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Essential Oils on Mixed Coccidia Vaccination and Infection in Broilers[1]

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Abstract: One trial was conducted to evaluate the effects of two specific essential oil (EO) blends in broilers infected with viable oocysts of mixed *Eimeria* spp. Eight treatments were evaluated which consisted of three controls, two unvaccinated treatments and three treatments vaccinated at day of hatch with Advent® coccidia vaccine. The three controls were: 1) Unmedicated-Uninfected (UU), 2) Unmedicated-Infected (UI), and 3) antibiotic plus ionophore (AI). The two unvaccinated treatments were fed diets supplemented with either Crina® POULTRY (CP) or Crina® ALTERNATE (CA) at 100 ppm. Cocci-vaccinated treatments included one group fed diets without feed additives (WFA), and two fed diets supplemented with the two EO products (CP and CA) at the same concentration. At 19 d of age, all birds except those in the UU control were infected with *E. acervulina*, *E. maxima*, and *E. tenella*. Lesion scores (LS) and oocyst counts (OC) were performed 7 d post-infection and anticoccidial indexes (ACI) were calculated. The non-cocci-vaccinated chickens fed the EO blend CA, and the cocci-vaccinated chickens fed WFA diets had similar feed conversion ratios to the UU broilers, 7 d post infection. The cocci-vaccinated chickens fed diets containing EO had lower relative BWG than the cocci vaccinated group fed WFA diets. The lowest OC was observed in vaccinated birds fed WFA diets. Under the conditions of this experiment, the dietary inclusion of EO blends may serve as an alternative to antibiotic and/or ionophores in mixed *Eimeria* spp. infections in non-cocci-vaccinated broilers, but no benefits of EO supplementation were observed for vaccinated broilers against coccidia.

Key words: Broiler chicken, *Eimeria spp.*, essential oils, cocci-vaccination

Introduction

The effective use of anti-coccidial feed additives and growth promotant antibiotics (GPA) has played a major role in controlling enteric disease in the broiler industry over the past decades (Jones and Ricke, 2003; Thomke and Elwinger, 1998a). However, due to the rapid and continuous development of microbial drug resistance, higher costs for medication, consumer pressure for poultry products free of drug residues, and the ban of these products in several countries (Magner, 1991; Thomke and Elwinger, 1998b), there is an increasing interest in the search for alternative products to GPA and ionophores.

Coccidiosis is a common enteric parasitism that significantly influences broiler production, causing economic losses of about 1.5 billion USD every year worldwide (Stevens, 1998). Anticoccidial vaccination of broiler chickens has become a common alternative to achieve a sustainable control of coccidiosis (Chapman *et al*., 2002; Lillehoj and Lillehoj, 2000; Williams, 2002). The commercial cocci-vaccines available worldwide contain viable oocysts of at least three of the more common *Eimeria* species, such as *E. acervulina*, *E. maxima*, and *E. tenella* (Lillehoj and Lillehoj, 2000; Williams, 2002; Dalloul and Lillehoj, 2005). Under some commercial conditions, live vaccination generally causes a transitory early reduction in growth, which is generally associated with an increased incidence of secondary enteritis, and in very few occasions with necrotic enteritis (NE) (Chapman *et al*., 2002; Williams, 2002; Wages and Kenneth, 2003). Coccidiosis and *Clostridium perfringens* (Cp) type A or C are considered to be the main etiologies for NE (Wages and Kenneth, 2003; McDougald, 2003). The intestinal microflora also plays a role in acquired mucosal immunity and enteritis (Cebra, 1999; Kelly and Conway, 2005). Normal gut microflora in healthy birds inhibits the pathogenicity of Cp (Fukata *et al*., 1991), and modulates the immune

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

responses against coccidia (Lillehoj and Lillehoj, 2000; Dalloul and Lillehoj, 2005; Dalloul *et al.*, 2003).

