1-1-1998

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Interactive Effects of Sodium Bentonite and Coccidiosis with Monensin or Salinomycin in Chicks[1,2]

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ABSTRACT Three experiments (Exp.) were conducted to determine the interactive effects of sodium bentonite (NaB) with the efficacy of monensin (MON) or salinomycin (SAL) in coccidiosis-infected chicks. Male broiler chicks 5 to 14 d of age were used, and each treatment was replicated with eight (Exp. 1) or four (Exp. 2 and 3) pens of five chicks each. In Exp. 1, MON (80 ppm), NaB (0.50%), or MON+NaB were fed to uninfected and coccidiosis-infected (5 10^5 sporulated *Eimeria acervulina* oocysts on Day 2 of the Exp.) chicks in a 2 2 2 factorial arrangement of treatments. Experiment 2 was identical to Exp. 1, but SAL (30 ppm) replaced MON as the anticoccidial additive. In Exp. 3, MON (55 ppm) or SAL (22 ppm) were added individually or with NaB (0.50%) to diets for uninfected or coccidiosis-infected chicks. Coccidial infection reduced ($P < 0.01$) gain, feed intake, gain:feed, plasma carotenoids, and percentage tibia ash in all experiments. The MON and SAL additions increased these response criteria in infected chicks (coccidiosis by anticoccidial, $P < 0.07$), except MON did not increase ($P > 0.10$) feed intake or tibia ash in Exp. 3. In Exp. 3, NaB partially reduced the positive effect of MON on daily gain (NaB by MON, $P < 0.03$), and of SAL on feed intake (NaB by SAL, $P < 0.08$). the NaB addition also increased gain:feed ($P < 0.08$), and the increase was greater in infected chicks (coccidiosis by NaB, $P < 0.08$). Also in Exp. 3, SAL increased feed intake more in chicks not fed NaB than in chicks fed NaB (SAL by NaB, $P < 0.08$). Dietary NaB (0.5%) may reduce the efficacy of MON and SAL in coccidiosis-infected chicks when these additives are added at less than recommended levels.

(*Key words*: chick, sodium bentonite, coccidia, monensin, salinomycin)

1998 Poultry Science 77:600–604

INTRODUCTION

Anticoccidials are commonly used feed additives in the poultry industry. Another group of feed additives commonly added to poultry diets is agents that bind mycotoxins. Sodium bentonite (NaB), some forms of hydrated sodium calcium aluminosilicates (HSCA), and other similar dietary feed additives have been shown to decrease the severity of aflatoxicosis and other mycotoxicoses in chickens (Phillips *et al.*, 1988; Kubena *et al.*, 1990a,b; Huff *et al.*, 1992; Scheideler, 1993) and swine (Smith, 1984; Colvin *et al.*, 1989; Schell *et al.*, 1993). Some of these compounds even bind aflatoxin B_1 *in vitro* (Phillips *et al.*, 1988; Scheideler, 1993). The effectiveness of these additives apparently depends on their ability to bind aflatoxin in the intestine, resulting in the toxin being made unavailable for absorption.

Because of the unique nature of these clay feed additives, they have been increasingly incorporated into poultry and swine diets to prevent aflatoxicosis. However, many nutrients and anticoccidials are large-molecular-weight charged compounds that could also be rendered unavailable by these feed additives. Southern *et al.* (1994) reported that NaB did not adversely affect growth or tibia mineral concentrations in chicks fed nutrient-deficient diets. Chung *et al.* (1990) reported that HSCA did not affect liver vitamin A concentrations or the utilization of riboflavin. On the other hand, Schell *et al.* (1993) reported that NaB decreased the absorption and retention of some minerals in swine, and high levels of NaB have been shown to decrease the availability of vitamin A in chicks (Briggs and Fox, 1956; Laughland and Phillips, 1956). To our knowledge, there are no data

Received for publication August 5, 1997.
Accepted for publication November 19, 1997.

