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Primary Audience: Veterinarians, Nutritionists, Feed Manufacturers, Flock Supervisors

SUMMARY

Coccidiosis control may become a greater problem as the use of growth-promoting antibiotics (GPA) and ionophores declines. Vaccination with live oocysts may turn into a popular alternative to the use of coccidiostats in broilers, although cocci vaccination is frequently linked to temporary lower performance in young flocks. This experiment evaluates the dietary supplementation of 2 specific essential oil (EO) blends (Crina Poultry and Crina Alternate), either as alternatives to GPA and ionophores (BMD + Coban) or as feed additives that help to improve the performance of cocci-vaccinated broilers. Live performance and lesion scores were observed. These 2 specific EO blends differ in their efficacy to promote growth. Chickens that were not cocci vaccinated and were fed Crina Poultry had better feed conversion ratio (FCR) than the unmedicated control treatment in the starter period. The same EO improved FCR in cocci-vaccinated birds in the finisher period in comparison to the negative control group, but those responses were not significantly different from other treatments or significant at 49 d of age. No significant differences were observed in lesion scores at 37 d. Diets supplemented with a GPA-ionophore combination consistently supported the best BW gain and FCR in each period and the entire grow-out period. No significant beneficial or deleterious effects on live performance were observed due to these specific EO blends in cocci-vaccinated broilers.

Key words: broiler chicken, feed additive, cocci vaccination, essential oil, coccidiosis

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DESCRIPTION OF PROBLEM

The search for new alternatives to replace the beneficial effects of growth-promoting antibiotics (GPA) and ionophores is becoming more important because several products have been banned, and microbial drug-resistance appears to be increasing [1, 2, 3]. Coccidiosis is one of the most common enteric diseases to control in poultry production. This disease causes losses close to US$1.5 billion worldwide every year [4] and at least $300 million to the US poultry industry [5].

[1]The use of trade names in this publication does not imply endorsement of the products mentioned or criticism of similar products not mentioned.

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An alternative for the sustainable control of coccidiosis in broilers is live vaccination with viable oocysts. The commercial cocci vaccines available worldwide contain oocysts of at least the 3 more common species of *Eimeria* [6, 7, 8]. Vaccination with live oocysts has proven to be successful in commercial conditions and is currently more widely used in broilers [8, 9]. However, cocci vaccination generally reduces early live performance of broilers, which generally is associated with secondary enteritis, or even sporadically, necrotic enteritis (NE) incidence [6, 7]. Coccidiosis and *Clostridium perfringens* type A or C are considered to be the main etiologies for NE [9].

Intestinal microflora play a role in acquired immunity against coccidia [10, 11, 12, 13], although the exact mechanisms underlying this response are not clear. It seems that chickens perform better under *Eimeria* challenge when fed *Lactobacillis*-based probiotics; have higher subpopulations of gut T lymphocytes expressing the surface markers CD3, CD4, CD8 and α/β T-cell receptors; and have significantly higher IFN-γ and IL-2 at 3 d postinfection with *Eimeria acervulina* [12]. There is also evidence that the normal gut microflora in healthy birds inhibits the pathogenicity of *C. perfringens* [14], and coccidia infection favors *C. perfringens* proliferation [15].

Several alternatives, such as the use of coadjuvants [12, 16], antibiotics, probiotics [10], and other feed additives, have been used to reduce the deleterious effects of cocci vaccination, helping to modulate gut microflora [6, 12]. Because antibiotics may be banned from the poultry industry, new natural alternatives that reduce *C. perfringens* infection should be evaluated. Recently [17, 18] the essential oils (EO) thymol, carvacrol, and eugenol were reported to have specific properties against *Clostridium* spp. colonization and proliferation in the gut of broilers, reducing NE complications when using live vaccination. Other researchers have concluded that oregano EO (mainly carvacrol and thymol) also exerts specific anticoccidial effects against *Eimeria tenella* [19] or a mixed challenge of *Eimeria spp.* [20].