The supplementation of poultry diets with phytochemicals is an alternative to GPA and ionophores to modulate enteric microflora (Kamel, 2000; Young and Noh, 2001; Williams and Losa, 2001). However, the effects of these phytochemicals on bird live performance are highly variable, which may be due to their various effects not only on gut microbiota dynamics (Dorman and Deans, 2000; Paster *et al.*, 1990), but also on animal metabolism (Lee *et al.*, 2004). A detailed understanding of the bioactive effects of feeding plant products and phytochemical blends to reduce the incidence of diseases in birds remains a challenge. Herbs, spices and their essential oils (EO) extracted by steam distillation comprise more than 30 chemically active compounds, most of which are phenolic compounds (carvacrol, thymol, eugenol, curcumin and peppermint) with varying anti-oxidative, antimicrobial or antifungal activity (Lee *et al.*, 2004; Botsoglou *et al.*, 2002; Economou *et al.*, 1991). In reference to NE, Mitsch *et al.* (2004) concluded that EO thymol, carvacrol, and eugenol can control the proliferation of Cp in the broiler intestines and could potentially reduce the effects of complications associated with coccidiosis such as NE. However, the authors also concluded that different EO blends may have different efficacies in this respect. Additionally, Giannenas *et al.* (2003) observed that oregano EO, mainly carvacrol and thymol, also exerted an anticoccidial effect against *E. tenella*.

The purpose of this study was to evaluate the dietary supplementation of two commercially available EO blends, Crina® POULTRY (CP) and Crina® ALTERNATE (CA) as alternatives to GPA and ionophores for non-cocci-vaccinated broilers, or as immunomodulators in cocci-vaccinated broilers during a mixed *Eimeria* spp. infection.

Materials and Methods

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

Treatments and bird husbandry: A total of 288 one-day-old Cobb-500 male chickens were used to compare eight treatments; five non-coccidia-vaccinated and three coccidia-vaccinated. The unvaccinated treatments included three control groups with chickens fed basal diets. Two of these control groups were negative controls fed diets without feed additives: (1) Unmedicated-Uninfected (UU) and (2) Unmedicated-Infected (UI). A third treatment was included as a positive control group (3) with chickens fed the basal diets supplemented with the antibiotic bacitracin methylene disalicilate as BMD® that provides 50 g/lb (110 g/kg) of bacitracin activity (Alpharma, Inc., Ft. Lee, NJ 07024)., and the ionophore monensin as Coban 60® (Elanco Animal Health division of Eli Lilly & Co. Indianapolis, IN 46825), which provides 60 g/lb (132 g/kg) of monensin activity (AI). The other two non-coccidia-vaccinated groups were fed the basal diet supplemented with the specific EO blends CP and CA (DSM CRINA, DSM Nutritional Products SA., Switzerland). Chickens in the last three treatments were vaccinated with viable attenuated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella* at 1 d of age with Advent®. These cocci-vaccinated chickens were given the basal diets without feed additives (WFA) (6), and two treatments were fed the two EO blends CP (7) and CA (8). These EO blends were added to the basal diets in powder form at 100 ppm, with a total concentration of active EO compounds of about 30%. The main compounds are thymol (*thymus vulgaris),* eugenol (*Syzygium aromaticum*), also part of (*Cinnamonum zeylanicum*), curcumin (*Curcuma zanthorrhiza*) and piperin (*Piper nigrum*).

Broilers were placed in 48 floor pens (6 birds/pen) in a tunnel-ventilated black out house and randomly assigned among eight dietary treatment groups with 6 replicates per treatment. Bedding consisted of used litter top-dressed with two inches of fresh pine wood shavings. Birds were reared to 13 d of age in floor pens and then moved to battery cages. This management was done to guarantee that birds had natural contact with litter microflora and recycling of vaccinal oocysts as well as to evaluate the effects of treatments after stress of transportation, and adaptation to a new environment and diet. Another six additional replicates of the negative control treatments (UU and UI) were raised in battery cages (6 birds/cage) from the first day of age to avoid cross contamination with field or vaccinal oocysts. These additional replicates were used for the challenge and to make comparisons with the other treatments after 13 days of age.

