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J. O. Matthews  
*LSU Agricultural Center*

T. L. Ward  
*LSU Agricultural Center*

L. L. Southern  
*LSU Agricultural Center*

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# Interactive Effects of Betaine and Monensin in Uninfected and *Eimeria acervulina*-Infected Chicks<sup>1,2</sup>

J. O. MATTHEWS, T. L. WARD,<sup>3</sup> and L. L. SOUTHERN<sup>4</sup>

Department of Animal Science, Louisiana Agricultural Experiment Station,  
Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803-4210

**ABSTRACT** Three experiments (Exp.) were conducted to evaluate the interactive effects of dietary betaine (BET) and monensin (MON) in uninfected or *Eimeria acervulina*-infected chicks. The treatments were replicated with six (Exp. 1) or five (Exp. 2 and 3) pens of five chicks each. The experimental periods lasted 9 (Exp. 1 and 2) or 10 (Exp. 3) d each and the coccidiosis infections were established on Day 2 (Exp. 1 and 2) or Day 3 (Exp. 3) of the experiment. Average initial weight of the chicks was 101, 73, and 68 g in Exp. 1 to 3, respectively, and the initial age of the chicks was 5 (Exp. 1) or 4 (Exp. 2 and 3) d. A corn-soybean meal basal diet was used in each experiment. In Exp. 1, the effect of dietary BET (0, 0.1, or 0.5%) in uninfected or coccidiosis-infected (COC; 5 × 10<sup>5</sup> sporulated *E. acervulina* oocysts) chicks was investigated. In Exp. 2, the interactive effects of BET (0 or 0.1%) and MON (0 or 55 ppm) in uninfected or COC chicks were investigated in a 2 × 2 × 2 factorial arrangement of treatments. Experiment 3 was identical to Exp. 2, except the level of MON was 110 rather than

55 ppm. In Exp. 1, 2, and 3, COC reduced ( $P < 0.01$ ) gain, feed intake (FI), feed efficiency (GF), and plasma carotenoid concentration (CAR) and increased ( $P < 0.01$ ) lesion score (LS). In Exp. 1, gain and FI were decreased in uninfected chicks fed 0.1% BET but gain and FI were increased in COC chicks fed 0.1% BET (COC × BET quadratic,  $P < 0.01$ ). Dietary BET linearly increased ( $P < 0.05$ ) GF. In Exp. 2 and 3, MON increased ( $P < 0.01$ ) gain, FI, GF, and CAR and decreased ( $P < 0.01$ ) LS of COC chicks, but MON had no effect in uninfected chicks (COC × MON,  $P < 0.01$ ). In Exp. 2, GF was increased more in chicks fed both MON and BET than in chicks fed MON (BET × MON,  $P < 0.06$ ). In Exp. 3, BET increased GF of uninfected chicks fed MON and of COC chicks not fed MON (COC × BET × MON,  $P < 0.02$ ). Betaine may have an effect on *E. acervulina*-infected chicks, but there is no conclusive evidence to indicate that the efficacy of MON is improved when fed in combination with BET.

(Key words: chicks, coccidia, monensin, betaine, growth)

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## INTRODUCTION

The use and evaluation of betaine (trimethyl glycine) as a feed additive in the diets of pigs (Smith *et al.*, 1994; Matthews *et al.*, 1995), chicks (Saunderson and MacKinnlay, 1990; Virtanen *et al.*, 1996; Remus and Virtanen, 1996; Zimmermann *et al.*, 1996), and salmon (Virtanen *et al.*, 1989) has increased recently. It is used to replace a portion of the sulfur amino acid requirement, which has been a topic of interest among nutritionists for some time (McKittrick, 1947; Pesti *et al.*, 1979; Remus and Virtanen, 1996; Zimmermann *et al.*, 1996). In pigs,

betaine was investigated and used as a means to increase lean and decrease fat in the carcass (Smith *et al.*, 1994; Matthews *et al.*, 1995).

