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Effect of Ammonium Chloride Ingestion on *Eimeria acervulina*-Infected Chicks Fed Excess Copper[1]

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ABSTRACT Four experiments were conducted with broiler chicks to investigate the effect of dietary NH_4Cl on gain, feed efficiency, duodenal pH, and liver Cu concentration of *Eimeria acervulina*-infected chicks. Experimental coccidial infection reduced chick gain, feed efficiency, and duodenal pH, but it increased liver Cu concentration in chicks fed excess Cu. Ammonium chloride had no effect on gain, feed efficiency, liver Cu concentration, or on duodenal pH.
(*Key words:* chicks, gain, feed efficiency, liver Cu, coccidiosis, ammonium chloride).

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INTRODUCTION

Eimeria acervulina infections (duodenal coccidiosis) are a major concern of the poultry industry. Economic losses due to depressed growth rate and feed efficiency, as well as additional adverse pathological symptoms (Stephens *et al.*, 1974; Allen and Danforth, 1984), are observed in infected chicks. For example, duodenal pH is reduced (Ruff and Reid, 1975) and trace element retention is increased during coccidial infections (Southern and Baker, 1982a,b; 1983a,b; Brown and Southern, 1985a, 1986). Hungerford and Linder (1983) and Menard *et al.* (1983) have suggested that reduced duodenal pH may be important in trace mineral availability. Therefore, coccidiosis-induced uptake of trace elements may be the result of an enhanced availability of the element due in part to the reduction in duodenal pH.

Ammonium chloride has been used to acidify diets (Barzel, 1975; Petito and Evans, 1981, 1984). The reduction in dietary pH may enhance trace mineral absorption in a manner similar to that observed during *E. acervulina* infection. The purpose of this investigation was to determine the interaction between dietary NH_4Cl and experimental *E. acervulina* infection in broiler chicks fed high levels of Cu. Gain, feed efficiency, duodenal pH, and liver Cu concentration were used as response criteria. Gain and feed efficiency are sensitive tools for evaluating *E. acervulina* infections (Hill *et al.*, 1985) and liver Cu concentration increases dramatically in *E. acervulina*-infected chicks fed high Cu diets (Southern and Baker, 1983a).

MATERIALS AND METHODS

Four experiments were conducted with Arbor Acre × Peterson crossbred broiler chicks. From hatching to four days posthatching, the chicks received a corn-soybean meal diet (Table 1). After an overnight fast (feed and water), chicks were inspected for fecal pasting and navel infection and discarded if either condition existed. Chicks were then weighed, wingbanded, and randomly assigned to treatment groups. Chicks were provided continuous light and housed in heated, thermostatically controlled starter batteries (mean temperature of 35 C) with raised wire floors. Three replicates of five (Experiment 1) or six (Experiments 2, 3, and 4) chicks were assigned to each treatment. Average initial weights of chicks were 64.7, 103.0, 68.5, and 70.7 g in Experiments 1 to 4, respectively. The experimental periods were 5 to 13 (Experiment 1) or 5 to 15 (Experiments 2, 3, and 4) days posthatching and chicks were allowed *ad libitum* access to experimental diets and tap water.

The basal diet (Table 1) was a corn-soybean meal diet formulated to meet or exceed the nutrient requirements of growing chicks (National Research Council, 1984). Dietary additions

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TABLE 1. *Composition of the basal diet.*[1]

Ingredient	(%)
Dextrose	to 100.00
Corn (8.6% CP)	45.94
Soybean meal (44% CP)	42.50
Corn oil	5.00
Alfalfa leaf meal	2.00
Defluorinated rock phosphate	2.10
Oyster shell flour	.40
NaCl	.40
Vitamin mix[2]	.25
DL-Methionine	.15
$MnSO_4 \cdot H_2O$ (32.51% Mn)	.05
$ZnCO_3$	.01

[1] Calculated composition of diet: crude protein (CP), 23%; lysine, 1.37%; methionine, .52%; cystine, .37%; Ca, .99% total P, .79%; metabolizable energy, 3,000 kcal/kg.

[2] Roche Chemical Division, Nutley, NJ. Provided the following per kilogram of diet: retinyl acetate, 6,614 IU; cholecalciferol, 1,653 IU; dl-alpha-tocopheryl acetate, 7 IU; vitamin B_{12}, 11 μg; riboflavin, 6.6 mg; niacin, 33.1 mg; d-pantothenic acid, 11.0 mg; choline, 551 mg; menadione, 1.5 mg; folic acid, .7 mg; pyridoxine, 1.1 mg; thiamin, 1.1 mg; d-biotin, 55 μg.

were made to the basal diet at the expense of dextrose and Cu was supplied by $CuSO_4 \cdot 5H_2O$.

Coccidial infections were established by crop intubation of a 1 mL aqueous inoculum containing 4×10^5 sporulated *E. acervulina* oocysts on Days 0, 3, and 6 of the experiments. Infections were administered as previously described (Brown and Southern, 1986).