[1]Approved for publication by the director of the Louisiana Agricultural Experiment Station as Manuscript Number 97-11-0264.

[2]Research supported in part by Prince Agri Products, Quincy, IL 62301.

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Abbreviation Key: B = basal diet; Exp. = experiment; HSCA = hydrated sodium calcium aluminosilicate; MON = monensin; NaB = sodium bentonite; SAL = salinomycin.

TABLE 1. Basal diet composition

Ingredients	Percentage
Corn	46.18
Soybean meal, 44% CP	41.99
Alfalfa leaf meal	2.00
Corn oil	6.00
Dicalcium phosphate	1.53
Oyster shell flour	1.35
Salt	0.40
Vitamin-mineral premix[1]	0.25
Selenium premix[2]	0.10
DL-methionine	0.20
Calculated composition	
CP	22.86
Lysine	1.26
Methionine plus cystine	0.91
Calcium	1.00
Total phosphorus	0.69

[1]Provided the following per kilogram of diet: vitamin A, 11,000 IU (retinyl acetate); cholecalciferol (d-activated animal sterol), 1,650 IU; vitamin E (dl-α-tocopheryl acetate), 8 IU; menadione (menadione sodium bisulfite complex), 0.72 mg; thiamine (mononitrate), 1 mg; riboflavin, 4.4 mg; niacin, 33 mg; Ca d-pantothenate, 8.1 mg; folic acid, 0.33 mg; d-biotin, 0.055 mg; vitamin B_{12}, 0.011 mg; choline (as choline chloride), 382 mg; manganese (oxide), 60 mg; zinc (sulfate), 44 mg; iron (ferrous sulfate), 20 mg; copper (oxide) 2 mg; iodine (ethylenediamine dihydrodide), 1.2 mg; cobalt (carbonate), 0.2 mg; ethoxyquin, 125 mg.

[2]Provided 0.10 mg Se/kg of diet.

regarding clay feed additives and their effect on the efficacy of anticoccidials. Therefore, the purpose of this study was to determine whether NaB affects the efficacy of monensin (MON) or salinomycin (SAL) in coccidiosis-infected broiler chicks.

MATERIALS AND METHODS

Three experiments were conducted with male Arbor Acres broiler chicks from the Louisiana State University Poultry Science Research Farm [Experiments (Exp.) 1 and 2] or Ross Peterson[5] broiler chicks (Exp. 3). From 0 to 4 d posthatching, the chicks were fed a corn-soybean meal diet (Table 1) formulated to meet or exceed the nutrient requirements for growing chicks (National Research Council, 1994). The chicks were held without feed and water for 16 h, weighed, wing-banded, and randomly allotted to treatments. They were provided continuous light and were housed in thermostatically controlled starter batteries maintained at 35 C. The experiments were completely randomized designs, and each treatment was replicated eight (Exp. 1, two trials of four replicates each) or four (Exp. 2 and 3) times with five chicks per replicate. Average initial weight of the chicks was 88.7 (Trial 1, 86.1; Trial 2, 91.3), 64.8, and 79.8 g in Exp. 1 to 3, respectively, and each experiment lasted 9 d.

Experiment 1 consisted of eight treatments in a 2 2 2 factorial arrangement. Monensin (80 ppm), NaB (0.5%), or both were included in the diet and fed to uninfected chicks or chicks infected with 5 10^5 sporulated *Eimeria acervulina* oocysts on Day 2 of the experiment. The coccidia were given via crop intubation of a 1-mL aqueous inoculum. Uninfected chicks received 1-mL of tap water on Day 2. Experiment 1 was replicated in time, resulting in a total of eight replications per treatment. Experiment 2 was identical to Exp. 1 except the coccidiostat used was SAL included at 30 ppm, and the experiment was not replicated in time. The coccidiostats are known to have very high efficacy at levels below the recommended inclusion levels, which is the reason for the lower-than-recommended levels used in Exp. 1 and 2. The dietary levels that are approved for use in poultry are 100 to 121 ppm for MON, and 44 to 66 ppm for SAL.