Coccidiosis is one of the major disease problems in the poultry industry. Coccidiosis results in reduced growth and feed efficiency, and causes ionic and osmotic disorders of the gut, which are evident by reduced duodenal pH (Ruff and Reid, 1975; Fox and Southern, 1987) and absorption of nutrients (Turk and Stephens, 1970; Ruff *et al.*, 1976). Betaine has been shown to be an osmoprotectant for *Bacillus subtilis* (Boch *et al.*, 1994), and betaine accumulation in the renal medulla cells allows the cells to tolerate osmotic stress (Bagnasco *et al.*, 1986).

Currently, literature concerning betaine fed in combination with coccidiosis infections in chicks is limited. Remus and Virtanen (1996) reported that betaine and methionine were equally efficacious in diets for coccidiosis-infected chicks. Zimmermann *et al.* (1996) reported similar results for chicks fed 0.05 mg/kg betaine, but not in chicks fed 0.1 mg/kg betaine. In both

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<sup>3</sup>Present address: Consolidated Nutrition, L. C., Ft. Wayne, IN 46801-2508.

<sup>4</sup>To whom correspondence should be addressed.

TABLE 1. Basal diet composition

Ingredients and composition	Percentage
Corn	45.57
Soybean meal, 44% CP	42.34
Alfalfa leaf meal	2.00
Corn oil	6.00
Monocalcium phosphate	1.40
Oyster shell flour	1.62
Salt	0.40
Vitamin-mineral premix <sup>1</sup>	0.25
DL-methionine	0.25
Selenium premix <sup>2</sup>	0.10
Choline premix <sup>3</sup>	0.07
Calculated composition	
CP	23.00
Lysine	1.27
Methionine	0.59
Methionine and cystine	0.96
Calcium	1.00
Total phosphorus	0.70
Choline, mg/kg diet	2,213
Metabolizable energy, kcal/kg	2,975

<sup>1</sup>Provides the following per kilogram of diet: vitamin A, 11,000 IU (retinyl acetate); cholecalciferol (d-activated animal sterol), 1,650 IU; vitamin E (dl- $\alpha$ -tocopheryl acetate, 8 IU; menadione (menadione sodium bisulfite complex), 0.72 mg; thiamine (mononitrate), 1 mg; riboflavin, 4.4 mg; niacin, 33 mg; d-pantothenic acid (d-Ca pantothenate), 8.1 mg; folic acid, 0.33 mg; d-biotin, 0.055 mg; vitamin B<sub>12</sub>, 0.011 mg; choline (as chloride), 382 mg; manganese (oxide), 60 mg; zinc (sulfate), 44 mg; iron (ferrous sulfate), 20 mg; copper (oxide) 2 mg; iodine (ethylenediamine dihydride), 1.2 mg; cobalt (carbonate), 0.2 mg; ethoxyquin, 125 mg.

<sup>2</sup>Provides 0.1 mg Se/kg of diet as Na<sub>2</sub>SeO<sub>3</sub>.

<sup>3</sup>Provides 365 mg/kg of diet (total choline of 747 mg/kg from choline and vitamin premix).

of these studies, all chicks were infected with coccidiosis, and in the report by Zimmermann *et al.* (1996), all chicks received an anticoccidial medication. Virtanen *et al.* (1996) reported that betaine in combination with salinomycin was more effective in preventing the signs of coccidiosis (lesion scores and decreased feed efficiency) than salinomycin alone. As in the aforementioned research, all chicks were infected with coccidiosis.

To our knowledge, the independent and combined effects of betaine, coccidiosis, and anticoccidials have not been investigated in chicks. Thus, the purpose of this research was to determine whether betaine or betaine in combination with monensin would diminish the adverse effects of an *E. acervulina* infection in chicks.

## MATERIALS AND METHODS

Three experiments were conducted with male Arbor Acres (Exp. 1 and 2) or Hubbard Peterson (Exp. 3)

chicks. From 5 (Exp. 1) or 4 (Exp. 2 and 3) 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). Additional choline and DL-methionine were added to exceed the choline and sulfur amino acid requirements to ensure adequate methyl groups. The chicks were held without feed and water for 16 h prior to experiment initiation, then 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. Each treatment was replicated six (Exp. 1) or five (Exp. 2 and 3) times with five chicks per replicate. Average initial weight of the chicks was 101, 73, and 68 g in Exp. 1 to 3, respectively.

