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Dietary Cimetidine and Copper in *Eimeria acervulina*-Infected Chicks[1] (42264)

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Abstract. The effects of varying levels of cimetidine (*N''*-cyano-*N*-methyl-*N'*-{2-[(5-methylimidazol-4-yl)methylthio]-ethyl}guanidine) on *Eimeria acervulina* (duodenal coccidiosis)-induced changes in gain, efficiency, duodenal pH, and liver copper concentration of chicks were investigated. In a preliminary trial, gain, efficiency, and duodenal pH were significantly reduced in chicks inoculated one time with 1×10^6 sporulated oocysts. Dietary addition of 121 ppm monensin (2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decane-7-butyric acid) prevented these coccidiosis-induced aberrations. In subsequent trials, growth rate, feed efficiency, and duodenal pH were reduced by *E. acervulina* infection, but were unaffected ($P > 0.10$) by dietary addition of 0.01% cimetidine. Linear depressions ($P < 0.05$) in gain and efficiency, however, were observed from 0.05 and 0.10% cimetidine additions. Dietary addition of 500 ppm copper increased liver copper levels thirtyfold ($P < 0.01$) after 2 weeks. Significant coccidiosis × copper interactions were detected in gain, efficiency, duodenal pH reduction, and liver copper elevation of chicks repeatedly inoculated with 4×10^5 sporulated *E. acervulina* oocysts. Coccidiosis increased liver copper levels ($P < 0.01$) of chicks fed excess copper an additional threefold compared with uninfected chicks fed excess copper. Dietary additions of 0.01, 0.05 or 0.10% cimetidine were ineffective in preventing coccidiosis-associated performance and duodenal pH depressions as well as the coccidiosis-induced liver copper elevation. Apparently, host response to cimetidine is minor in comparison to effects of coccidia on duodenal pH. Increased copper solubility at low duodenal pH may explain high tissue copper levels and enhanced copper toxicity due to coccidiosis.

The mechanism by which infection with the sporozoan *Eimeria acervulina* (duodenal coccidiosis) increases tissue trace metal concentration in chicks (1–4) is not known. Recently reported trace metal interactions in *E. acervulina*-infected chicks (5, 6) are inconsistent with a proposed (1, 7) generalized increase in metal ion uptake by infusion across sporozoite-damaged intestinal mucosa. Moreover, gut permeability changes due to coccidiosis in general favor leakage *into* the intestinal lumen, and not across the serosa into the blood stream (8).

A well-documented intestinal pH decrease occurs during coccidiosis. Trace metal availability and/or absorption are thought to be altered by pH-mediated processes. For example, zinc uptake has been proposed to depend on variable, pH-dependent degrees of ionization of ligands in the intestinal lumen, rendering zinc more or less available for transport across membranes (9). Coccidiosis-exacerbated toxicities of trace metals might thus be alleviated simply by reversing intestinal pH changes resulting from the infection. Cimetidine (*N''*-cyano-N-methyl-*N'*-{2-[(5-methylimidazol-4-yl)methylthio]-ethyl}guanidine), a histamine H_2-receptor antagonist, is commonly used in treatment of human duodenal ulcers (10) and is a potent inhibitor of gastric acid secretion in chicks (11, 12). Also, Hall and Oddy (13) recently reported the anthelmintic action of cimetidine against abomasal parasites in sheep, and a concomitant increase in abomasal pH.

The present investigation was conducted to evaluate use of dietary cimetidine to counter coccidiosis-induced decreases in intestinal pH that may lead to increased tissue copper concentrations. Response variables examined included growth, efficiency of growth, duodenal pH, and liver copper concentration in control and *E. acervulina*-infected chicks.

