# Effect of Betaine on the Growth Performance of Chicks Inoculated with Mixed Cultures of Avian *Eimeria* Species and on Invasion and Development of *Eimeria tenella* and *Eimeria acervulina In Vitro* and *In Vivo*

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**ABSTRACT** At 7 d postinoculation (DPI) with a mixed culture of avian *Eimeria* species, 21-d-old chicks maintained in batteries and floor pens on a diet containing 0.15% (3 lb/ton) betaine plus 66 ppm (60 g/ton) salinomycin were significantly heavier and had significantly lower feed conversion ratios and mortality than chicks fed diets containing 0.15% betaine or 66 ppm salinomycin alone, or the control diet. At 31 DPI, when the chicks were 45 d old, the differences between the diet groups were not as great as at 7 DPI. *In vitro*, except at high concentrations, betaine was nontoxic to sporozoites of *Eimeria tenella* or *Eimeria acervulina* and had little effect on their invasion and development in cultured cells. *In vivo*, invasion by *E. tenella* and *E. acervulina* sporozoites was significantly reduced in all chicks fed diets containing betaine or salinomycin compared with that in control chicks. There was a significant interaction between betaine and salinomycin that impacted on invasion by both species. Overall development of *E. tenella* did not appear to be adversely affected by addition of betaine to diets containing salinomycin. Conversely, development of *E. acervulina* was reduced in chicks fed diets containing 0.075% (1.5 lb/ton) betaine plus 66 ppm salinomycin as compared with that in chicks fed salinomycin alone.

(*Key words*: betaine, coccidiosis, growth performance, chicken, parasite development)

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## INTRODUCTION

Betaine, or glycine betaine, is widely found in nature and is synthesized by a variety of plants and organisms (Boch *et al*., 1994). The accumulated betaine protects the cells from osmotic stress and allows them to continue regular metabolic activities in conditions that would normally inactivate the cell (Rudolph *et al*., 1986; Petronini *et al*., 1992; Ko *et al*., 1994). In mammals, betaine is well known for its ability to help cells tolerate osmotic stress that occurs as a result of the production of hyperosmotic urine (Bagnasco *et al*., 1986). The osmoprotection occurred not only in animals that accumulated betaine through choline oxidation, but also through synthesis from betaine added to the feed (Virtanen, 1995).

Coccidiosis in avian species is an enteric disease, and the infection is associated with osmotic and ionic disorders (Crompton, 1976; Virtanen, 1995). These disorders are probably caused by dehydration and diarrhea that are characteristic of coccidial infection, but may be exacerbated by ionophorous anticoccidial drugs that are widely used to prevent coccidiosis (Virtanen, 1995). Based on its osmoprotective properties, it was hypothesized that betaine might have a stabilizing effect on the intestinal cells in coccidia-infected chickens and reduce the pathogenic effects of the infection. To test the hypothesis, growth performance (BW, feed conversion efficiency, and mortality) in infected chicks that were fed diets containing betaine alone or in combination with salinomycin, an ionophorous anticoccidial drug, was compared with that of chicks fed unsupplemented diets. Concomitantly, the direct effect of betaine on the coccidia was determined by examining invasion and development by *Eimeria tenella* and *Eimeria acervulina* in cell culture and in chicks fed the same diets that were used to measure growth performance.

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## MATERIALS AND METHODS

### *Growth Performance in Chicks*

***Battery Studies.*** Two replicate studies were conducted in a heat-controlled facility (forced-air heaters) equipped with incandescent lighting and negative pressure ventilation. The chicks were housed in 46 × 61 cm battery cages with 281 $cm^2$ per bird. In each study, male

Peterson × Arbor Acres chicks were obtained from a commercial hatchery at 1 d of age and a representative group was weighed. Chicks within 5 g of the mean BW were randomly distributed into four diet groups. Each diet group consisted of 12 cages with 10 chicks per cage. The diet groups were fed 1) a basal diet (crumbled form) that met or exceeded nutritional recommendations established by the National Research Council (NRC, 1994) (control), 2) the basal diet supplemented with 0.15% purified betaine[1] (15bet), 3) the basal diet supplemented with 66 ppm salinomycin (66sal), and 4) the basal diet supplemented with 0.15% betaine and 66 ppm salinomycin (15bet+66sal). Beginning on Day 1, the chicks were provided *ad libitum* access to feed and water.