In Exp. 1, a 2  $\times$  3 factorial arrangement of treatments was used; three levels of betaine<sup>5</sup> (0, 0.1, or 0.5%) were fed to uninfected or *E. acervulina*-infected chicks. In Exp. 2 and 3, a 2  $\times$  2  $\times$  2 factorial arrangement of treatments was used consisting of two levels of betaine (0 or 0.1%) and two levels of monensin (Exp. 2, 0 or 55 ppm; Exp. 3, 0 or 110 ppm), which were fed to uninfected or *E. acervulina*-infected chicks. Analysis of dietary treatments for betaine<sup>6</sup> in Exp. 1 indicated levels of 0, 0.09, and 0.47%, respectively. Analysis of the diets for betaine and monensin<sup>7</sup> in Exp. 2 indicated levels of 0 and 0.09% betaine, and 0 and 51 ppm monensin. Analysis of the diets for betaine and monensin in Exp. 3 indicated levels of 0 and 0.09% betaine, and 0 and 103 ppm monensin. However, betaine and monensin levels will be referred to as the calculated levels. Coccidial infections were established by crop intubation of a 1-mL aqueous solution containing 5  $\times$  10<sup>5</sup> sporulated *E. acervulina* oocysts on Day 2 of Exp. 1 and 2, and on Day 3 of Exp. 3. Uninfected chicks received crop intubations of tap water.

Weight gain and feed intake were determined on Day 9 of Exp. 1 and 2, and on Day 10 of Exp. 3. On Day 9 of Exp. 1 and 2 and on Day 10 of Exp. 3, all chicks were bled via cardiac puncture for determination of plasma carotenoid concentrations. Blood was pooled among chicks within each pen, plasma was harvested, and frozen for subsequent analysis for carotenoid concentration. After collection of blood, chicks were killed by carbon dioxide asphyxiation, intestines were removed, and the duodenal section of the intestine was lesion-scored by methods described by Johnson and Reid (1970). Lesion scoring was conducted by the same individual for all experiments and the individual was not aware of the specific treatments imposed.

## Carotenoid Analysis

Carotenoid compounds were approximated by the methods of Allen (1987) with the following modifications. Two milliliters of acetone were added to a 0.5-mL plasma sample to precipitate proteins from the sample. Samples

<sup>5</sup>Betafin -BCR; Finnsugar Bioproducts Inc., Schaumburg, IL 60173-5008.

<sup>6</sup>Betafin analysis conducted by Finnsugar Bioproducts Inc., Schaumburg, IL 60173-5008.

<sup>7</sup>Monensin analysis conducted by Elanco Animal Health Customer Service Laboratories, Indianapolis, IN 46285.

TABLE 2. Weight gain, feed intake, gain:feed, lesion score, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed betaine (BET), (Experiment 1)<sup>1</sup>

Dietary additions	Weight gain			Feed intake			Gain:feed ratio			Lesion score <sup>2</sup>		Plasma carotenoids		
	- <sup>3</sup>	+	$\bar{x}$	-	+	$\bar{x}$	-	+	$\bar{x}$	-	+	-	+	$\bar{x}$
	(g)						(g:g)					( $\mu\text{g/mL}$ )		
Basal (B)	335	237	286	432	335	384	0.775	0.708	0.742	0	2.73	3.82	0.81	2.32
B + 0.1% BET	323	248	286	422	354	388	0.764	0.702	0.733	0	2.83	3.48	0.70	2.09
B + 0.5% BET	338	233	286	425	328	377	0.796	0.712	0.754	0	2.63	3.83	0.73	2.28
$\bar{x}$	332	239		426	339		0.778	0.707		0	2.73	3.71	0.75	
Pooled SEM	5			5			0.008			0.12		0.12		
Source of variation							Probability							
Coccidiosis (COC)							0.01			...		0.01		
BET linear							0.05			NS		NS		
BET quadratic							NS			NS		0.06		
COC BET linear							NS			...		NS		
COC BET quadratic							NS			...		NS		

<sup>1</sup>Data are means of six replicates of five chicks from 5 to 14 d posthatching; average initial weight was 101 g.