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Materials and Methods. *Animals and diet.* Male Cobb chicks from the Louisiana State University Agricultural Center were fed a corn–soybean meal diet (Table I) from hatching to 4 days posthatching. After an overnight fast, the chicks were inspected for navel infection and fecal pasting, and then weighed, wing-banded, and randomly assigned to treatments. The chicks were maintained on a 24-hr constant light schedule in stainless-steel, heated, thermostatically controlled starter batteries (mean temperature was 35°C) with raised wire floors.

The control diet was a corn–soybean meal diet (Table I) formulated to meet the nutrient requirements of growing chicks (14). Dietary treatment additions, specified by trial below, were made to the control diet at the expense of glucose · H_2O. Feed and water were provided *ad libitum*.

Coccidial infections. Experimental coccidial infections were established by 1-ml crop intubation of an aqueous inoculum containing 1×10^6 sporulated *E. acervulina* oocysts once on Day 4 (trial 1) or Day 2 (trials 2 and 3), or 4×10^5 oocysts every third day (trials 4 and 5) of the experimental periods. Previous experience (6) indicated inoculation at these rates results in moderate coccidial infections with 20 to 50% reductions in gain. Sporulated oocysts were enumerated by microscopic examination of the inoculum. Presence of infection was verified by recovery of viable oocysts shed in the feces, and by patent intestinal lesions (typical scores 1–2) characteristic of *E. acervulina* infection (15). Uninfected chicks received tap water sham inoculations.

TABLE I. COMPOSITION OF BASAL DIET[a]

Ingredient	%
Glucose · H_2O[b]	to 100.00
Ground corn[c] (8.6% CP)	46.58
Soybean meal[c] (44.0% CP)	42.50
Vegetable oil[d]	5.00
Alfalfa meal, dehyd.[e] (17.0% CP)	2.00
Defluorinated rock phosphate[f]	2.10
Oyster shell flour[g]	0.40
NaCl[h]	0.40
Vitamin premix[i]	0.25
DL-methionine[j]	0.15
$MnSO_4 \cdot H_2O$[h]	0.05
$ZnCO_3$[h]	0.01

[a] Contains 23.0% crude protein (CP).
[b] A. E. Staley Manufacturing Co., Decatur, Ill.
[c] Rex Milling Co., Inc., New Iberia, La.
[d] Bunge Oil, Atlanta, Ga.
[e] Bert and Wetta Sales, Inc., Maize, Kans.
[f] Amax Phosphate Inc., Plant City, Fla.
[g] Southern Industry Corp., Mobile Ala.
[h] Mallinckrodt, Inc., Paris, Ky.
[i] Roche Chemical Division, Nutley, N.J. Provided the following per kilogram of diet: retinyl acetate, 6614 IU; cholecalciferol, 1653 IU; *dl*-α-tocopherol 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, 0.7 mg; pyridoxine, 1.1 mg; thiamin, 1.1 mg; *d*-biotin, 55 μg.
[j] Degussa Corp., Teterboro, N.J.

Duodenal pH. For measurement of duodenal pH[3], chicks were euthanized by cervical dislocation. The duodenal loop, twice the length of the pancreas, was excised and its contents expressed into a test tube with a minimum volume of distilled water. The pH of the contents was determined immediately with an electronic pH meter and semimicro combination electrode suitable for *in vitro* diagnostic use (16, 17).

Trials. A preliminary study (trial 1) was conducted to confirm the effects of *E. acervulina* infection in chicks on duodenal pH, gain, and efficiency, and to assess the effects of a conventional anticoccidial on the infection. Zero or 121 ppm of the ionophore monensin[4] (2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decane-7-butyric acid) were added to the control diet, and fed in the presence and absence of coccidiosis, constituting a 2 × 2 factorial arrangement of treatments (18). Three replications of six chicks were allotted to each treatment. On each of Days 4 through 7 postinoculation (PI), one chick was randomly selected from each replicate for duodenal pH measurement. Gain and efficiency data were taken on Day 8 of the experiment (4 days PI).