At 14 d of age, the chicks were inoculated, via the drinking water, with a mixed culture of *Eimeria* species to simulate a natural coccidiosis challenge. The coccidial oocyst suspension was adjusted to deliver approximately $1 \times 10^5$ oocysts of *E. acervulina*, $5 \times 10^4$ oocysts of *E. maxima*, and $1 \times 10^4$ oocysts of *E. tenella* per chick. At 21 d of age (7 DPI), the surviving birds were weighed individually. Total feed consumption per cage was measured, and feed conversion ratios (FCR; feed:gain) were calculated after correction for mortality. Total mortality and day of death were recorded for each treatment group, and all birds that died were examined for coccidiosis.

***Floor Pen Study.*** A floor pen facility was prepared by removing wet litter from the previous trial and top-dressing the remainder with approximately 2.5 cm of new litter. Each pen was 1.3 × 4.7 m and contained one tube feeder and one waterer per pen. Air exchange was enhanced by wall fans. Artificial lighting was provided continuously. Male and female Peterson × Arbor Acres chicks were obtained as for the battery study and randomly distributed to pens. Each diet group consisted of eight pens and contained 44 males and 44 females. Beginning on Day 1, the chicks were assigned to the same diets as in the battery studies and to two additional diets: the basal diet supplemented with 44 ppm salinomycin (44sal) and with 0.15% betaine plus 44 ppm salinomycin (15bet+44sal). All diets were provided for *ad libitum* consumption as crumbles from 1 to 21 d and pellets from 22 to 45 d.

At 14 d of age, chicks were inoculated via the drinking water with the same three species of *Eimeria* as in the battery studies. Data were collected at 7 and 31 d PI, when the chicks were 21 and 45 d of age, respectively. At each interval, the chickens were weighed individually; total feed consumption per pen was determined and FCR were calculated. Mortality was recorded for each treatment group, and all birds that died during the study were examined for coccidiosis.

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## Coccidial Invasion and Development

***Toxicity to Coccidial Sporozoites and Cultured Cells.*** Sporozoites of *E. tenella* and *E. acervulina* were excysted (Danforth, 1982), suspended in concentrations of betaine from 0 to 4.3 *M*, and incubated for 45 min at 22 C. The sporozoites were washed twice and resuspended in Medium 199 (M199)[2] without betaine. An aliquot of the sporozoite suspension was examined by phase microscopy for changes in morphology; the remaining sporozoites were inoculated onto cultured primary chick kidney cell cultures (PCK) (Doran, 1970) to evaluate their ability to invade. At 2.5 h postinoculation (HPI) at 41 C, the cultures were fixed in 10% buffered formalin and stained with hematoxylin and eosin. The cell confluence and the number of intracellular sporozoites in 20 equally spaced microscopic fields (500×) was determined. To examine the effect of betaine on host cells, 3-d-old uninfected cultures of PCK were incubated in M199 containing 5% fetal bovine serum (M199+5%) and from 0 to 500 m*M* betaine at 41 C for 2 h. The cultures were fixed and stained as above and examined for changes in culture morphology.

***Invasion and Development in Cultured Cells.*** Sporozoites of each species were suspended in M199+5% containing 0 to 107 m*M* betaine. Each suspension was inoculated immediately into PCK at $3 \times 10^5$ sporozoites per culture and incubated at 41 C. Cultures inoculated with *E. tenella* were fixed at 2.5, 48, and 72 HPI and stained as described above. Cultures inoculated with *E. acervulina* were fixed only at 2.5 HPI because this species develops poorly, if at all, in cell culture (Doran, 1973). Invasion was quantified as above. Development was based on the ratio of developmental stages to total parasite stages (sporozoites plus developmental stages) in 40 equally spaced microscopic fields at 500×. Data were collected from a total of five or six cultures per treatment group per interval in two replicate experiments.

***Invasion and Development in Chicks.*** Male Peterson × Arbor Acres chicks were obtained as for the battery and floor pen studies at 1 d of age. At 2 wk, groups of 15 chicks were fed the same four diets used in the battery trials (control, 15bet, 66sal, and 15bet+66sal) and two additional diets: the basal diet supplemented with 0.075% betaine (075bet) and with 0.075% betaine plus 66 ppm salinomycin (075bet+66sal). Twenty-four hours after the diets were initiated, chicks were inoculated with $1.5 \times 10^7$ oocysts (to examine invasion) or $3 \times 10^5$ oocysts (to examine development). The isolates of *E. tenella* or *E. acervulina* were the same as those used in the battery and floor pen studies. For each species of *Eimeria*, six chicks from each diet group were sampled for invasion at 6 HPI, and three chicks from each diet group were sampled at 48 and 96 HPI for development. Tissues were taken from the middle of the cecal pouch (*E. tenella*) or the duodenal loop (*E. acervulina*), fixed in cold Carnoy's solution (60% methanol, 30% chloroform, 10% glacial acetic acid) for 2 h, embedded in paraffin, sectioned, and stained with parasite-specific

monoclonal antibodies (mAb) (Augustine and Danforth, 1984). Invasion was quantified by counting the number of intracellular sporozoites per cross-section of intestine at 6 HPI. Development was based on the ratio of developmental stages to total parasites and on the maturity of the stages. Counts from three cross-sections of intestine from each bird were averaged for each data point.