<sup>2</sup>Data for uninfected chicks was not used for analysis of lesion scores, thus only linear and quadratic contrasts were used for analysis.

<sup>3</sup>Uninfected (-) or coccidiosis-infected (+) chicks.

were then vortexed, centrifuged for 10 min at 2,800 *g*, and then covered to avoid deterioration by light. Absorbance was determined 1 h later by spectrophotometric analysis at a wavelength of 456 nm using a  $\beta$ -carotene standard.<sup>8</sup>

## Statistical Analysis

Data were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for factorially arranged treatments. Orthogonal, single degree of freedom contrasts were used to evaluate all main effects and interactions, as well as linear and quadratic effects, when appropriate. The pen of chicks served as the experimental unit for all data.

## RESULTS

In each of the three experiments, the coccidial infection reduced ( $P < 0.01$ ) weight gain, feed intake, feed efficiency, and plasma carotenoid concentrations, and increased ( $P < 0.01$ ) lesion score (Tables 2, 3, 4).

In Exp. 1, the 0.1% betaine addition decreased weight gain and feed intake in uninfected chicks, but increased weight gain and feed intake in coccidiosis-infected chicks (COC BET quadratic,  $P < 0.01$ ). The 0.5% betaine addition did not affect ( $P > 0.10$ ) gain or feed intake. Feed efficiency was increased by the 0.5% betaine addition in both coccidiosis-infected and uninfected chicks (BET linear,  $P < 0.05$ ), but the response was greater in uninfected chicks. Lesion score was not affected by the addition of betaine to the diet. Plasma carotenoid concentrations were decreased (BET quad-

atic,  $P < 0.06$ ) in chicks fed 0.1% betaine in both coccidiosis-infected and uninfected chicks.

In Exp. 2, the 55 ppm monensin increased ( $P < 0.01$ ) gain, feed intake, feed efficiency, and plasma carotenoid concentrations in coccidiosis-infected chicks, but not in uninfected chicks (COC MON,  $P < 0.01$ ). Addition of betaine increased feed efficiency in the presence of monensin, but not in the absence of monensin (BET MON,  $P < 0.06$ ). Betaine addition tended to increase feed efficiency of uninfected chicks, but tended to decrease feed efficiency of coccidiosis-infected chicks (COC BET,  $P = 0.14$ ). Betaine addition also tended to decrease (BET MON,  $P = 0.10$ ) feed intake of chicks fed monensin. Lesion score was reduced ( $P < 0.01$ ) in coccidiosis-infected chicks receiving 55 ppm monensin.

In Exp. 3, the 110 ppm monensin increased ( $P < 0.01$ ) gain, feed intake, feed efficiency, and plasma carotenoid concentrations in coccidiosis-infected chicks, but not in uninfected chicks (COC MON,  $P < 0.01$ ). Betaine addition decreased feed efficiency of uninfected and coccidiosis-infected chicks, but in combination with monensin, betaine increased feed efficiency of uninfected chicks (COC BET MON,  $P < 0.02$ ). Lesion score was reduced ( $P < 0.01$ ) in coccidiosis-infected chicks receiving 110 ppm monensin.

## DISCUSSION

In all three experiments, the coccidial infection reduced gain, feed intake, feed efficiency, and plasma carotenoid concentrations and increased lesion score. Similar results have been reported numerous times (Turk, 1981; Willis and Baker, 1981; Allen, 1987; Conway *et al.*, 1993; Fox and Southern, 1987; Tyczkowski and Hamilton, 1991; Ward *et al.*, 1990). In Exp. 2 and 3, monensin increased gain, feed intake, and feed efficiency in coccidiosis-infected chicks, but not in unin-

<sup>8</sup>Sigma Catalog no. C 0126; Sigma Chemical Co., St. Louis, MO 63178-9916.