Broilers were fed with corn-soybean meal starter (1 to 13 d) and grower (13 to 33 d) diets previously described (Oviedo-Rondón *et al.*, 2005). Diets were formulated to guarantee or exceed recommended nutrient requirements (NRC, 1994) with 22.4% crude protein and 3,050 kcal/kg for the starter diets and 19.6% protein and 3,120 kcal/kg for the grower diets. One basal diet was mixed for each dietary period and dietary additives were blended in at later time according to treatment distribution. Diets were pelletized, starter diets fed as crumbles and grower diets as pellets.

Vaccination and infection: Broilers in the treatments 6, 7 and 8 were vaccinated by spray with Advent® cocci-vaccine at 1 d of age in a cabinet. The Advent vaccine contains viable oocysts of *E. acervulina* (strain VND-A10), *E. maxima* (strain VND-M27), and *E. tenella* (strain VND-T49). (Viridus Animal Health, LLC. – Novus International, Inc. St. Louis, MO 63141). All broilers

except those in the UU treatment were infected at 19 d of age with a standard oral inoculum of sporulated oocysts from field isolates of *E. acervulina*, *E. maxima*, and *E. tenella* at 200, 100, and 50 x 10^3 viable oocysts/ml, respectively. The oocysts used in the challenge inoculum were unrelated to those in the vaccine.

Data collection: Body weight gain (BWG) and feed intake (FI) were recorded when broilers were 13, 19, and 26 d of age. Feed conversion ratio (FCR) was calculated and corrected for body weight of mortality. Mortality was recorded twice a day. Temperature and light were controlled. Variations from the programmed and relative humidity were recorded.

Lesion scores (LS) in the duodenum, midgut, and ceca were evaluated in two birds per cage 7 d post infection according to Johnson and Reid (1970). Oocyst counts (OC) were performed also at 7 d post infection from duplicate fecal samples taken from each cage group. Fecal samples were homogenized and diluted in a saturated NaCl solution at a ratio of 1:10 before counting in McMaster chambers to determine the number of oocysts per g of feces (Hodgson, 1970). The oocyst index (OI) was calculated as compared to UI control. An anti-coccidial index (ACI) was calculated for each treatment taking into consideration survival rate, relative BWG, and indexes of lesion scores and oocysts.

Statistical analyses: Pen means were used as experimental units. A completely randomized statistical design was used. Percentage mortality data were transformed by the arcsine method before analysis and final data are presented as natural numbers. Statistical significance was based on a probability of $P \leq 0.05$. Data were subjected to ANOVA using the GLM procedure of SAS system (SAS, 2001). Mean separation was accomplished using Tukey's multiple range tests when a significant F statistic was indicated by ANOVA. Because the oocyst yields were not normally distributed, they were transformed into ln(x+1). Lesion scores were analyzed using a chi-square test (Kruskal–Wallis test) (Sall and Lehman, 1996).

Results and Discussion

Different intestinal stresses were evaluated in this experiment. Coccidia vaccination was evaluated from 1 to 13 d, transport and adaptation to new diet and environment from 14 to 19 d of age, and the mixed cocci-infection from 19 to 26 d.

Pre-infection periods: Data of live performance from two pre-infection periods are presented in Table 1. During the first 13 d in floor pens, only FCR was significantly ($P < 0.05$) affected by treatments. The EO blend CP improved FCR significantly in non-vaccinated birds as compared to the controls UU and UI raised in the floor pens and in the battery cages. However, this improvement was not significantly ($P > 0.05$) different from the other treatments (Table 1).