Consequently, the purpose of this project was to evaluate the dietary supplementation of 2 different specific EO blends (Crina Poultry and Crina Alternate) [21] as alternatives to GPA and ionophores for broilers that are not cocci vaccinated or as an immunomodulator in cocci-vaccinated broilers during a grow-out period of 49 d.

MATERIALS AND METHODS

All procedures involving animals were approved by the Stephen F. Austin State University Institutional Animal Care and Use Committee.

Birds, Diets and Experimental Design

A total of 1,728 1-d-old Cobb-500 male chickens were placed in 48 floor-pens (36 birds/pen) in a tunnel-ventilated dark-house and randomly distributed among 7 treatment groups (4 groups not cocci vaccinated and 3 cocci-vaccinated groups). There were 7 replicates per treatment, except for the negative control (unmedicated) that had 6 replicates. Used litter, top-dressed with 2 in. of fresh pine wood shavings was used as bedding. The previous flock housed in this same facility was challenged with coccidia. The lighting program used during this experiment consisted of continuous lighting (3 ft-c = 32 lx) with 5 min of blackout training from 1 to 7 d of age, 14 h of light (11 lx)/d up to 35 d, followed for 21 h light/d up to 42 d of age, and finally increasing light intensity (21.52 to 32 lx) and photoperiod (23 light/d) during the withdrawal phase (42 to 49 d). All changes in photoperiod length and light intensity were made gradually.

Broilers were raised to 49 d and fed starter (1 to 13 d), grower (13 to 34 d), finisher (35 to 42 d), and withdrawal (42 to 49 d) diets (Table 1). Diets were formulated to guarantee or exceed recommended nutrient requirements [22]. One basal diet was mixed for each dietary period, and the additives were blended in according to treatment distribution at a later time.

Seven treatments were compared: 4 not cocci vaccinated and 3 cocci vaccinated. The not cocci-vaccinated treatments included chickens fed 1) basal diets without feed additives (WFA), which was the negative control or unmedicated group; 2) basal diets supplemented with an antibiotic (BMD) [23] at 50 g/ton, and ionophore (Coban 60) [24] at 90 g/ton as positive control group (AI). Two treatments were fed the basal

TABLE 1. Composition (%) of the basal diets and nutrient concentrations

Ingredient	Starter 0–13 d	Grower 13–35 d	Finisher 35–42 d	Withdrawal 42–49 d
Yellow corn	58.48	63.67	67.99	71.30
Poultry fat	2.42	2.66	2.41	2.75
Soybean meal	35.66	30.61	26.86	23.34
Calcium carbonate	0.85	0.77	0.65	0.84
Tricalcium phosphate	1.80	1.62	1.48	1.29
Sodium chloride	0.38	0.27	0.25	0.2
Sodium bicarbonate	0.01	0.01	0.02	0.05
HCL-lysine	0.10	0.12	0.12	0.07
DL-Methionine	0.21	0.18	0.13	0.07
Vitamin-mineral premix[1,2]	0.09	0.09	0.09	0.09
Total	100.00	100.00	100.00	100.00
Nutrient concentration				
ME, kcal/kg[3]	3,050	3,120	3,150	3,200
Crude protein, %[4]	22.40	19.60	18.30	17.1
Calcium, %[3]	1.00	0.90	0.80	0.80
Available phosphorus %[3]	0.45	0.41	0.38	0.34
Lysine, %[4]	1.21	1.08	0.98	0.89
Methionine, %[4]	0.45	0.43	0.36	0.31
TSAA, %[4]	0.82	0.77	0.67	0.62
Tryptophan, %[4]	0.28	0.26	0.24	0.22
Threonine, %[4]	0.80	0.71	0.67	0.61
Sodium, %[3]	0.26	0.21	0.20	0.18
Chlorine, %[3]	0.28	0.21	0.20	0.17
DEB, mEq/kg[5]	264	242	225	209