**TABLE 3. Weight gain, feed intake, gain:feed, lesion score, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed betaine (BET), or monensin (MON) or both (Experiment 2)<sup>1</sup>**

Dietary additions	Weight gain			Feed intake			Gain:feed ratio			Lesion score <sup>2</sup>		Plasma carotenoids			
	– <sup>3</sup>	+	$\bar{x}$	–	+	$\bar{x}$	–	+	$\bar{x}$	–	+	–	+	$\bar{x}$	
	(g)						(g:g)			(μg/mL)					
Basal (B)	294	204	249	364	289	327	0.807	0.706	0.757	0	2.68	4.01	1.15	2.58	
B + 0.1% BET	292	201	247	359	294	327	0.814	0.683	0.749	0	2.08	4.27	1.03	2.65	
B + 55 ppm MON	298	246	272	375	334	355	0.796	0.737	0.767	0	1.56	4.34	1.50	2.92	
B + BET + MON	296	243	270	361	324	343	0.820	0.750	0.785	0	1.68	4.54	1.54	3.04	
$\bar{x}$	295	224		365	310		0.809	0.719		0	2.00	4.29	1.31		
Pooled SEM	5			5			0.009			0.22		0.14			
Source of variation <sup>4</sup>							Probability								
Coccidiosis (COC)	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			...		NS			
BET MON	NS			0.10			0.06			NS		NS			

<sup>1</sup>Data are means of five replicates of five chicks from 4 to 13 d posthatching; average initial weight was 73 g.

<sup>2</sup>Data for uninfected chicks was not used for analysis of lesion scores, thus only contrasts for betaine, monensin, and betaine by monensin were used for analysis.

<sup>3</sup>Uninfected (-) or coccidiosis-infected (+) chicks.

<sup>4</sup>No significant (NS;  $P > 0.10$ ) effects were observed for betaine, coccidiosis by betaine, and coccidiosis by betaine by monensin, thus, they are not included in the table.

fed chicks, resulting in coccidiosis by monensin interactions, and our data are in agreement with previous reports (Bafundo, 1986; Ward *et al.*, 1990).

The purpose of Exp. 1 was to determine whether betaine would affect growth in uninfected or in coccidiosis-infected chicks. The 0.1% addition of betaine (but not the 0.5% addition) increased weight gain and feed intake in coccidiosis-infected chicks, but it decreased weight gain and feed intake of uninfected chicks. Because 0.1% betaine was more effective in alleviating the effects of reduced gain and feed intake of

coccidiosis-infected chicks in Exp. 1, this level was used in the following two experiments. In Exp. 2, betaine increased feed efficiency in uninfected chicks, but decreased feed efficiency in infected chicks. In Exp. 3, betaine decreased feed efficiency in both uninfected and infected chicks. When the common treatments (consisting of 0.1% betaine in uninfected and coccidiosis infected chicks) among these three experiments were pooled with experiment in the statistical model as a blocking factor, there were no significant main effects of betaine (Table 5). However, addition of 0.1% betaine

**TABLE 4. Weight gain, feed intake, gain:feed, lesion score, and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed betaine (BET) or monensin (MON) or both (Experiment 3)<sup>1</sup>**

Dietary additions	Weight gain			Feed intake			Gain:feed ratio			Lesion score <sup>2</sup>		Plasma carotenoids		
	– <sup>3</sup>	+	$\bar{x}$	–	+	$\bar{x}$	–	+	$\bar{x}$	–	+	–	+	$\bar{x}$
	(g)						(g:g)					( $\mu\text{g/mL}$ )		
Basal (B)	304	219	262	367	305	336	0.829	0.808	0.819	0	2.36	3.37	1.43	2.40
B + 0.1% BET	287	227	257	355	308	332	0.808	0.735	0.772	0	2.36	3.63	1.44	2.54
B + 110 ppm MON	296	287	292	357	347	352	0.830	0.828	0.829	0	0.50	3.43	2.85	3.14
B + BET + MON	306	289	298	359	354	357	0.852	0.817	0.835	0	0.40	3.58	2.87	3.23
$\bar{x}$	298	256		360	329		0.830	0.797		0	1.41	3.50	2.15	
Pooled SEM	8			9			0.010			0.15		0.13		
Source of variation <sup>4</sup>							Probability							
Coccidiosis (COC)	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			...		0.01		
COC BET MON	NS			NS			0.02			...		NS		

<sup>1</sup>Data are means of five replicates of five chicks from 4 to 14 d posthatching; average initial weight was 68 g.