In trials 2 and 3, graded levels of cimetidine[5] were added to the basal diet to assess their ef-

[3] For brevity and simplicity, acidity of the intestinal milieu, rigorously the mean hydrogen ion activity of heterogenous nondilute suspensions, is commonly referred to in terms of pH.
[4] Eli Lilly and Co., Indianapolis, Ind. 46285.
[5] Sigma Chemical Co., St. Louis, Mo. 63178.

fect on performance and duodenal pH of uninfected and *E. acervulina*-infected chicks. Cimetidine was added at 0 and 0.01% (trial 2) and at 0, 0.05, and 0.10% (trial 3) of basal diet. Treatments were arranged factorially with four replications of six (trial 2) or four (trial 3) chicks each. Duodenal pH measurements were as in trial 1, and gain and efficiency data are for Day 6 of the experiment (4 days PI).

Trials 4 and 5 were conducted to investigate the effect of cimetidine on the *E. acervulina*-induced increase in tissue copper concentration. Uninfected and infected chicks were fed either of two levels of added cimetidine (0 or 0.01% in trial 4; 0 or 0.05% in trial 5) and/or two levels of added copper (0 or 500 ppm, from sulfate pentahydrate) constituting $2 \times 2 \times 2$ factorially arranged treatments. There were three replications of six (trial 4) and five (trial 5) chicks each. An extended 14-day experimental period was necessary for optimum detection of liver copper concentration differences due to treatments. The repeated inoculation schedule (4×10^5 oocysts on Days 0, 3, 6, 9, and 12 of each trial) was calculated to overcome any immunity the chicks might develop, and to prevent postimmune compensatory effects. At the termination of each trial, three chicks most uniform by weight within replicate were euthanized for duodenal pH measurement. Individual liver samples from these three chicks were pooled within replicate and analyzed for copper concentration as previously described (1).

Statistical analyses. Data from all experiments were analyzed by analysis of variance procedures appropriate for factorially arranged treatments (19). Treatment differences were tested with meaningful single degree of freedom comparisons. The pH values were analyzed statistically without transformation, as discussed by Ruff *et al.* (17).

Results. In trial 1, coccidial infection decreased chick weight gain 13.3% ($P < 0.01$). Monensin partially (37%) alleviated ($P < 0.09$) this coccidiosis-associated decrease in growth. As expected, coccidiosis reduced ($P < 0.02$) duodenal pH on Days 5, 6 and 7 PI, generally 1 pH unit lower than uninfected controls. Monensin effectively prevented ($P < 0.02$) this coccidiosis-induced duodenal pH decrease with no effect on duodenal pH of uninfected chicks.

In trials 2 and 3 (Table II), uninfected and infected chicks exhibited a linear depression in growth and growth efficiency with increasing levels of cimetidine greater than 0.01%. Dietary cimetidine did not affect duodenal pH of uninfected chicks, and was ineffective at all levels tested in influencing duodenal pH of infected chicks. The pH response over this time period in trials 2 and 3 was similar to that in

TABLE II. GAIN, EFFICIENCY, AND DUODENAL pH OF CONTROL(−) AND *Eimeria acervulina*-INFECTED(+) CHICKS FED VARYING LEVELS OF CIMETIDINE (TRIALS 2 AND 3)[a]

Trial	Dietary cimetidine (%)	Gain[d] (g)		Gain/feed[e] (g/g)		Duodenal pH[f]	
		−	+	−	+	−	+
2[b]	0	110	97	0.775	0.715	6.44	5.37
	0.01	110	96	0.761	0.725	6.51	5.81
	Pooled SEM	3.0		0.018		0.10	
3[c]	0	155	138	0.767	0.762	6.33	4.99
	0.05	121	106	0.693	0.618	6.44	5.58
	0.10	89	84	0.585	0.539	6.48	5.16
	Pooled SEM	4.8		0.026		0.18	