### Statistical Analyses

Data from growth performance (battery and floor pen studies) and invasion in chicks were subjected to ANOVA using the General Linear Model of SAS Institute (1991) in a factorial arrangement of treatments with betaine and salinomycin as the main effects. Data from invasion in cultured cells was subjected to ANOVA and Duncan's multiple range procedure. Differences at or less than $P = 0.05$ were considered to be significant.

## RESULTS

### Growth Performance in Chicks

***Battery Study.*** Results of the two replicate experiments were similar (Table 1). At 21 d of age (7 DPI), chicks fed 15bet+66sal had significantly greater BW and significantly lower FCR than chicks fed diets containing 15bet or 66sal, alone, or the control diet (Table 1). The BW and mortality of inoculated chicks fed 15bet+66sal did not differ significantly from those of uninoculated control chicks. Although the FCR of the inoculated chicks fed 15bet+66sal was significantly higher than the uninoculated control chicks, it was significantly lower than that of chicks fed the other diets (Table 1). In addition, mortality among the chicks fed 15bet+66sal was significantly decreased as compared with the inoculated controls and the chicks fed 15bet, and differed little from that of uninoculated controls (Table 1). There were no significant interactions between betaine and salinomycin.

***Floor Pen Study.*** At 21 d of age (7 DPI), the BW and FCR of the chicks on diets containing all combinations of betaine and salinomycin were significantly better than the BW and FCR of the inoculated controls (Table 2, 1 to 21 d). The BW of chicks fed 15bet+66sal was significantly greater than that of chicks fed 15bet or 66sal alone or fed 15bet+44sal (Table 2, 1 to 21 d). Feed conversion ratios generally followed the pattern of BW, being lowest in the heavier chicks and highest among the controls (Table 2, 1 to 21 d). Betaine alone in the diet did not reduce mortality; however, the addition of betaine to diets containing 44 and 66 ppm salinomycin reduced mortality by 42 and 44%, respectively, over diets containing only salinomycin (Table 2, 1 to 21 d).

At 45 d of age (31 DPI), the BW of chickens fed 15bet+66sal and 15bet+44sal did not differ significantly; however, both were significantly greater than the BW of the controls (Table 2, 1 to 45 d). The BW of chickens fed 66sal was also significantly greater than the controls, but the BW of chickens fed 15bet and 44sal were not. The FCR of chickens fed 15bet+66sal and 15bet+44sal did not differ significantly from the FCR of chickens fed 66sal or 44sal alone; however, all were significantly better than the FCR of the controls (Table 2, 1 to 45 d). Mortality was significantly reduced among all groups fed betaine plus salinomycin or salinomycin alone as compared with controls. Betaine alone had no statistically significant effect on mortality. However, the differences in mortality among chickens fed both betaine and salinomycin was numerically lower than among chickens fed diets with salinomycin alone (Table 2, 1 to 45 d). There were no significant interactions between betaine and salinomycin.

### Coccidial Invasion and Development

***Toxicity to Coccidial Sporozoites and Cultured Cells.*** Sporozoites of both species of *Eimeria* that were incubated for 45 min in 850 m*M* betaine remained motile, refractile, and morphologically indistinguishable from sporozoites incubated in medium without betaine (Figure 1.1); invasion of cultures cells by the treated sporozoites did not differ from that of untreated sporozoites (data not shown). Sporozoites incubated in concentrations of 2.4 *M* betaine and greater were longer, thinner, and less refractile than untreated sporozoites (Figure 1.2), and did not invade PCK (data not shown). Uninfected PCK incubated in 107 m*M* betaine were similar morphologically to cultures incubated in medium without betaine (Figure 1.3). However, PCK incubated in concentrations of betaine from 214 to 640 m*M* partially detached from the coverslips and displayed nuclear changes (Figure 1.4), but remained susceptible to invasion by sporozoites.

***Invasion and Development in Cultured Cells.*** The inclusion of betaine in the inoculation medium at levels up to 107 m*M* did not significantly decrease invasion by either *E. tenella* or *E. acervulina* (Table 3). Subsequent development by *E. tenella* in cultures containing up to 107 m*M* betaine did not differ significantly from that in control cultures at any interval PI (data not shown).