<sup>2</sup>Data for uninfected chicks was not used for analysis of lesion scores, thus only contrasts for betaine, monensin, and betaine by monensin were used for analysis.

<sup>3</sup>Uninfected (-) or coccidiosis-infected (+) chicks.

<sup>4</sup>No significant (NS;  $P > 0.10$ ) effects were observed for betaine, coccidiosis by betaine, or betaine by monensin, thus, they are not included in the table.

TABLE 5. Pooled analysis of Experiments 1, 2, and 3 for weight gain, feed intake, gain:feed, lesion score and plasma carotenoids of uninfected or *Eimeria acervulina*-infected chicks fed betaine (BET)<sup>1</sup>

Dietary additions	Weight gain		Feed intake		Gain:feed ratio		Lesion score <sup>2</sup>		Plasma carotenoids	
	– <sup>3</sup>	+	–	+	–	+	–	+	–	+
	(g)				(g:g)				(μg/mL)	
Basal (B)	334	236	401	320	0.831	0.737	0	2.59	3.75	1.13
B + 0.1% BET	324	242	393	329	0.823	0.735	0	2.44	3.79	1.05
Pooled SEM	4		4		0.006		0.10		0.09	
Source of variation <sup>4</sup>					Probability					
Coccidiosis (COC)	0.01		0.01		0.01		...		0.01	
COC BET	0.05		0.04		NS		...		NS	

<sup>1</sup>Data are means of sixteen replicates (Experiments 1, 2, and 3) of five chicks per replicate.

<sup>2</sup>Data for uninfected chicks was not used for analysis of lesion scores, thus only the main effect of betaine was used for analysis.

<sup>3</sup>Uninfected (-) or coccidiosis-infected (+) chicks.

<sup>4</sup>No significant (NS;  $P > 0.10$ ) effects were observed for betaine, thus, they are not included in the table.

increased average daily gain and feed intake in coccidiosis-infected chicks but decreased these response criteria in uninfected chicks, resulting in a significant coccidiosis by betaine interaction. The percentage change in these response variables from betaine, whether it be a decrease or an increase in uninfected or infected chicks, respectively, was approximately the same, ranging from 2 to 3%.

In Exp. 2 and 3, the objective was to determine whether betaine would enhance the efficacy of monensin in coccidiosis-infected chicks. Experiment 2 used 55 ppm monensin, which we have found in previous experiments to provide less than 100% efficacy (Gray *et al.*, 1995). Thus, if betaine were going to improve the efficacy of monensin, a response should have been observed at the 55 ppm level. In Exp. 3, a level of 110 ppm monensin was used, which is the recommended inclusion level in the diet of chicks. The common treatments also were pooled among Exp. 2 and 3 with the different levels of monensin being treated as one level. The results of this analysis indicated no significant main effects of betaine or betaine interactions. Betaine did not enhance the efficacy of monensin as assessed by weight gain, lesion score, or plasma carotenoids, but betaine improved feed efficiency of infected chicks fed monensin in Exp. 2. However, Virtanen *et al.* (1996:149) reported that betaine "potentiated the action of salinomycin" as assessed by feed efficiency and lesion score. We do not know the reason for the discrepancy between our data and those of Virtanen *et al.* (1996), although there are many differences in the experimental protocol, including the age of chicks when the lesion scores were determined and the use of floor pens by Virtanen *et al.* (1996) vs our use of raised-wire floors. The aforementioned authors did not reveal the severity of the coccidiosis infections used in their studies, which also could explain a portion of the difference in the data. In addition, betaine did not affect plasma carotenoids in any of the experiments. Currently we do not have any knowledge of literature to support or refute the effect of betaine on plasma carotenoid concentrations.

The results of these experiments indicate that betaine may have an effect on *E. acervulina*-infected chicks, but no conclusive evidence indicates an improvement of the efficacy of monensin when fed in combination with betaine. Additional research needs to be conducted to further determine the role of dietary betaine in coccidiosis-infected chicks.

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