The stress of transportation at 13 d of age, and the changes in diet and environment from floor pens into cages significantly ($P < 0.001$) affected the BWG and FCR of all cocci-vaccinated treatments in comparison to birds from UU control groups raised in batteries or AI groups previously housed in floor pens, during the period from 14 to 19 d of age. Cocci-vaccinated broilers fed WFA diets had the lowest FI, but this was only significantly ($P < 0.001$) lower than that of UU controls. Although, feed additives did not improve significantly the performance of cocci-vaccinated or non-cocci-vaccinated broilers in comparison to controls, the relative BWG of cocci-vaccinated birds during the pre-infection period was improved by at least 8% by either EO blend supplement (67 and 68% vs. 59%) and depressed by at least 6% in non-coccivacinated broilers in relation to the AI group. Additionally, non-cocci-vaccinated broilers fed diets supplemented with either CP or CA had BWG and FCR not significantly different from the three control groups (Table 1).

Even though cocci-vaccination with viable attenuated oocysts had no negative effects on broiler performance at 13 d of age, the stress associated with movement into cages and change to grower diets caused shifts on gut microflora as it was demonstrated by data presented in the companion papers (Oviedo-Rondón *et al.*, 2005, Hume *et al.*, 2006). These shifts in microflora affected more the cocci-vaccinated treatments ($P < 0.001$) at 19 d of age (Table 1). Apajalahti (2004) reported that *Eimeria maxima* infection caused temporary changes in ileal and cecal microbial populations and patterns of fermentation. It would be expected that these effects are more clear when three *Eimeria* spp. are utilized. The effect of multiple specie vaccination might explain the sporadic reductions in performance and higher incidence of enteritis in cocci-vaccinated broilers under commercial conditions, where some stressful conditions or concomitant diseases are present, without real field coccidia challenge (Williams, 2005).

Live performance post-infection: The mixed oocyst *Eimeria* infection reduced ($P < 0.001$) BWG by 45%, FI by 30%, and increased FCR by 29% in the UI control groups in comparison with the UU groups (Table 2). Cocci-vaccinated broilers fed WFA or CP diets, and non-vaccinated broilers fed CA diets had significantly better BWG than the UI during the period from 19 to 26 d of age. All cocci-vaccinated treatments had significantly higher FI than the UI control, and not significantly different from the UU control. Only the cocci-vaccinated birds fed WFA diets and the non-vaccinated broilers fed CA diets had significantly better FCR than the UI control group, and even similar to the UU control group. Survival

Table 1: Effect of essential oils, medication, and vaccination on live performance of broilers pre-infection (1 – 19 d of age)[1]

Treatment[2, 3]	BWG[5] (g/bird/day)		Relative BWG[6] (%)		FI[7] (g/bird/day)		FCR[8] (g/g)	
	1-13d	14-19 d	1-13 d	14-19d	1-13 d	14-19d	1-13 d	14-19 d
UU	27.6	50.5^{a}	100	100	34.6	71.0^{ab}	1.25^{a}	1.41^{c}
UI	27.7	42.7^{abc}	100	85	34.7	67.2^{abc}	1.25^{a}	1.57^{c}
AI	27.5	48.3^{ab}	100	96	34.1	73.8^{a}	1.23^{ab}	1.53^{c}
Crina Poultry	28.4	45.3^{ab}	103	90	34.6	68.7^{ab}	1.21^{b}	1.53^{c}
Crina Alternate	28.4	39.7^{bc}	103	79	35.0	62.2^{bc}	1.23^{ab}	1.59^{bc}
V4 + WFA	27.2	29.7^{d}	98	59	33.5	56.3^{c}	1.23^{ab}	1.90^{a}
V + Crina Poultry	27.4	34.0^{cd}	99	67	33.5	62.5^{bc}	1.22^{ab}	1.85^{a}
V +Crina Alternate	27.8	34.0^{cd}	101	68	34.5	60.5^{bc}	1.23^{ab}	1.79^{ab}
CV %	3.3	12.5	_	_	1.5	9.4	1.6	6.9
Pooled SEM	0.4	2	_	_	0.4	2.3	0.01	0.05
P-value	0.176	< 0.001	_	_	0.153	< 0.001	0.049	< 0.001