[a] Infected chicks (+) were inoculated once on Day 2 with 1×10^6 sporulated oocysts.
[b] Four replicates of six chicks. Average initial weight was 53.5 g.
[c] Four replicates of four chicks. Average initial weight was 77.1 g.
[d] Four days PI. Trial 2: coccidiosis effect ($P < 0.01$). Trial 3: coccidiosis and cimetidine linear effects ($P < 0.01$).
[e] Four days PI. Trial 2: coccidiosis effect ($P < 0.03$). Trial 3: cimetidine linear effect ($P < 0.01$).
[f] Five days PI. Trials 2 and 3: coccidiosis effect ($P < 0.01$).

trial 1; consequently, only the representative values from Day 5 PI are presented in Table II.

The results of trials 4 and 5 are presented in Table III. Gain and efficiency were reduced by experimental coccidiosis and the 0.05% cimetidine addition (trial 5), but not the 0.01% cimetidine addition (trial 4). Excess copper had no effect ($P > 0.10$) on gain or efficiency of uninfected chicks. As expected, however, gains and efficiencies of infected chicks fed excess Cu were depressed to an extent greater than that attributable to coccidiosis alone, as a result of coccidiosis × copper interactions ($P < 0.01$). The combination of 0.05% cimetidine and excess copper had a tendency to depress gain and efficiency of infected chicks more than cimetidine or copper fed separately.

Duodenal pH changes in infected chicks were significant across treatments when measured after 14 days on the repeated-inoculation schedule, but they were not as pronounced as when measured 5 days PI. A coccidiosis × copper interaction ($P < 0.01$), coincident to that in the performance data, was observed; of chicks fed excess copper, infected chicks had lower duodenal pH values than uninfected chicks, and uninfected chicks fed excess copper had duodenal pH values similar to chicks fed the control diet.

The liver copper concentrations observed (Table III) confirm the previously reported (1, 5) inducement of excess copper uptake or retention during coccidiosis. Excess dietary copper increased ($P < 0.01$) liver copper concentration thirtyfold. Again, a coincident coccidiosis × copper interaction ($P < 0.01$) was detected. There was an additional threefold increase in liver copper concentration when copper was fed to infected chicks compared

TABLE III. GAIN, EFFICIENCY, DUODENAL pH, AND LIVER COPPER CONCENTRATION OF CONTROL(−) AND *Eimeria acervulina*-INFECTED(+) CHICKS FED EXCESS COPPER AND/OR VARYING LEVELS OF CIMETIDINE (TRIALS 4 AND 5)[a]

	Dietary additions		Gain[d] (g)		Gain/feed[e] (g/g)		Duodenal pH[f]		Liver copper[g] (μg/g dry tissue)	
Trial	Cu (ppm)	Cim. (%)	−	+	−	+	−	+	−	+
4[b]	0	0	379	202	0.726	0.535	6.32	6.08	13	13
	500	0	363	154	0.732	0.518	6.26	5.00	696	1986
	0	0.01	364	219	0.686	0.571	6.53	6.18	13	13
	500	0.01	375	135	0.719	0.483	6.45	5.25	487	1955
	Pooled SEM		11		0.017		0.16		158	
5[c]	0	0	443	375	0.690	0.638	6.82	6.59	15	13
	500	0	426	255	0.722	0.572	6.84	5.57	471	1550
	0	0.05	385	313	0.670	0.626	6.82	6.70	14	12
	500	0.05	391	218	0.664	0.518	6.61	6.11	565	1679
	Pooled SEM		10		0.018		0.23		162	

[a] Infected chicks (+) were inoculated on Days 0, 3, 6, 9, and 12 with 4×10^5 sporulated oocysts. All data are for Day 14 of the trial.

[b] Three replications of six chicks each. Average initial weight was 60.1 g.

[c] Three replications of five chicks each. Average initial weight was 86.6 g.

[d] Trial 4: coccidiosis and copper effects and coccidiosis × copper interaction ($P < 0.01$). Trial 5: coccidiosis, copper, and cimetidine effects and coccidiosis × copper interaction ($P < 0.01$).