[1]Provided per kilogram of diet: vitamin A (from vitamin A acetate), 7,714 IU; cholecalciferol, 2,204 IU; vitamin E (from DL-α-tocopheryl acetate), 16.53 IU; vitamin B_{12}, 0.013 mg; riboflavin, 6.6 mg; niacin, 39 mg; pantothenic acid, 10 mg; menadione (from menadione dimethylpyrimidinol), 1.5 mg; folic acid, 0.9 mg; thiamin (from thiamine mononitrate), 1.54 mg; pyridoxine (from pyridoxine hydrochloride), 2.76 mg; D-biotin, 0.066 mg; ethoxyquin, 125 mg; Se, 0.1 mg.
[2]Provided per kilogram of diet: Mn (from $MnSO_4{\cdot}H_2O$), 100 mg; Zn (from $ZnSO_4{\cdot}7H_2O$), 100 mg; Fe (from $FeSO_4{\cdot}7H_2O$), 50 mg; Cu (from $CuSO_4{\cdot}5H_2O$), 10 mg; I (from $Ca(IO_3)_2{\cdot}H_2O$), 1 mg.
[3]Calculated values from NRC [22].
[4]Analyzed values.
[5]DEB = dietary electrolyte balance (Na + K − Cl).

diets supplemented with the specific EO blends: 3) Crina Poultry and 4) Crina Alternate. Chickens in the other 3 treatments were vaccinated at 1 d of age with Advent [25] by spray. These treatments were 5) vaccinated and fed WFA diets, 6) vaccinated and fed diets including Crina Poultry, and 7) vaccinated and fed a diet including Crina Alternate. The EO blends were added to all 4 diets at 100 ppm. The AI combination was not added to the withdrawal diet (42 to 49 d of age).

Measurements and Statistical Analysis

Body weight gain (BWG) and feed intake (FI) were recorded at 13, 35, 42, and 49 d of age. Feed conversion ratio (FCR) was calculated and corrected by FI and mortality weights. Relative BWG was calculated in relation to the WFA negative control group. The temperature, humidity, and lighting program were controlled, and variations from the programmed parameters were recorded.

Mortality was recorded twice a day. Verification of oocyst shedding was performed at 7 d of age in all treatments. Lesion scores in the duodenum, midgut (jejunum-ileum), and ceca were evaluated at 37 d of age [26] in 2 broilers per pen.

Pen means were used as experimental units. The data were analyzed as a completely randomized design. Percentage mortality data were transformed to arcsine for analysis, and final data are presented as natural numbers. All variables of statistical significance were based on a probability of $P \leq 0.05$. Data were subjected to ANOVA using GLM procedure of SAS system

[27]. Mean separation was accomplished using Tukey's multiple range tests. Lesion scores were analyzed using a chi-squared test (Kruskal-Wallis test) [27].

RESULTS AND DISCUSSION

Live Performance of Non-Cocci-Vaccinated Broilers

No significant ($P > 0.05$) differences were observed for BWG and FI during the starter period (Tables 2 and 3). The positive control treatment AI did not significantly improve broiler BWG when compared with their cohorts in the negative WFA control group during the starter and grower periods, but it significantly ($P < 0.01$) promoted growth during the finisher period. This finding might have been due to low bacterial challenge during these initial feeding phases, because used litter was top-dressed with fresh pine wood shavings. Broilers fed diets containing Crina Alternate had significantly ($P < 0.01$) less FI than the broilers in the WFA control treatment during the finisher period without negatively affecting BWG or FCR (Tables 2 and 3). The BWG of non-cocci-vaccinated broilers fed Crina Poultry was not significantly different from those broilers fed diets supplemented with the AI combination or from the WFA negative control. No significant ($P > 0.05$) treatment effects were observed during the withdrawal period.

The relative BWG improvements were not higher than 8% when compared to WFA negative control (Table 2). These results are consistent with extensive literature reviews [3] that concluded the average benefit of GPA is 3 to 4% with a range that goes from no benefit to 8%. The AI diet (positive control) significantly improved FCR (Table 3) during the grower ($P < 0.001$), and finisher ($P < 0.05$) periods and during the entire grow-out period ($P < 0.001$). The EO blend Crina Poultry significantly improved FCR ($P < 0.05$) in nonvaccinated birds when compared with the WFA negative control treatment but were not significantly different from the other treatments (Table 3).