$^{a-d}$Means within columns and lacking common lowercase superscripts are significantly different (P < 0.05). [1]Means represent 6 replicates of pens during 1-13 d of 36 male broilers each or battery cages of 6 broilers each for 36 birds per treatment.
[2]UU= Unmedicated - Uninfected; UI = Unmedicated Infected at 19 days of age; AI = BMD® at 50 g/ton + Coban® – 60 at 90 g/ton.
[3]Essential oils, Crina® Poultry and Crina® Alternate were added at 100 ppm. [4]V = cocci-vaccinated with Advent® at 1 d of age by spray; WFA = fed diets without feed additives. [5]BWG = body weight gain. [6]Relative BWG (%) = BWG per group x 100 / BWG of UU birds. [7]FI = feed intake [8]FCR = feed conversion ratio (feed:gain ratio)

Table 2: Effect of infection, essential oils, medication, and vaccination on live performance of broilers post infection (20-26 d)[1]

Treatment[2,3]	BWG[4]	FI[6]	FCR[7]	Oocyst count[8]	
	-------- (g/bird/day) ---------		(g/g)	(10^3/g of excreta)[9]	Ln (x+1)[10]
UU	61.4^{a}	107.1^{a}	1.75^{b}	0.15 ± 0.04	4.87 ± 0.26^{c}
UI	33.7^{c}	75.0^{c}	2.25^{a}	7.74 ± 2.68	8.56 ± 0.43^{ab}
AI	47.7^{abc}	89.6^{bc}	1.90^{ab}	10.96 ± 1.82	9.23 ± 0.17^{a}
Crina Poultry	45.6^{bc}	89.1^{bc}	2.00^{ab}	5.05 ± 1.86	7.86 ± 0.62^{b}
Crina Alternate	49.6^{ab}	85.0^{bc}	1.73^{b}	6.73 ± 2.87	8.43 ± 0.38^{ab}
V4 + WFA	55.9^{ab}	97.7^{ab}	1.77^{b}	2.89 ± 0.61	7.80 ± 0.30^{b}
V + Crina Poultry	52.7^{ab}	98.3^{ab}	1.88^{ab}	6.21 ± 3.50	8.03 ± 0.54^{b}
V+ Crina Alternate	46.9^{bc}	97.7^{ab}	2.13^{ab}	4.45 ± 1.89	8.03 ± 0.37^{b}
CV %	15.5	10.0	12.1	3.1	12.7
Pooled SEM	3.1	5.0	0.09	1.9	0.38
P-value	< 0.001	<0.001	0.002	0.055	< 0.001

$^{a-c}$Means within columns and lacking common lowercase superscripts are significantly different (P < 0.05).
[1]Means represent 6 replicates of 6 broilers each for 36 birds per treatment.
[2]UU= Unmedicated - Uninfected; UI = Unmedicated Infected, orally inoculated with a mixed solution of approximately 2 x 10^5 oocyst of *E. acervulina,* 1x10^5 oocysts of *E. maxima* and 5x10^4 oocysts of *E. tenella* per bird on day 19; AI = BMD® at 50 g/ton + Coban® – 60 at 90 g/ton. [3]Essential oils, Crina® Poultry and Crina® Alternate were added at 100 ppm.
[4]V = cocci-vaccinated with Advent® at 1 d of age by spray; WFA = fed diets without feed additives. [5]BWG = body weight gain
[6]FI = feed intake. [7]FCR = feed conversion ratio (feed:gain ratio). [8]Oocyst counts are given as mean ± SEM (n = 24),
[9]Total oocyst output per gram of excreta. [10]Transformation of oocyst outputs to Ln (x+1) was used due to lack of normality in OC data.

was not affected significantly (P > 0.05) by infection or any of the treatments evaluated.

The specific EO blends CP and CA had significant but variable beneficial effects in non-cocci-vaccinated broilers according to the period evaluated. Dietary supplementation of CP, but not CA, supported improved FCR in the two pre-infection periods, while the EO blend CA, but not CP, maintain BWG and FCR similar to the UU control group in spite of the mixed cocci-infection. This differential effect among specific EO blends was also observed by Lee *et al.* (2003) who concluded that the EO thymol and its isomer carvacrol have significantly different effects on live performance and triglyceride metabolism. They concluded that carvacrol, but not thymol, was observed to improve FCR, even though FI and BWG were reduced.