***Invasion and Development in Chicks.*** By 6 HPI, sporozoites of both *E. tenella* and *E. acervulina* had invaded intestinal cells of chicks fed each of the diets (Figure 1.5). However, the number of intracellular sporozoites was significantly reduced in chicks fed all of the diets containing betaine and salinomycin as compared with chicks fed the control diet (Table 4). There was a significant interaction between betaine and salinomycin. In the absence of salinomycin, invasion by each species decreased with increasing concentrations of betaine. Conversely, in the presence of salinomycin, invasion increased over invasion in chicks on the control diet (Figures 2 and 3). At 48 HPI, there were fewer developmental stages of *E. tenella* in chicks fed 075bet+66sal and 15bet+66sal than in chicks fed either level of betaine without salinomycin (Table 5), however, the number of developmental stages of *E. acervulina* in chicks fed the different diets was similar (Table 5). By 96 h PI,

**TABLE 1. Effect of dietary betaine and salinomycin on growth parameters (mean ± SEM) of uninfected control chicks[1] and chicks inoculated with a mixed culture of avian *Eimeria* species at 2 wk of age, battery experiments**

| Supplement[2] | | Experiment 1 | | | Experiment 2 | | |
|---|---|---|---|---|---|---|---|
| Betaine | Salinomycin | Body weight | Feed conversion | Mortality | Body weight | Feed conversion | Mortality |
| (%) | (ppm) | (kg) | (g:g) | (%) | (kg) | (g:g) | (%) |
| 0 | 0 | 0.593 ± 0.010$^{a}$ | 1.339 ± 0.022$^{e}$ | 3.3 ± 4.7$^{b}$ | 0.652 ± 0.045$^{a}$ | 1.279 ± 0.028$^{d}$ | 5.9 ± 11.1$^{b}$ |
| 0 | 0 | 0.593 ± 0.025$^{c}$ | 1.456 ± 0.054$^{a}$ | 34.2 ± 10.4$^{a}$ | 0.485 ± 0.045$^{c}$ | 1.625 ± 0.068$^{a}$ | 41.7 ± 23.0$^{a}$ |
| 0.15 | 0 | 0.691 ± 0.025$^{c}$ | 1.458 ± 0.050$^{a}$ | 32.5 ± 9.2$^{a}$ | 0.558 ± 0.068$^{b}$ | 1.582 ± 0.077$^{a}$ | 32.5 ± 10.9$^{a}$ |
| 0 | 66 | 0.611 ± 0.015$^{b}$ | 1.411 ± 0.033$^{c}$ | 10.8 ± 9.5$^{c}$ | 0.563 ± 0.036$^{b}$ | 1.399 ± 0.054$^{b}$ | 15.8 ± 13.8$^{b}$ |
| 0.15 | 66 | 0.630 ± 0.019$^{a}$ | 1.376 ± 0.023$^{d}$ | 5.8 ± 6.4$^{b}$ | 0.625 ± 0.004$^{a}$ | 1.334 ± 0.036$^{c}$ | 5.8 ± 8.6$^{b}$ |
| Betaine × salinomycin | *P* | 0.690 | 0.596 | 0.926 | 0.130 | 0.159 | 0.561 |
| Interaction | *F* | NS | NS | NS | NS | NS | NS |

$^{a-e}$Means within experiments and columns with no common superscript differ significantly ($P \leq 0.05$); n = 120 chicks per diet group per experiment.

[1]First line of table contains data from uninfected control chickens; other four lines of data are from infected chickens.

[2]Diet unsupplemented (0) or supplemented with 0.15% betaine, 66 ppm salinomycin, or both.

**TABLE 2. Effect of dietary betaine and salinomycin on growth parameters (mean ± SEM) of chicks at 21 and 45 d of age after inoculation with a mixed culture of avian *Eimeria* species at 2 wk of age, floor pen experiment**