The results observed with Crina Poultry are not surprising. Other specific EO blends have been shown to be as effective as the antibiotics virginiamycin [28] and BMD [17] in promoting growth and in the prevention of NE and as the ionophores lasolacid [19] and salinomycin [20] in reduction of coccidiosis. The EO blends from thyme and garlic do not improve the nutritional value of soybean meal, and any improvements observed with EO might be attributed to their antimicrobial effects, especially toward *C. perfringens* [6, 28, 29].

Live Performance of Cocci-Vaccinated Broilers

All cocci-vaccinated treatments had significantly ($P < 0.01$) less BWG during the grower period (13 to 35 d) than the positive control AI but were not significantly different from the WFA negative control (Table 2). The BWG during the entire period (0 to 49 d) was significantly ($P < 0.01$) lower for cocci-vaccinated broilers fed WFA diets than for broilers fed AI diets but not lower than the broilers in the WFA negative control.

No significant ($P > 0.05$) deleterious effects on FCR were observed in any of the cocci-vaccinated groups as compared to the WFA negative control (Table 3). The supplementation of Crina Poultry in diets fed to cocci-vaccinated chickens significantly improved FCR in the finisher period when compared with the WFA control (Table 3). In contrast, broilers fed diets supplemented with Crina Alternate had significantly worse FCR than the broilers fed AI or Crina Poultry supplemented diets.

The specific blends of EO evaluated in the present experiment did not consistently promote better performance in cocci-vaccinated broilers. On the other hand, the dietary supplementation of oregano products has shown significant benefits to increase BWG and FI of cocci-vaccinated broilers up to 48 d of age, whereas FCR was not significantly affected [7, 30]. It is necessary to consider that there is high variability among specific EO blends with respect to contents of phenolic compounds that might also affect the performance, metabolism, and immunity of broilers [31, 32].

No significant ($P > 0.05$) differences due to treatment effects were observed in mortality (data not shown). At 7 d of age, oocyst shedding was observed only in cocci-vaccinated treatments. Lesion scores were not affected ($P > 0.05$) by treatments (Table 4) at 37 d. However,

TABLE 2. Effects of essential oils, medication, and vaccination on body weight gain (BWG; g) and relative BWG (%) of male broiler chickens[1]

	BWG					Relative BWG[4]				
Treatment[2,3]	0–13 d	13–35 d	35–42 d	42–49 d	0–49 d	0–13 d	13–35 d	35–42 d	42–49 d	0–49 d
NV − WFA	359	1,641[abc]	624[b]	617	3,241[ab]	100	100	100	100	100
NV − AI	358	1,712[a]	677[a]	597	3,344[a]	100	104	108	97	103
Crina Poultry	369	1,670[ab]	638[ab]	593	3,269[ab]	103	102	102	96	101
Crina Alternate	369	1,631[bc]	613[b]	586	3,199[b]	103	99	98	95	99
V − WFA	353	1,585[c]	653[ab]	611	3,202[b]	98	97	105	99	99
V + Crina Poultry	356	1,612[bc]	652[ab]	615	3,259[ab]	99	98	104	100	101
V + Crina Alternate	362	1,619[bc]	625[b]	610	3,217[ab]	101	99	100	99	99
CV %	3.3	2.8	4.5	8.2	2.1					
Pooled SEM	5	17	11	19	25					
P-value	0.178	0.001	0.004	0.880	0.003					

[a–c]Means within the same column with different superscripts differ significantly ($P < 0.05$).
[1]Means represent 7 replicates and 36 male broilers each for 252 birds per treatment except for nonvaccinated and without feed additives, the negative control, which had 6 replicates and 216 birds.
[2]NV = nonvaccinated; WFA = without feed additives; V = cocci-vaccinated with Advent [25] at 1 d of age by spray; AI = BMD [23] at 50 g/ton + Coban 60 [24] at 90 g/ton, except for the withdrawal phase (42 to 49 d).
[3]Essentials oils [21], Crina Poultry and Crina Alternate, were added at 100 ppm.
[4]Relative body weight gain (%) = $\frac{\text{BWG per group} \times 100}{\text{BWG of unmedicated birds}}$.