Oocyst counts, Lesion Scores and Anticoccidial Index: The highest OC was observed in the AI group, but this was not significantly different from the OC observed in the UI control, and the non-cocci-vaccinated broilers fed CA diets (Table 2). All cocci-vaccinated groups (WFA, CP and CA), and the non-coccivaccinated groups fed CP diets had OC significantly (P < 0.001) lower than the AI control group, but not different from the UI control.

Oocyst counts per gram of excreta are a very difficult measurement to evaluate in a mixed *Eimeria* spp. infection since every specie cycles at different rates (McDougald, 2003). However, since under field conditions the existence of single specie cocci-vaccines or infections are unusual, it is important to study this pathology using various *Eimeria* spp. in the infection experiments. Unexpectedly, the highest OC was

Table 3: Effect of infection, essential oils, medication, and vaccination on intestinal lesion scores 7 d post infection[1]

Treatment[3,4]	Lesion Scores (Mean rank)[2]			Lesion Scores[9] (Mean rank)
	Duodenum[6]	Jejunum-Ileum[7]	Cecum[8]	
UU	0.1 ± 0.0 (13.2) [c]	0.2 ± 0.1 (13.8)	0.1 ± 0.1 (10.6) [c]	0.7 ± 0.4 (8.0) [b]
UI	0.9 ± 0.1 (25.8) [a]	0.8 ± 0.3 (26.9)	1.8 ± 0.2 (41.9) [a]	3.4 ± 0.5 (37.6) [a]
AI	1.0 ± 0.1 (28.1) [a]	0.8 ± 0.3 (28.3)	0.8 ± 0.3 (26.3) [ab]	2.5 ± 0.6 (26.0) [ab]
Crina Poultry	1.3 ± 0.2 (33.8) [a]	0.8 ± 0.3 (27.4)	0.7 ± 0.3 (23.7) [b]	2.7 ± 0.4 (30.5) [ab]
Crina Alternate	0.5 ± 0.2 (15.0) [b]	1.1 ± 0.2 (35.0)	1.0 ± 0.3 (30.6) [ab]	2.6 ± 0.4 (29.7) [ab]
V5 + WFA	1.3 ± 0.3 (32.6) [a]	0.4 ± 0.2 (21.6)	0.3 ± 0.1 (18.3) [bc]	2.0 ± 0.4 (23.1) [ab]
V + Crina Poultry	1.2 ± 0.2 (31.1) [a]	0.7 ± 0.5 (20.3)	0.7 ± 0.4 (23.7) [b]	2.5 ± 0.8 (19.9) [ab]
V + Crina Alternate	0.6 ± 0.2 (16.4) [b]	0.5 ± 0.2 (22.8)	0.5 ± 0.2 (21.0) [b]	1.6 ± 0.2 (16.6)[ab]
Chi-square	16.9	9.4	19.4	19.3
Probability	0.01	0.227	0.007	0.007

[a-c]Means within the same column lacking common lowercase superscripts are significantly different ($P < 0.05$). [1]Means represent 2 birds per pen and 12 replicates per treatment. [2]Kruskal-Wallis test was used to evaluate lesion score data from each gut section ($P < 0.05$). Results are presented as mean ± SEM (Mean rank). [3]UU = Unmedicated - Uninfected; UI = Unmedicated Infected, orally inoculated with a mixed solution of approximately $2x10^5$ oocyst of *E. acervulina*, $1x10^5$ oocysts of *E. maxima* and $5x10^4$ oocysts of *E. tenella* per bird on day 19; AI = BMD® at 50 g/ton + Coban® – 60 at 90 g/ton. [4]Essential oils, Crina Poultry and Crina Alternate were added at 100 ppm. [5]V = cocci-vaccinated with Advent® at 1 d of age by spray; WFA = fed diets without feed additives. [6]Area most affected by E. acervulina. [7]Area most affected by E. maxima. [8]Area most affected by E. tenella. [9]Total of lesion scores in each bird at 7 d post infection.

