and Buresh (1987) found a positive growth response at 500 ppm Cu and decreased growth at 750 ppm Cu. Much of this variation may be due to level of Cu supplementation, growing conditions, type of diet, and length of treatment. Excess dietary Cu also has been implicated in oral lesions (Jensen *et al.*, 1991) and gizzard erosion (Robbins and Baker, 1980) in chickens.

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, as well as supplementing the diet with Cu. The beneficial effect of combining dietary and water Cu supplementation is not supported by experimental evidence. In fact, the potential for toxicosis may be increased by simultaneously adding the two sources of Cu. Therefore, this investigation was conducted to determine the effect of dietary Cu, water Cu, or both on growth performance, water intake, whole-blood hemoglobin (Hb), and plasma and liver Cu concentrations.

MATERIALS AND METHODS

An experiment was conducted using 232 Nicholas Large White toms[4] from 9 to 19 d of age. From hatching to 8 d posthatching, all poults were fed a basal diet formulated to meet the nutrient requirements of the growing poult (NRC, 1984; 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 183 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 in 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 (32.5 g water/d) was determined using an additional water container placed in the same room as the batteries. Pen feed intake was determined on Days 5 and 10. Group pen poult weights were determined on Day 5 and individual poult weights were determined on Day 10.

Experimental Treatments

Poults were assigned to either the basal diet (Table 1) or the basal diet supplemented with .08% $CuSO_4 \cdot 5H_2O$ (204 mg Cu/kg diet) and four concentrations of Cu

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	2,881.46
Copper (analyzed),[3] mg/kg	16.53

[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 copper were analyzed and found to contain 237.5 mg Cu/kg of diet.

[4]Janssen Farms, Zeeland, MI 49464.

in the drinking water in a 2 × 4 factorial arrangement of treatments. Each treatment was replicated six times with five (Replicates 1 to 5) or four (Replicate 6) poults each. Water Cu treatments were obtained by 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, 51.5, 103, or 206 mg Cu/kg water represent 0, .5, 1, and 2 times the recommended dosage.[5] Water Cu levels were confirmed by atomic absorption spectrophotometric analysis[6] (Anonymous, 1982); Cu concentration in tap water was not detectable by atomic absorption spectrophotometry. Dietary Cu analysis revealed that the basal diet and Cu-supplemented diet contained 16.5 and 237.5 mg Cu/kg diet, respectively. Water Cu treatments were imposed from Day 6 to 10 of the experiment (six replicates). Therefore, from Day 1 to 5, only the two dietary Cu treatments were imposed (24 replicates).

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. Blood samples were kept on ice until Hb analysis[8] was completed (within 6 h). Blood samples were centrifuged (1,600 × *g* for 20 min at 4 C), and plasma was harvested and frozen for later analysis of Cu concentration 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]

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

Statistical Analyses

Growth performance data for Day 1 to 5 of the experiment were analyzed by one-way analysis of variance procedures appropriate for 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 effects with 24 replicates per dietary treatment. Growth performance data for Days 6 to 10 were analyzed as a factorial arrangement of treatments in a completely random design. In addition, due to a reduction in growth by dietary Cu from Day 1 to 5, Day 5 body weight was used as a covariate in the model for Day 6 to 10 growth data. Whole-blood Hb and plasma and liver Cu data were analyzed as a factorial arrangement of treatments in a completely random design, without covariate 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.

RESULTS AND DISCUSSION

Gain was decreased ($P < .02$) in poults consuming supplemental dietary Cu from Days 1 to 5 of the experiment (Table 2). However, feed intake, gain:feed, and water intake were not affected ($P > .10$) by supplemental dietary Cu. Weeks and Sullivan (1972) reported that poult growth was depressed by 500 or 1,000 ppm dietary Cu supplementation when poults were reared in battery brooders to 4 wk of age, but these same dietary Cu levels improved gain when added in the diet of poults to 24 wk of age under commercial conditions. Christmas and Harms (1979) also reported reduced growth performance when poults were fed 500 or 750 ppm Cu to 21 d of age in battery brooders. Kashani *et al.* (1986) reported improved growth performance in poults fed 60 ppm dietary Cu to 8 wk of age, but 120 and 240 ppm dietary Cu reduced growth. Poults were reared in floor pens using wood shavings for litter. However, Guenthner *et*

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

Supplemental dietary Cu	Daily gain	Daily feed intake	Gain: feed	Daily water intake
(mg/kg)	(g per poult)		(g:g)	(g per poult)
0	36.2	41.9	.865	119.2
204	34.8	41.2	.846	119.3
SEM	.4	.3	.009	1.4
Source of variation	Probability > F			
Dietary Cu	.0194	NS	NS	NS

[1]Data are means of 24 replicates of 5 (20 replicates) or 4 (4 replicates) Nicholas Large White toms. Average initial weight was 183 g.