TABLE 3. Effects of essential oils, medication, and vaccination on feed intake (g) and feed conversion (feed:gain ratio, g:g) of male broiler chickens[1]

	Feed intake					Feed conversion ratio				
Treatment[2,3]	0–13 d	13–35 d	35–42 d	42–49 d	0–49 d	0–13 d	13–35 d	35–42 d	42–49 d	0–49 d
NV − WFA	450	2,824	1,488[a]	1,476	6,238	1.25[a]	1.72[a]	2.36[a]	2.40	1.92[a]
NV − AI	443	2,810	1,472[ab]	1,489	6,213	1.23[ab]	1.64[b]	2.17[b]	2.50	1.86[b]
Crina Poultry	450	2,865	1,458[ab]	1,528	6,300	1.21[b]	1.71[a]	2.28[ab]	2.58	1.93[a]
Crina Alternate	455	2,835	1,408[b]	1,445	6,143	1.23[ab]	1.74[a]	2.30[ab]	2.47	1.92[a]
V^2 − WFA	436	2,788	1,496[a]	1,479	6,198	1.23[ab]	1.76[a]	2.29[ab]	2.43	1.93[a]
V + Crina Poultry	436	2,840	1,473[ab]	1,546	6,295	1.22[ab]	1.76[a]	2.18[b]	2.51	1.93[a]
V + Crina Alternate	448	2,824	1,443[ab]	1,487	6,202	1.23[ab]	1.74[a]	2.31[a]	2.45	1.93[a]
CV %	1.5	2.2	2.8	4.3	2.1	1.6	1.9	4.7	5.9	1.5
Pooled SEM	6	23	15	24	49	0.01	0.01	0.04	0.05	0.01
P-value	0.153	0.380	0.005	0.117	0.291	0.049	0.001	0.043	0.345	0.001

[a,b]Means within the same column with different superscripts differ significantly ($P < 0.05$).
[1]Means represent 7 replicates and 36 male broilers each for 252 birds per treatment except for nonvaccinated and without feed additives (NV-WFA), the negative control, which had 6 replicates and 216 birds).
[2]V = cocci-vaccinated with Advent [25] at 1 d of age by spray; AI = BMD [23] at 50 g/ton + Coban 60 [24] at 90 g/ton, except for the withdrawal phase (42 to 49 d).
[3]Essential oils [21], Crina Poultry and Crina Alternate, were added at 100 ppm.

TABLE 4. Effects of essential oils, medication, and vaccination on intestinal lesion scores of male broilers at 37 d of age[1]

Treatment[2,3]	Lesion scores[4]			Total lesion score[5]
	Duodenum	Jejunum-ileum	Cecum	
NV – WFA	1.17 ± 0.30 (23.3)	0.0 ± 0.14 (21.5)	0.33 ± 0.21 (23.5)	1.50 ± 0.22 (20.8)
NV – AI	1.43 ± 0.27 (27.3)	0.14 ± 0.13 (24.9)	0.57 ± 0.19 (29.2)	2.14 ± 0.51 (29.6)
Crina Poultry[6]	1.43 ± 0.27 (28.6)	0.00 ± 0.13 (21.5)	0.43 ± 0.19 (25.8)	1.86 ± 0.26 (26.3)
Crina Alternate[6]	1.14 ± 0.27 (23.4)	0.14 ± 0.13 (24.9)	0.43 ± 0.19 (25.8)	1.71 ± 0.29 (23.9)
V[4] – WFA	1.43 ± 0.27 (26.8)	0.14 ± 0.13 (24.9)	0.29 ± 0.19 (22.4)	1.86 ± 0.40 (25.6)
V + Crina Poultry	1.28 ± 0.27 (26.2)	0.28 ± 0.13 (28.4)	0.29 ± 0.19 (22.4)	1.86 ± 0.90 (27.3)
V + Crina Alternate	0.71 ± 0.27 (15.8)	0.14 ± 0.13 (24.9)	0.29 ± 0.19 (22.4)	1.14 ± 1.21 (17.5)
Chi-squared	4.95	3.52	2.04	3.94
P-value	0.550	0.742	0.916	0.685