| Supplement[1] | | 1 to 21 d | | | 1 to 45 d | | | 21 d |
|---|---|---|---|---|---|---|---|---|
| Betaine | Salinomycin | Body weight | Feed conversion | Mortality | Body weight | Feed conversion | Mortality | Lesion score |
| (%) | (ppm) | (kg) | (g:g) | (%) | (kg) | (g:g) | (%) | |
| 0 | 0 | 0.580 ± 0.010$^{e}$ | 1.443 ± 0.074$^{a}$ | 16.6 ± 2.4$^{a}$ | 1.948 ± 0.029$^{c}$ | 1.977 ± 0.077$^{a}$ | 19.0 ± 2.8$^{a}$ | 3.63$^{a}$ |
| 0.15 | 0 | 0.592 ± 0.010$^{d}$ | 1.397 ± 0.033$^{b}$ | 14.1 ± 2.7$^{a}$ | 1.953 ± 0.031$^{bc}$ | 1.954 ± 0.067$^{ab}$ | 15.9 ± 2.5$^{a}$ | 3.47$^{a}$ |
| 0 | 44 | 0.604 ± 0.007$^{c}$ | 1.381 ± 0.031$^{bc}$ | 8.4 ± 2.9$^{b}$ | 1.968 ± 0.025$^{b}$ | 1.936 ± 0.052$^{abc}$ | 10.6 ± 3.5$^{b}$ | 2.88$^{b}$ |
| 0.15 | 44 | 0.617 ± 0.007$^{b}$ | 1.346 ± 0.024$^{cd}$ | 4.7 ± 3.3$^{c}$ | 1.982 ± 0.026$^{ab}$ | 1.898 ± 0.050$^{bc}$ | 7.1 ± 4.6$^{bc}$ | 2.22$^{c}$ |
| 0 | 66 | 0.617 ± 0.007$^{b}$ | 1.349 ± 0.024$^{cd}$ | 4.7 ± 3.4$^{c}$ | 1.981 ± 0.030$^{ab}$ | 1.920 ± 0.054$^{abc}$ | 7.0 ± 3.7$^{bc}$ | 2.34$^{c}$ |
| 0.15 | 66 | 0.640 ± 0.013$^{a}$ | 1.311 ± 0.022$^{d}$ | 2.7 ± 2.6$^{c}$ | 2.004 ± 0.025$^{a}$ | 1.877 ± 0.046$^{c}$ | 4.3 ± 2.5$^{c}$ | 1.81$^{c}$ |
| Betaine × salinomycin | *P* | 0.305 | 0.941 | 0.758 | 0.675 | 0.903 | 0.963 | 0.353 |
| Interaction | *F* | NS | NS | NS | NS | NS | NS | NS |

$^{a-e}$Means within ages and columns with no common superscript differ significantly ($P \leq 0.05$); n = 704 chicks per diet group.

[1]Diet supplemented with nothing (0) or with 0.15% betaine, 44 or 66 ppm salinomycin, or betaine plus each level of salinomycin.