TABLE 3. Growth performance, whole-blood hemoglobin, and plasma Cu concentrations of poults fed 0 or 204 ppm supplemental dietary Cu with 0, 51.5, 103, or 206 ppm water Cu from Day 6 to 10 of the experiment[1]

Cu treatment		Daily Cu intake[2]		Daily gain	Daily feed intake	Gain: feed	Whole-blood hemoglobin	Plasma Cu
Dietary	Water	Dietary	Water					
(mg/kg)		(mg per poult)		(g per poult)		(g:g)	(g/dL)	(μg/mL)
0	0	1.0	0	46.4	60.9	.762	8.9	.29
0	51.5	1.0	8.3	45.9	59.0	.777	8.8	.32
0	103	1.0	16.7	45.1	58.0	.778	9.0	.32
0	206	.8	24.9	32.1	47.6	.674	9.3	.35
204	0	14.3	0	46.3	60.0	.772	8.9	.29
204	51.5	14.3	8.1	46.5	60.2	.780	8.1	.34
204	103	13.5	16.5	44.6	57.0	.783	8.8	.32
204	206	11.1	25.5	33.7	46.9	.715	9.0	.43
SEM				1.6	1.6	.025	.4	.05
Main effects								
Dietary Cu								
0				42.4	56.4	.748	9.0	.32
204				42.8	56.0	.763	8.7	.35
Water Cu								
0				46.4	60.5	.767	8.9	.29
51.5				46.2	59.6	.779	8.5	.33
103				44.9	57.5	.781	8.9	.32
206				32.9	47.3	.695	9.2	.39
Source of variation[3]				Probability > F				
Day 5 BW[4]				.0730	.0594	NS		
Dietary Cu				NS	NS	NS	NS	NS
Cu water linear				.0001	.0001	.0030	NS	.0352
Cu water quadratic				.0003	.0092	.0263	NS	NS

[1]Data are means of six replicates of five (Replicates 1 to 5) or four (Replicate 6) Nicholas Large White toms each. Average initial weight was 183 g.

[2]Copper intake calculated using analyzed Cu levels of 16.53 and 237.5 mg Cu/kg diet and 0, 51.5, 103, and 206 mg Cu/kg water.

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

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

al. (1978) reported that 120 ppm dietary Cu stimulated poult growth in a study using floor pens with corn cobs for litter. Thus, it seems that the effect of excess dietary Cu is dependent on the level of dietary Cu as well as the growing conditions. However, the previous investigators did not evaluate both feed and water as sources of excess Cu.

Incongruent with the Day 1 to 5 gain, Day 6 to 10 poult gain was not affected ($P > .10$) by dietary Cu (Table 3). Feed intake, gain:feed, and water intake also were not affected ($P > .10$) by dietary Cu level. These observations agree with other data that have shown a growth reduction by dietary Cu early in the life of the poult, but not in later life (Weeks and Sullivan, 1972; Kashani *et al.*, 1986). The reduction in growth due to excess dietary Cu from Days 1 to 5 and the lack of response to dietary Cu from Days 6 to 10 may reflect a period of adaptation.

Day 6 to 10 gain, feed intake, and gain: feed were reduced (quadratic effect, $P < .03$) in poults fed supplemental water Cu and most of the response occurred at the highest (206 mg Cu/kg water) water Cu level (Table 3). Daily water intake of poults receiving either 0 or 51.5 mg Cu/kg water was less in poults fed 204 ppm dietary Cu than in poults fed the basal level of Cu (Figure 1A). However, daily water intake of poults receiving either 103 or 206 mg Cu/kg water was greater in poults fed 204 ppm Cu than in poults fed the basal level of Cu (dietary Cu by water Cu interaction, $P < .10$). Thus, it seems that poults were able to minimally adapt to excess Cu in the water if they previously had been fed Cu in the diet. The lack of a reduction in gain for Days 6 to 10 due to excess dietary Cu after reduced Day 1 to 5 gain points to an adaptive response. Also, the greater water intake of poults when fed two sources of excess Cu further indicates this adaptation response.