[1]Means represent 14 broilers per treatment and are presented as mean ± SEM (mean rank).
[2]NV = nonvaccinated; V = cocci-vaccinated with Advent [25] at 1 d of age by spray; WFA = without feed additives; AI = BMD [23] at 50 g/ton + Coban 60 [24] at 90 g/ton, except for the withdrawal phase (42 to 49 d).
[3]Essential oils [21], Crina Poultry and Crina Alternate, were added at 100 ppm.
[4]Kruskal-Wallis test ($P < 0.05$) was used to evaluate the effects of treatment on coccidia lesion scores [26] in each intestinal section.
[5]Total values of coccidial intestinal lesion scores [26].

the lesions and incidence of enteritis were observed mainly in the duodenum, indicating that some bacterial or protozoal challenge was still present at the time of evaluation. We hypothesized that there might not have been enough coccidial or bacterial challenge to observe significant effects.

The differences in responses between these 2 specific EO blends might be due to their different compositions. Lee et al. [31] observed that specific EO blends containing variable amounts of the active compounds carvacrol and thymol had significantly different effects on live performance. Blends of EO with carvacrol, but not thymol, improved FCR, although the researchers observed a reduction in BWG.

CONCLUSIONS AND APPLICATIONS

1. These 2 specific EO blends differ in their efficacy to promote growth in broilers.
2. The EO blend Crina Poultry promoted growth and maintained good FCR in young non-cocci-vaccinated broilers, but no significant improvements in live performance were observed at 49 d of age.
3. No significant effects of EO blends on live performance of cocci-vaccinated broilers were observed. The effects of EO blends on immunology of cocci-vaccinated broilers should be further studied.

REFERENCES AND NOTES

1. Thomke, S., and K. Elwinger. 1998. Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. Ann. Zootech. 47:153–167.

2. Huyghebaert, G. 2003. Replacement of antibiotics in poultry. In Proc. 12th Eastern Nutr. Conf., Québec, Canadá.

3. Thomke, S., and K. Elwinger. 1998. Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. Ann. Zootech. 47:245–271.

4. Stevens, D. A. 1998. Coccidiosis. Pages 591–59 in Encyclopedia of Immunology. 2nd ed. Vol I. P. J. Delves, and I. M. Roitt, ed. Acad. Press, London.

5. Magner, B. R. 1991. Anticoccidials. Pages 549–563 in Veterinary Applied Pharmacology and Therapeutics. 5th ed. G. C. Brander, D. M. Paugh, R. J. Bywater, and W. L. Jenkins, ed. ELBS, Bailliere Tindall, London.

6. Chapman, H. D., T. E. Cherry, H. D. Danforth, G. Richards, M. W. Shirley, and R. B. Williams. 2002. Sustainable coccidiosis control in poultry production; the role of live vaccines. Int. J. Parasitol. 32:617–629.

7. Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol. 31:317–353.

8. Danforth, H. D. 1998. Use of live oocyst vaccines for the control of avian coccidiosis: Experimental studies and field trials. Int. J. Parasitol. 28:1099–1109.

9. McDougald, L. R. 2003. Coccidiosis. Pages 974–991 in Diseases of Poultry. 11th ed. Y. M. Saif, ed. Iowa State Press-Blackwell Publ. Co. Ames, IA.