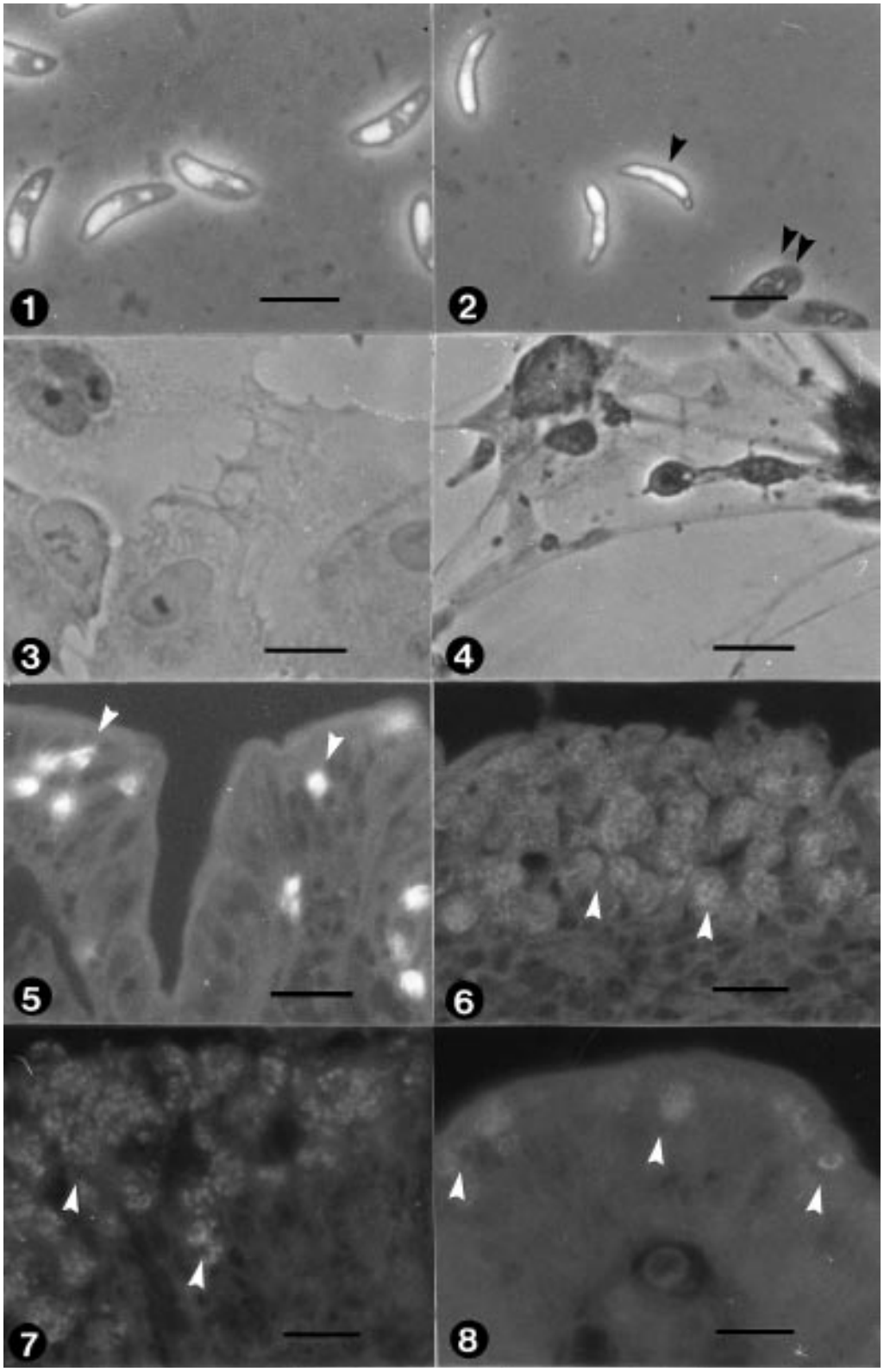


**FIGURE 1.** Effect of betaine on host cells and parasites. Frames 1 to 4 were taken by phase microscopy; 5 to 8 by epiflorescence microscopy. 1) sporozoites of *Eimeria tenella* incubated in control medium, 2) sporozoites of *E. tenella* incubated in medium containing 2.4 *M* betaine. Note elongated (arrowhead) and nonrefractile (double arrowhead) sporozoites, 3) primary chick kidney cells incubated in control medium, 4) primary chick kidney cells incubated in medium containing 214 m*M* betaine, 5) cross-section of intestine from chick fed no betaine or salinomycin, showing fluorescing sporozoites of *E. tenella* at 6 h postinoculation (arrowheads), 6) cross-section of intestine from chick fed no betaine or salinomycin, showing developmental stages of *E. acervulina* at 96 h postinoculation (arrowheads), 7) cross-section of intestine from chick fed 0.075% betaine and no salinomycin, showing developmental stages of *E. acervulina* at 96 h postinoculation (arrowheads), 8) cross-section of intestine from chick fed 0.075% betaine and 66 ppm salinomycin, showing developmental stages of *E. acervulina* at 96 h postinoculation (arrowheads). Scale bars = 10 $\mu$m.

**TABLE 3. Effect of betaine on invasion of cultured chick kidney cells (mean ± SEM) by sporozoites of *Eimeria tenella* and *Eimeria acervulina* at 3 h postinoculation**

| Betaine | n[1] | Cell confluency | Sporozoites per 20 microscopic fields at 500× | |
|---|---|---|---|---|
| | | | *E. tenella* | *E. acervulina* |
| (m*M*) | | (%) | | |
| 0 | 5 | 40 + 5[a] | 41 + 6[a] | 9 + 1[a] |
| 27 | 5 | 35 + 4[a] | 56 + 10[a] | ND |
| 54 | 5 | 37 + 4[a] | 82 + 12[a/b] | 9 + 1[a] |
| 71 | 5 | 34 + 8[a/b] | 72 + 8[a] | ND[2] |
| 107 | 4 | 37 + 5[a] | 43 + 11[a] | 9 + 1[a] |

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$). [a/b] = significant in one experiment but not in other.

[1]n = number of cultures per treatment group per coccidial species in two replicate experiments.

[2]ND = not done.

**TABLE 4. Effect of dietary betaine and salinomycin on invasion by *Eimeria tenella* and *Eimeria acervulina* sporozoites (mean ± SEM) in chicks at 6 h postinoculation**

| Betaine:salinomycin | n[1] | Sporozoites per cross-section of intestine | |
|---|---|---|---|
| | | *E. tenella* | *E. acervulina* |
| (%)/(ppm) | | | |
| 0/0 | 6 | 156 + 43[a] | 125 + 10[a] |
| 0/66 | 6 | 6 + 4[b] | 34 + 4[c] |
| 0.075/0 | 6 | 40 + 10[b] | 58 + 4[b] |
| 0.075/66 | 6 | 68 + 10[b] | 40 + 3[c] |
| 0.15/0 | 6 | 47 + 25[b] | 30 + 3[c] |
| 0.15/66 | 6 | 53 + 13[b] | 43 + 4[c] |
| Betaine × salinomycin | F | 8.53 | 61.09 |
| Interaction | P | 0.001 | <0.001 |

[a–c]Means within columns with no common superscripts differ significantly ($P < 0.05$).