Experiment 3 consisted of 12 treatments (with four replicates each) in factorial arrangement. The basal diet (B), B + MON (55 ppm), or B + SAL (22 ppm) were included with and without the addition of 0.5% NaB and these six dietary treatments were fed to uninfected or coccidiosis-infected chicks. The experimental coccidial infections were established as in Exp. 1 and 2.

At the termination of each experiment, all chicks were bled via cardiac puncture. Two milliliters of blood were collected from each chick (pooled by replicate) in tubes containing sodium heparin and the blood was immediately placed on ice. Samples were then centrifuged at 1,600 *g* for 10 min at 4 C, and the plasma was collected and frozen (–21 C). The plasma was analyzed for carotenoid concentration by the method of Allen (1987) with the following modification. Two milliliters of acetone were added to 0.5 mL of plasma to precipitate the plasma proteins. The samples were vortexed, centrifuged for 10 min at 1,500 *g*, and covered to avoid deterioration by light. Absorbance was determined 1 h later by spectrophotometric analysis at a wavelength of 456 nm using a β-carotene standard.[6]

After the chicks were bled, they were killed by cervical dislocation. The right leg of each chick was removed and the tibiatarsus was cleaned of adherent tissue, extracted (Soxhlet) continuously for 48 h in 90% ethanol and then for 48 h in anhydrous diethyl ether. The tibiae were dried at 100 C for 12 h in a forced-air oven. Dry fat-free tibias were weighed, dry ashed at 550 C for 20 h, and residual weights taken to determine percentage ash.

The data were analyzed by analysis of variance procedures appropriate for experiments with a factorial arrangement of treatments in a completely randomized design (Steel and Torrie, 1980). Orthogonal, single degree-of-freedom contrasts were used to evaluate all main effects and interactions. Trial (replicate in time) was included in the model for the data analysis of Exp. 1. Experiment 3 was analyzed as one experiment. The main effects of coccidiosis, SAL, MON, and NaB and the

[5]Sanderson Farms, Laurel, MS 39440.

[6]Sigma catalog No. C 0126; Sigma Chemical Co., St. Louis, MO 63178-9916.

TABLE 2. Weight gain, feed intake, gain:feed, tibia ash, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed monensin (MON, 80 ppm) or sodium bentonite (NaB, 0.50%), Experiment 1[1]

Dietary additions	Weight gain			Feed intake			Gain:feed ratio			Tibia ash			Plasma carotenoids		
	–[2]	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄
	(g)						(g:g)			(%)			(μg/mL)		
Basal (B)	33.4	25.1	29.3	45.2	39.5	42.4	0.74	0.63	0.69	51.3	48.0	49.7	3.08	0.57	1.83
B + MON	33.4	31.1	32.3	45.7	43.1	44.4	0.73	0.72	0.73	50.8	49.8	50.3	3.49	1.73	2.61
B + NaB	33.2	25.0	29.1	45.6	40.1	42.9	0.73	0.62	0.68	50.4	49.0	49.7	3.09	0.59	1.84
B + MON + NaB	33.3	32.5	32.9	44.6	45.1	44.9	0.75	0.72	0.74	51.3	50.3	50.8	3.03	1.48	2.26
x̄	33.3	28.4		45.3	42.0		0.74	0.67		51.0	49.3		3.17	1.09	
Pooled SEM		0.5			0.9			0.01			0.40			0.17	
Source of variation[3]	Probability														
Coccidiosis (COC)		0.01			0.01			0.01			0.01			0.01	
MON		0.01			0.01			0.01			0.01			0.01	
COC MON		0.01			0.01			0.01			0.02			0.01	

[1]Data are means of eight replicates of five chicks from 5 to 14 d posthatching; average initial weight was 88.7 g (Trial 1, 86.1; Trial 2, 91.3).