Poult Hb concentration was not affected ($P > .10$) by dietary Cu or by water Cu (Table 3). Lack of a decrease in Hb related to excess Cu indicates that the diet supplied sufficient Fe; excess Cu decreases Hb only in cases of Fe deficiency (Waibel *et al.*, 1964; Kline *et al.*, 1972). Plasma Cu concentration was not affected ($P > .10$) by dietary Cu level but was increased (linear effect, $P < .04$) by water Cu. Ledoux and co-workers (1987) reported that 800 ppm dietary Cu was required to increase plasma Cu concentration. Water intake on average was 2.7 times feed intake. Even though water intake was decreased at the higher water Cu concentrations, Cu intake was greater from water than from feed. Increased Cu intake from water consequently increased total daily Cu intake and thus may account for the increase in plasma Cu. The majority of the increase in

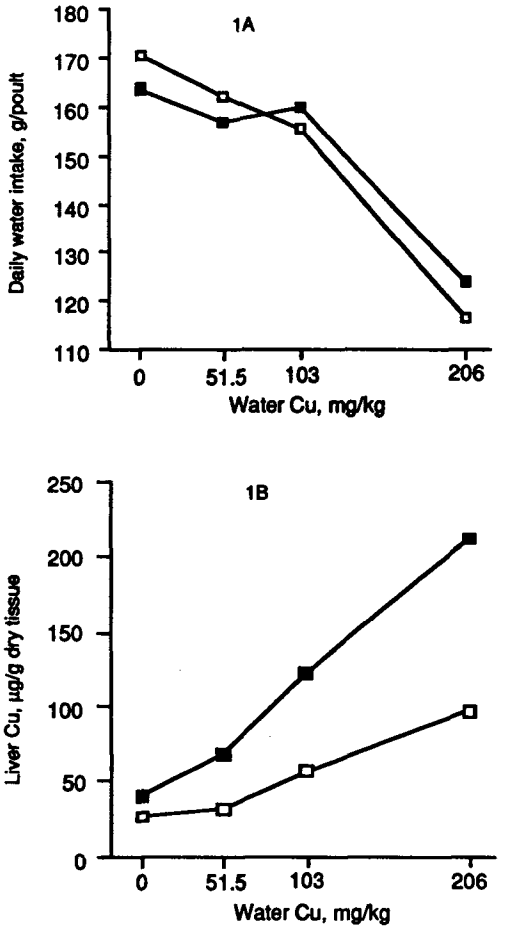

FIGURE 1. Daily water intake from Day 6 to 10 of the experiment (1A), and liver Cu concentration (1B) of poults fed 0 (□) or 204 (■) ppm supplemental dietary Cu with 0, 51.5, 103, or 206 ppm water Cu. The pooled SEM was 1.6 and 7.3 for daily water intake and liver Cu concentration, respectively. Liver Cu data were log-transformed [ln (y + 1)] for statistical analysis; transformed data SEM = .08.

plasma Cu occurred at the highest concentration of water Cu. The increase in plasma Cu indicates Cu absorption by an unsaturable (simple diffusion) mechanism (Davis and Mertz, 1987).

Liver Cu concentrations have been used to estimate Cu status and to estimate bioavailability of Cu sources (Baker *et al.*, 1991; Ledoux *et al.*, 1991). Liver Cu concentration was increased in poults fed supplemental dietary Cu ($P < .01$) and in poults fed supplemental water Cu (cubic effect, $P < .05$) (Figure 1B). Liver Cu concentration was increased more in poults receiving both supplemental dietary and water Cu than in poults receiving either treatment alone (dietary Cu by water Cu quadratic, $P < .10$).

The dietary Cu-induced increase in liver Cu concentration was expected and has been reported in poultry (Southern and Baker, 1983; Ledoux *et al.*, 1987) and swine (Cromwell *et al.*, 1989; Ward *et al.*, 1991). However, the greater than additive response in liver Cu concentration resulting from both dietary and water Cu supplementation has not been reported. Adding Cu to the drinking water of poults (particularly in excess of 100 mg Cu/kg water) may decrease growth. In addition, simultaneous supplementation of feed and water with Cu increases liver Cu accumulation in poults. The long-term effects of such tissue Cu accumulation are not known.

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