[1]n = number of chicks per treatment group.

**TABLE 5. Effect of dietary betaine and salinomycin on development of *Eimeria tenella* and *Eimeria acervulina* in 2-wk-old chicks at 48 h postinoculation**

| Betaine:salinomycin | n[1] | First and early second-generation schizonts | |
|---|---|---|---|
| | | *E. tenella* | *E. acervulina* |
| (%)/(ppm) | | | |
| 0/0 | 3 | 34 (22)[2] | 36 (60) |
| 0/66 | 3 | 49 (800) | 21 (80) |
| 0.075/0 | 3 | 61 (100) | 20 (80) |
| 0.075/66 | 3 | 25 (62) | 23 (74) |
| 0.15/0 | 3 | 67 (220) | 25 (68) |
| 0.15/66 | 3 | 20 (47) | 20 (65) |

[1]n = number of chicks per treatment group.

[2]Numbers in parentheses represent percentage development. Numbers greater than 100% represent the initiation of subsequent generations.

**TABLE 6. Effect of dietary betaine and salinomycin on development of *Eimeria tenella* and *Eimeria acervulina* in chicks at 96 h postinoculation[1]**

| Species[1] | Betaine:salinomycin | Development |
|---|---|---|
| | (%)/(ppm) | |
| *E. tenella* | All diet groups | Numerous stages in cross-sections. Primary stages: mature second generation schizonts and gamonts. |
| *E. acervulina* | 0/0 | Epithelium packed with stages. Primary stages: macrogamonts. |
| | 0/66 | A few villi heavily infected; majority more lightly infected. |
| | 0.075/0 | Similar to 0/0. |
| | 0.075/66 | Development 50% of that in 0/0 and lighter than that in 0/60; most villi sparsely infected. |
| | 0.15/0 | Similar to 0/0. |
| | 0.15/66 | A few villi heavily infected and similar to 0/60; majority of villi lightly infected or uninfected. |

[1]Three chicks per treatment group per species; three cross-sections per bird examined.

development in chicks of *E. tenella* fed all six diets was similar (Table 6). Conversely, although development by *E. acervulina* in chicks fed betaine alone was similar to that in the controls (Table 6; Figure 1.6 and 1.7), development in chicks fed both betaine and salinomycin was markedly reduced over that in controls (Table 6; Figure 1.8).

## DISCUSSION

Betaine in combination with salinomycin in the diet of Peterson × Arbor Acres chicks conferred protection against a moderate, mixed infection of *E. acervulina*, *E. tenella*, and *Eimeria maxima* that was significantly greater than that afforded by betaine or salinomycin alone. Thus, even when coccidiosis was reasonably well controlled by salinomycin, addition of betaine to the feed increased the performance of the chickens. Some compounds, such as tiamulin, have been shown to increase the efficacy of ionophores in terms of weight gain, lesion scores, and mortality, possibly by reducing the metabolic degradation of the drugs (Meingassner *et al.*, 1979).

In the present study, the effects of betaine alone or added to diets containing salinomycin were evaluated as contributors to the improved performance in coccidia-infected chicks. Coccidial invasion in the presence of betaine differed somewhat *in vitro* and *in vivo*, preventing a clear-cut interpretation of the data. In cell cultures, betaine, alone, at levels that were roughly two- to eight-fold higher than the levels used in the battery and floor pen trials, did not adversely effect the morphology or

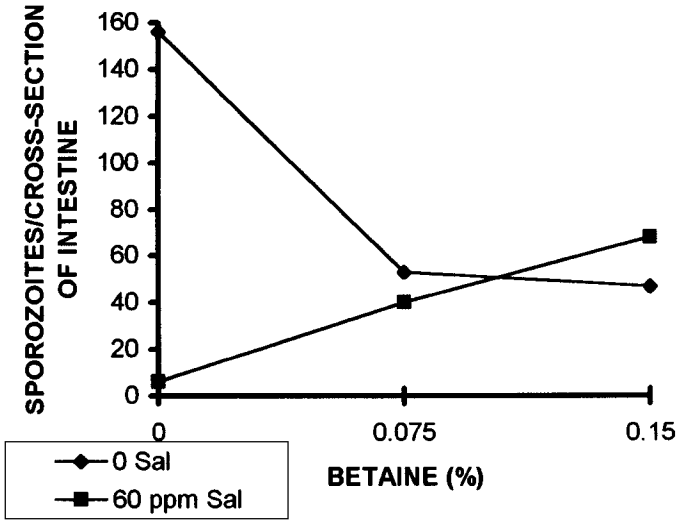


**FIGURE 2.** Effect of 0.075 and 0.15% dietary betaine on invasion of the ceca of 2-wk-old chicks by *Eimeria tenella* sporozoites, in the presence and absence of 66 ppm salinomycin. The specimens were taken at 6 h postinoculation, paraffin-embedded, sectioned, and subjected to fluorescent antibody labeling using a parasite-specific monoclonal antibody.