[e] Trial 4: coccidiosis effect and coccidiosis × copper interaction ($P < 0.01$). Trial 5: coccidiosis, copper, and cimetidine effects ($P < 0.02$), and coccidiosis × copper interaction ($P < 0.01$).

[f] Data are means of three uniform chicks per replicate. Trial 4: coccidiosis and copper effects and coccidiosis × copper interaction ($P < 0.01$). Trial 5: coccidiosis and copper effects ($P < 0.01$), and coccidiosis × copper interaction ($P < 0.05$).

[g] Data represent pooled samples from three uniform chicks per replicate. Treatment variances were heteroscedastic, therefore data were transformed ($\ln(x + 1)$) for analysis. Pooled SEM of transformed data = 0.093. Trials 4 and 5: coccidiosis and copper effects and coccidiosis × copper interaction ($P < 0.01$).

with uninfected chicks. A significant effect of cimetidine on liver copper was not demonstrated.

Discussion. Coccidiosis is known to increase trace metal retention (1–7) in chicks and oftentimes to exacerbate trace element toxicities. If the enhanced trace metal availability or absorption seen during coccidiosis is due to associated intestinal pH changes (decreases), reversing those pH changes should reduce trace metal toxicity during coccidiosis. Previous work (1) has demonstrated the effectiveness of the conventional anticoccidial, monensin, in maintaining performance and reducing tissue trace metal accumulation during coccidiosis. In trial 1 reported here, monensin was completely effective in ameliorating the coccidiosis-induced decrease in duodenal pH.

Cimetidine has been widely used as an oral antacid in treatment of human duodenal ulcers (10). Ward *et al.* (11) and Goto and Watanabe (12) report transitory intestinal pH increases in chicks after injection with cimetidine, as a result of its powerful antagonism of histamine-stimulated gastric acid secretion. In the present trials, daily intake of cimetidine when fed at 0.05% of diet was about equivalent to that injected by Ward *et al.* (11). Administration of cimetidine via dietary addition, however, did not produce a prolonged duodenal pH change of the magnitude reported after injecting cimetidine. The poor gains and feed efficiencies reported here from the 0.05 and 0.1% cimetidine addition, however, are consistent with impaired digestive processes resulting from reduced gastric secretions.

Cimetidine was ineffective in reversing the coccidiosis-associated reduction in pH, as well as other adverse effects attributable to coccidiosis. The reason may be that coccidia produce large amounts of lactic acid and other acids as well (8), and since cimetidine is not a buffer, it would not neutralize the acidic environment produced by coccidiosis. These results suggest that coccidia make the major contribution affecting duodenal pH decrease.

The duodenum is a major site of copper absorption (21). Acute copper poisoning in humans can result from consumption of acidic food or drink held in copper containers (22). The reduced pH increases copper solubility; thus, more copper is available for absorption. Availability of dietary copper has also been shown to be reduced by a human gastric antacid (a gel of the oxides of bismuth, aluminum, silica, magnesium, and sodium) (23). Failure of dietary cimetidine to prevent increased liver copper concentrations in *E. acervulina*-infected chicks fed excess dietary copper is thus consistent with its inability to influence intestinal tract pH.

The 500 ppm copper addition is just below the toxicity borderline for chicks. Thus, the additional performance depression attributed to coccidiosis when excess copper was fed was probably due to coccidiosis enhancing copper availability by reducing duodenal pH rather than changing direct effects of the infection. The end result is precipitation of copper toxicities by coccidiosis.

Cimetidine was totally ineffective in reducing copper absorption regardless of the infective state of the chicks. In fact, performance (trials 4 and 5) and liver copper means (trial 5) suggest cimetidine actually may have enhanced copper toxicity in infected chicks. This is in contrast to the effect on copper availability of the antacid mentioned above, and the previously reported (24, 25) inhibitory effect of cimetidine on iron absorption.

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