[2]Uninfected (–) or coccidiosis-infected (+) chicks.

[3]Main effects and interactions not listed were not significant ($P > 0.10$).

interactions of coccidiosis by NaB, coccidiosis by MON, coccidiosis by SAL, NaB by MON, NaB by SAL, coccidiosis by MON by NaB, and coccidiosis by SAL by NaB were evaluated. The pen of chicks was the experimental unit for all data.

RESULTS AND DISCUSSION

The experimental coccidial infections decreased ($P < 0.01$) gain, feed intake, gain:feed, tibia ash, and plasma carotenoids in all three experiments (Tables 2, 3, and 4), which is in agreement with numerous reports in the literature. In Exp. 1 and 2, the adverse effects of the coccidial infection were reversed by the addition of MON (coccidiosis by MON, $P < 0.02$; Table 2) or SAL (coccidiosis by SAL, $P < 0.01$; Table 3) administered at 80 or 30 ppm, respectively. In these two Exp., there was no main effect of NaB, and NaB did not interact with SAL, MON, or coccidiosis.

In Exp. 3 (Table 4), MON and SAL were added at approximately one-half the recommended level. The SAL addition increased (coccidiosis by SAL, $P < 0.03$) gain, feed intake, gain:feed, and plasma carotenoids in coccidiosis-infected chicks, but it was slightly less efficacious in preventing the decrease in tibia ash resulting from coccidiosis (coccidiosis by SAL, $P < 0.07$). The MON addition increased (coccidiosis by MON, $P < 0.03$) gain, gain:feed, and plasma carotenoids in coccidiosis-infected chicks, but it did not prevent the decrease ($P > 0.10$) in feed intake or tibia ash resulting from coccidiosis.

The results of Exp. 3 suggest that the levels of SAL and MON used were below the levels necessary for optimum protection against coccidiosis. Thus, if NaB was going to interfere with or accentuate the efficacy of either compound, it should have been evident in this experiment. The NaB addition increased gain:feed ($P <$

TABLE 3. Daily gain, feed intake, gain:feed, tibia ash, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed salinomycin (SAL, 30 ppm) or sodium bentonite (NaB, 0.50%), Experiment 2[1]

Dietary additions	Daily gain			Daily feed intake			Gain:feed			Tibia ash			Plasma carotenoids		
	–[2]	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄
	(g)						(g:g)			(%)			(μg/mL)		
Basal (B)	27.2	19.8	23.5	38.6	32.9	35.8	0.71	0.60	0.66	51.8	49.5	50.7	2.74	0.28	1.51
B + SAL	26.8	26.2	26.5	37.4	37.2	37.3	0.72	0.71	0.72	51.7	51.1	51.4	2.55	1.22	1.89
B + NaB	26.6	19.5	23.1	38.0	32.5	35.3	0.70	0.60	0.65	51.5	49.4	50.5	2.78	0.21	1.50
B + SAL + NaB	27.9	26.0	27.0	38.7	37.1	37.9	0.72	0.70	0.71	51.9	51.7	51.8	2.59	1.42	2.01
x̄	27.1	22.9		38.2	34.9		0.71	0.65		51.7	50.4		2.67	0.78	
Pooled SEM		0.7			0.6			0.01			0.4			0.21	
Source of variation[3]	Probability														
Coccidiosis (COC)		0.01			0.01			0.01			0.01			0.01	
SAL		0.01			0.01			0.01			0.01			0.01	
COC SAL		0.01			0.01			0.01			0.01			0.01	

[1]Data are means of four replicates of five chicks from 5 to 14 d posthatching; average initial weight was 64.8 g.

[2]Uninfected (–) or coccidiosis-infected (+) chicks.

[3]Main effects and interactions not listed were not significant ($P > 0.10$).