In Experiment 1, two levels of NH_4Cl (0 and 1%) and two levels of Cu (0 and 500 ppm) were fed in a 2×2 factorial arrangement of treatments. The design of Experiment 2 was similar to that of Experiment 1 except that experimental diets were fed to both healthy and to *E. acervulina*-infected chicks in a $2 \times 2 \times 2$ factorial arrangement of treatments. The design of Experiments 3 and 4 was identical to that of Experiment 2.

On Day 8 of Experiment 1 and day 10 of Experiments 2, 3, and 4, all chicks were weighed and three intermediate weight chicks per replicate were killed by cervical dislocation. The duodenum was immediately excised and pH of the contents determined as previously described (Brown and Southern, 1985b). Liver samples were taken from these three chicks and dried at 100 C, wet ashed with NHO_3 and H_2O_2 and analyzed for Cu by atomic absorption spectrophotometry.

Data were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for factorially arranged treatments. Orthogonal single degree-of-freedom comparisons were used to test treatment differences. Treatment variances for liver Cu data were heterogeneous; therefore, one was added to each observation and the data were log-transformed (natural) for statistical analysis. The results of Experiments 2, 3, and 4 were combined for statistical analysis after error variances were found to be homogeneous ($P>.10$). Error variances attributed to the treatment × experiment interactions were used as the error term to test treatment effects.

RESULTS AND DISCUSSION

In Experiment 1 (data not shown) gain, feed efficiency, and duodenal pH were not affected ($P>.10$) by NH_4Cl or by the addition of excess Cu to the diet. Liver Cu concentration, however, was increased ($P<.01$) by the addition of 500 ppm Cu.

The combined results of Experiments 2, 3, and 4 are presented in Table 2. Coccidial infection reduced ($P<.01$) gain, feed efficiency, and duodenal pH, but it increased ($P<.01$) liver Cu concentration. Similarly, the addition of 500 ppm Cu to the diet reduced gain ($P<.01$), duodenal pH ($P<.01$), and feed efficiency ($P<.07$) but it increased liver Cu accumulation. A coccidiosis × Cu interaction was detected in all response criteria. Infected chicks fed the high Cu diets had lower ($P<.03$) gain, feed efficiency, and duodenal pH, but higher liver Cu concentration than uninfected chicks fed the same high Cu diets. Dietary NH_4Cl had no significant effect on any of the response criteria studied. There was a tendency, however, for duodenal pH to be lower in coccidiosis-infected chicks fed NH_4Cl (coccidiosis × NH_4Cl interaction, $P<.08$).

The results of this investigation confirm previous research that duodenal coccidiosis increases Cu retention in chicks (Southern and Baker, 1983a; Brown and Southern, 1986). Ammonium chloride had little affect on any of the cirteria assessed in this investigation. This would indicate that the homeostatic mechanisms involved in controlling intestinal tract pH are

TABLE 2. *Gain, feed efficiency, duodenal pH, and liver copper concentration of control (−) and E. acervulina-infected (+) chicks fed 500 ppm Cu and/or 1% NH_4Cl (Experiments 2, 3 and 4)*[1]

Dietary additions	Gain[2]		Gain:feed[2]		Duodenal pH[2,3]		Liver Cu[2,4]	
	−	+	−	+	−	+	−	+
	(g)		(g/g)				(ppm)	
Basal (B)	285	170	.763	.592	6.42	5.87	14	15
B+1% NH_4Cl	273	169	.753	.606	6.51	5.30	13	14
B+500 ppm Cu	265	124	.749	.508	6.39	4.83	259	1,334
B+NH_4Cl+Cu[5]	266	112	.798	.498	6.34	4.48	288	1,399
Pooled SEM	11.1		.029		.18		169	

[1]Data are means of nine replicates of six (gain and gains:feed) or three (duodenal pH and liver Cu) chicks each on Day 10 of the experiments; combined average initial weight was 80.7 g (103.0, 68.5, and 70.7 g for Experiments 2 to 4, respectively).

[2]Coccidiosis effect was significant at $P<.01$; Cu effect for gain, duodenal pH, and liver Cu was significant of $P<.01$ and for gain:feed at $P<.07$; coccidiosis × Cu interaction effect was significant at $P<.03$.

[3]Coccidiosis × NH_4Cl interaction was significant at $P<.08$.

[4]Treatment variances were heterogeneous; therefore, data were log (natural) transformed for statistical analysis. Pooled standard error of the mean (SEM) for log-transformed data was .159.

[5]1% NH_4Cl + 500 ppm Cu.

capable of neutralizing dietary insults. Hurwitz and Bar (1968) reported that the chick intestine was capable of remarkable adjustment to perfusion of HCl and NaOH. Duodenal pH was reduced by the coccidial infection, however, and the intestinal tract appeared less capable of neutralizing dietary insults during the infection. This was indicated by the lower duodenal pH of infected chicks fed NH_4Cl. This effect of NH_4Cl on duodenal pH of coccidiosis-infected chicks was not conveyed to liver Cu accumulation; i.e., NH_4Cl did not affect liver Cu concentration. Results of this investigation indicate that NH_4Cl does not affect intestinal tract pH of uninfected chicks and that it does not affect the *E. acervulina*-induced increase in Cu absorption.

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