observed in the AI group, but this was not significantly different from the OC observed in the UI control, and the non - cocci - vaccinated broilers fed CA diets. We hypothesized that the strains of oocysts used in the infection might have developed resistance to the ionophore monensin and the recommended dose of 90 g/ton is not enough to control mixed infection with this specific inoculum. We observed high OC in parallel experiments performed at this lab with the same AI combination (Oviedo-Rondón *et al.*, 2005). Then, the most appropriate comparisons are made with the negative control UU. These EO blends did not significantly ($P > 0.05$) reduce the OC in this mixed cocci-infection. In contrast, Giannenas *et al.* (2003) observed that oregano and carvacrol EO reduced OC in birds infected with *E. tenella*, but in their experiment the ionophore treatment had the lowest OC.

Significant effects of treatments over LS were observed in duodenum ($P < 0.01$) and cecum ($P < 0.001$), but not ($P > 0.05$) in the jejunum-ileum section (Table 3). The LS scores observed in chickens fed diets with the AI combination were not significantly different from the UI control group. Dietary supplementation with the EO blend CA significantly reduced LS in duodenum in cocci-vaccinated and non-cocci-vaccinated birds, but this effect was not significant in LS observed in jejunum-ileum and cecum. In contrast, the EO blend CP reduced significantly the cecal LS in non-cocci-vaccinated birds in comparison to the UI control group, but a similar response was observed in all vaccinated groups. These results indicate differential effects of specific EO blends over upper and lower intestinal environments and/or enhancement of specific responses against *E. acervulina* and *E. tenella*.

The best ACI was observed in cocci-vaccinated chickens fed WFA diets (Table 4). Under the conditions of this experiment where apparently field oocsyst were resistant to monensin, all birds fed diets supplemented with EO blends had better ACI values than the AI - positive control group. In contrast, cocci-vaccinated birds fed EO blends had lower ACI values than the cocci-vaccinated fed WFA diets. Since LS index and survivability rates per individual pens are clearly not representing the whole treatment effect, it is not adequate to perform statistical analyses with these ACI values. However, the ACI provide an idea of overall broiler response to treatments.

It is of interest to observe that there are differences in the responses observed with these two specific EO blends according to the type of intestinal stress. These EO blends did not show any significant benefits in cocci-vaccinated birds, and, after the mixed *Eimeria* infection, the cocci-vaccinated broilers fed WFA diets had the best live performance, the lowest OC and LS, and the best ACI. In contrast, Waldenstedt (2003) observed significant improvements in BWG and FI in cocci-vaccinated broilers supplemented with dietary oregano (EO) up to 48 d of age, while FCR was not significantly affected (Williams and Losa, 2001). Additionally, Walter and Bilkei (2004) reported that dietary oregano EO can stimulate CD_4^+, CD_8^+, double positive T lymphocytes in peripheral blood, and mesenteric lymph nodes in pigs. If the effects were similar in birds, the present EO blends should enhance non-specific mucosal immunity against coccidia, which depends mainly on cellular CD_8^+ T cells (Dalloul *et al.*, 2003). Conversely, in the present experiment immunostimulatory effect of the EO blends CP and CA was not observed in birds vaccinated with Advent® cocci-vaccine. However, it is necessary to consider that specific EO blends vary considerably with respect to contents of phenolic compounds that might also exert cytotoxic effects on the villus tips of the

Table 4: Effect of infection, essential oils, medication and vaccination on relative body weight gain, lesion score and oocysts count indexes 7 d post infection[1]

Treatment[2,3]	Survival rate[5] (%)	RBW gain[6](%)	Lesion Score Index[7]	Oocyst Index[8]	Anti-coccidial Index[9]
UU	100	100	0.56	0.8	198.7
UI	100	55	2.85	40.0	112.2
AI	97	78	2.08	56.6	119.3
Crina Poultry	92	74	2.22	26.1	142.9
Crina Alternate	100	81	2.15	34.8	135.7
V[4] + WFA	97	91	1.67	14.9	174.4
V + Crina Poultry	100	86	2.08	32.1	149.0
V + Crina Alternate	100	76	1.32	23.0	151.7