TABLE 4. Daily gain, feed intake, gain:feed, tibia ash, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed monensin (MON, 55 ppm) salinomycin (SAL, 22 ppm), or sodium bentonite (NaB, 0.50%), Experiment 3[1]

Dietary additions	Daily gain			Daily feed intake			Gain:feed			Tibia ash			Plasma carotenoids		
	–[2]	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄	–	+	x̄
	(g)						(g:g)			(%)			(μg/mL)		
Basal (B)	30.8	21.4	26.1	38.5	34.0	36.3	0.80	0.63	0.72	49.8	47.3	48.6	3.66	1.02	2.34
B + SAL	30.6	28.0	29.3	39.3	40.1	39.7	0.78	0.70	0.74	49.8	48.6	49.2	3.59	1.39	2.49
B + MON	30.5	28.7	29.6	38.8	38.4	38.6	0.79	0.75	0.77	50.1	48.8	49.5	3.62	1.55	2.59
B + NaB	32.4	23.7	28.1	40.5	34.9	37.7	0.80	0.68	0.74	50.6	47.0	48.8	3.33	0.99	2.16
B + SAL + NaB	30.9	28.9	29.9	38.6	38.3	38.5	0.80	0.76	0.78	49.6	49.8	49.7	3.30	1.65	2.48
B + MON + NaB	30.1	26.8	28.5	37.9	36.4	37.2	0.79	0.74	0.77	49.9	48.5	49.2	3.39	1.48	2.44
x̄	30.9	26.3		38.9	37.0		0.79	0.71		50.0	48.3		3.48	1.35	
Pooled SEM		0.7			1.0			0.02			0.6			0.14	
Source of variation[3]							Probability								
Coccidiosis (COC)		0.01			0.01			0.01			0.01			0.01	
SAL		0.01			0.01			0.02			0.07			0.03	
MON		0.01			NS			0.01			0.11			0.02	
NaB		NS			NS			0.08			NS			NS	
COC SAL		0.01			0.01			0.02			0.07			0.03	
COC MON		0.01			NS			0.01			0.11			0.02	
COC NaB		NS			NS			0.08			NS			NS	
SAL NaB		NS			0.08			NS			NS			NS	
MON NaB		0.03			NS			NS			NS			NS	

[1]Data are means of four replicates of five chicks from 5 to 14 d posthatching; average initial weight was 79.8 g.

[2]Uninfected (–) or coccidiosis-infected (+) chicks.

[3]Main effects and interactions not listed were not significant ($P > 0.10$).

0.08), but the increase only occurred in coccidiosis-infected chicks (coccidiosis by NaB, $P < 0.08$). On the other hand, NaB partially reduced the positive effect of MON on daily gain (NaB by MON, $P < 0.03$) and of SAL on feed intake (NaB by SAL, $P < 0.08$). These results suggest that NaB may interfere with the efficacy of MON and SAL, but the levels of these anticoccidials included in poultry diets used in commercial practice are such that the effects of NaB would not be deleterious.

In other research evaluating the effect of NaB on dietary nutrients, Southern *et al.* (1994) reported that NaB did not adversely affect growth or tibia mineral concentrations in chicks fed nutrient deficient diets, and Chung *et al.* (1990) reported that HSCA did not affect liver vitamin A concentrations or the utilization of riboflavin. These results differ from the results of the present study, wherein NaB was shown to slightly decrease the efficacy of MON and SAL. However, the negative effects of NaB were minor and evident only when the anticoccidials were included at levels approaching one-half of the recommended levels. Briggs and Fox (1956) and Laughland and Phillips (1956) reported that high levels of NaB decreased the availability of vitamin A in diets for chicks. These reports used much higher levels of NaB than used in the present study.

The results of this study suggest that NaB may interfere with the efficacy of MON and SAL when these compounds are added of 50% at the minimum level approved for dietary inclusion. However, the approved levels of inclusion of these coccidiostats are high enough such that a practical problem should not occur.

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