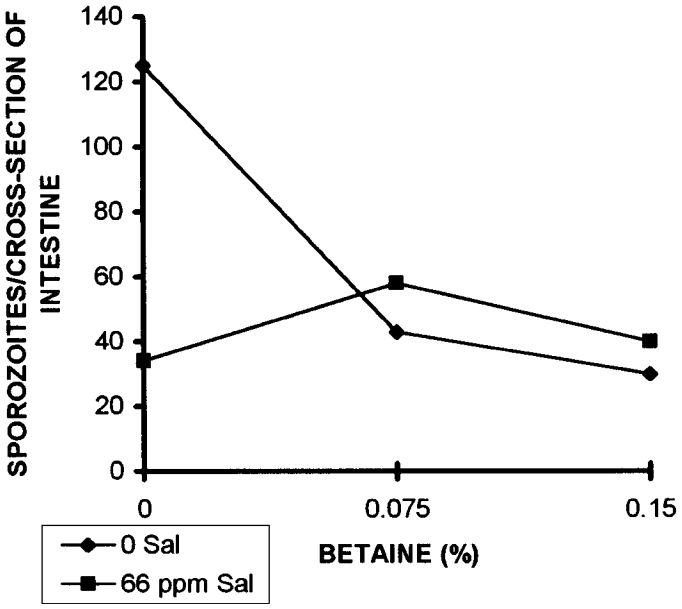


**FIGURE 3.** Effect of 0.075 and 0.15% dietary betaine on invasion of the ceca of 2-wk-old chicks by *Eimeria acervulina* sporozoites, in the presence and absence of 66 ppm salinomycin. The specimens were taken at 6 h postinoculation, paraffin-embedded, sectioned, and subjected to fluorescent antibody labeling using a parasite-specific monoclonal antibody.

the ability to invade by either *E. tenella* or *E. acervulina* sporozoites or to develop by *E. tenella* sporozoites. These data suggested that betaine probably did not enhance performance through overt toxicity toward the parasite. However, when ingested by chicks, betaine, alone, caused a significant reduction in invasion by both species of *Eimeria* as compared with invasion in the control chicks. Apparently, characteristics of betaine that are capable of inhibiting sporozoite invasion are functional in the intestinal environment but not in cell culture.

The effect of betaine with added salinomycin was not examined in cell culture. However, in chicks fed diets containing the supplements, there was a significant interaction between betaine and salinomycin that impacted on invasion. In the absence of salinomycin in the diet, invasion by both *E. tenella* and *E. acervulina* decreased as the concentration of betaine increased from 0 to 0.15%. When salinomycin was added to the diet along with betaine, invasion by *E. tenella* increased as the concentration of betaine increased from 0 to 0.15%. Under the same conditions, invasion by E. acervulina increased in the presence of 0.075% betaine and then decreased slightly with 0.15% betaine (but remained greater than invasion in the absence of salinomycin). The reason for the increase in invasion when salinomycin was added to feed containing betaine is unknown at this time. However, the data provide additional evidence that the improved performance in chicks fed both betaine and salinomycin was not caused by marked decreases in invasion as compared with invasion in chicks fed either of the supplements alone.

Betaine, by itself, had little effect on development of *E. tenella* in PCK and chicks or *E. acervulina* in chicks. The addition of betaine to diets containing salinomycin caused a transitory delay in the early development by *E. tenella* that was not evident at 6 DPI. In contrast, by 96 HPI, betaine plus salinomycin in the diet reduced development by *E. acervulina* to a level that was lower than that in chicks fed betaine or salinomycin alone. Thus, the increase in growth performance in chicks fed both betaine and salinomycin may have been due, in part, to a decrease in the development of *E. acervulina.*

In addition to its influence on coccidial invasion and development, betaine also has diverse physiological properties that could enhance the ability of the chicks to withstand coccidial infection. For example, betaine has been shown to stabilize cell membranes through interaction with membrane phospholipids during dehydration (Rudolph *et al.*, 1986), and to reduce fecal water content and increase the digestibility of several nutrients (Virtanen, 1995). These properties could have a direct impact on the intestinal membrane damage, dehydration, diarrhea, and maldigestion that are characteristic of coccidial infection (Crompton, 1976). Therefore, betaine may have contributed to the improved performance of coccidia-infected chicks directly, by partial inhibition of coccidial invasion and development, and, indirectly, by support of intestinal structure and function in the presence of coccidial infection.

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