10. Dalloul, R. A., H. S. Lillehoj, T. A. Shellem, and J. A. Doerr. 2003. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. Poult. Sci. 82:62–66.

11. Lillehoj, H. S., and E. P. Lillehoj. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis. 44:408–425.

12. Dalloul, R. A., and H. S. Lillehoj. 2005. Recents advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49:1–8.

13. Cebra, J. J. 1999. Influences of microbiota on intestinal immune system development. Am. J. Clin. Nutr. 69(Suppl.):1046S–1051S.

14. Fukata, T., Y. Hadate, E. Baba, and A. Arakawa. 1991. Influence of bacteria on *Clostridium perfringens* infections in young chickens. Avian Dis. 35:224–227.

15. Apajalahti, J. 2004. Microbial management: A new approach for development in animal nutrition. Pages 1–24 in Proc. 25th Int. Seminar of AMEVEA-Colombia, Bogotá, Colombia.

16. Allen, P. C., H. D. Danforth, S. A. Gregory, and P. Comens-Keller. 1997. Assessment of recombinant bovine somatropin® as in immunomodulator during avian coccidiosis: Immunization with living oocysts. Poult. Sci. 76:1150–1155.

17. Saini, R., S. Davis, and W. Dudley-Cash. 2003a. Oregano essential oil reduces necrotic enteritis in broilers. Pages 95–97 in Proc. 52th Western Poult. Dis. Conf., Sacramento, CA.

18. Mitsch, P., K. Zitterl-Eglseer, B. Köhler, C. Gabler, R. Losa, and I. Zimpernik. 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult. Sci. 83:669–675.

19. Giannenas, I., P. Florou-Paneri, M. Papazahariadou, E. Christaki, N. A. Botsoglou, and A. B. Spais. 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Arch. Anim. Nutr. 57:99–106.

20. Saini, R., S. Davis, and W. Dudley-Cash. 2003b. Oregano essential oil reduces the expression of coccidiosis in broilers. Pages 97–98 in Proc. 52th Western Poult. Dis. Conf., Sacramento, CA.

21. Crina Poultry and Crina Alternate are specific essential oil blends produced for CRINA S.A. a company of Akzo-Nobel/Intervet Group, Gland, Switzerland.

22. National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. National Acad. Press, Washington, DC.

23. BMD (Alpharma, Inc., Ft. Lee, NJ) provided 50 g/lb (110 g/kg) bacitracin activity as Bactitracin methylene disalicylate.

24. Coban 60 (Elanco Animal Health Division of Eli Lilly & Co., Indianapolis, IN) provided 60 g/lb (132 g/kg) monensin activity.

25. Advent contained viable oocysts of *E. acervulina* (strain VND-A10), *E. maxima* (strain VND-M27), and *E. tenella* (strain VND-T49). Viridus Animal Health, LLC (Novus Int., Inc. St. Louis, MO).

26. Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.

27. SAS User's Guide. 2001. Version 8 ed. SAS Inst. Inc. Cary, NC.

28. Piva, G., M. Morlacchini, R. Riceardi, F. Musoero, A. Prandini, and F. Mandolini. 1991. Probiotic effect of essential oils in animal feeding. Microbiol. Aliment. Nutr. 9:161–169.

29. Shanmugavelu, S., J. D. Brooker, T. Acamovic, and A. J. Cowieson. 2004. Effect of thyme oil and garlic powder on the nutritive value of soybean meal. Br. Poult. Sci. 45(Suppl. 1):S9–S10. (Abstr.)

30. Waldenstedt, L. 2003. Effect of vaccination against coccidiosis in combination with an antibacterial oregano (*Origanum vulgare*) compound in organic broiler production. Acta Agric. Scand. A 53:101–109.

31. Lee, K. W., H. Everts, H. J. Kappert, K. H. Yeom, and A. C. Beynen. 2003. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res. 12:394–399.

32. Lee, K. W., H. Everts, and A. C. Beynen. 2004. Essential oils in broiler nutrition. Int. J. Poult. Sci. 3:738–752.

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