[1]Values represent the relative value per treatment in relation to the UU or UI controls.
[2]UU= Unmedicated - Uninfected; UI = Unmedicated Infected, orally inoculated with a mixed solution of approximately $2x10^5$ oocyst of *E. acervulina*, $1x10^5$ oocysts of *E. maxima* and $5x10^4$ oocysts of *E. tenella* per bird on day 19;
AI = BMD® at 50 g/ton + Coban® – 60 at 90 g/ton. [3]Essential oils, Crina® Poultry and Crina® Alternate were added at 100 ppm.
[4]V = cocci-vaccinated with Advent® at 1 d of age by spray; WFA = fed diets without feed additives
[5]Survival rate, % = [No. of chickens p.i x 10 / No. of chickens housed (Pre-Infection)] x 100
[6]Relative BWG, % = [BWG per group / BWG of UU birds] x 100
[7]Lesion scores index = Lesion Score per group x 10 / No. of birds
[8]Oocyst count Index = Oocyst Count per group x .4 x 100 / Oocyst Count for Unmedicated-Infected birds
[9]Anti-coccidial Index= (% Survival + % Relative BWG) – (Index of Lesion Score + Index of Oocyst Count).

intestinal mucosa (Giannenas *et al.*, 2003; Lee *et al.*, 2004). This type of cytotoxic effect might affect part of the development of immune responses in cocci-vaccinated birds, which could partially explain the lower performance and higher LS and OC observed in cocci-vaccinated birds fed with these EO blends in the present experiment. It is important to determine whether or not these specific EO blends affect cocci-vaccine efficacy.

Results of our studies in gut microbial ecology (Oviedo-Rondón *et al.*, 2006; Hume *et al.*, 2006) by PCR-DGGE analyses of pre- and post infection samples collected in this experiment indicated that these EO blends modulate intestinal microbial communities, and this might explain partially the responses presented herein. The EO blends have been linked to antibacterial effects, stimulatory effects on digestive enzymes, antioxidant properties (Lee *et al.*, 2004), and might inactivate *Clostridium perfringens* toxins (Mitsch *et al.*, 2004). Shanmugavelu *et al.* (2004) concluded that EO from thyme and garlic do not improve the nutritional value of soybean meal, and they concluded that any improvements observed with EO could be due only to their antimicrobial effects. Further research should be conducted to clarify the specific effects over mucosal immunity or host metabolism under conditions of mixed *Eimeria* challenge.

Some EO blends had been shown to be as effective as the antibiotics virginiamycin (Piva *et al.*, 1991), and BMD (Saini *et al.*, 2003a), in the prevention of NE, and to the ionophores lasolacid (Giannenas *et al.*, 2003) and salinomycin (Saini *et al.*, 2003b) in reducing the expression of single specie coccidial infection. Although, a more complex cocci-infection was evaluated in the present experiment, the live performance, LS and ACI results compared to UU and UI controls indicate that these two EO blends show some benefits in responses against mixed *Eimeria* spp. infection, and could be considered as alternatives to GPA and ionophores, especially when there is drug-resistance and coccidia vaccination is not an option. The differences in beneficial effects between products indicate potential for further improvements in EO blend formulation to obtain more uniform results.

In conclusion the two specific EO blends evaluated had similar efficacy to maintain growth, reduce intestinal lesions and oocyst shedding after an induced mixed coccidia infection. Cocci-vaccinated broilers may show lower performance under some conditions of stress such as transportation and change of feed. However, cocci-vaccinated broilers fed diets without feed additives had the best live performance, lowest oocyst shedding and anticoccidial index responses seven days after a challenge with mixed *Eimeria* spp. The supplementation of these two specific EO blends to coccidia vaccinated broilers did not show any benefits under the present experimental conditions.

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