Journal of Animal and Feed Sciences, 21, 2012, 133–142

The effect of a dietary herbal extract blend on the performance of broilers challenged with *Eimeria* oocysts

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(Received 28 September 2011; revised version 15 November 2011; accepted 12 March 2012)

ABSTRACT

The aim of the experiment was to evaluate the anticoccidial efficacy of supplementing feeds with herbal extract blend containing garlic (*Allium sativum*), sage (*Salvia officinalis*), echinacea (*Echinacea purpurea*), thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare)* extracts in broiler chickens experimentally infected with 170.000 sporulated oocysts of *Eimeria acervulina, E. tenella, E. maxima and E. necatrix* at 12 days of age. A total of 280 Ross 308 broilers were randomly allocated into 5 groups with 7 replicates of 8 chickens per replicate. The experimental dietary treatments were formulated by supplementing the basal maize-soyabean meal diet. The experimental design allocated the groups as follows: 1. uninfected birds and an unsupplemented diet; 2. uninfected birds and the diet supplemented with the herbal extract blend at a level of 1 g/kg feed (200 mg of each herbal extract/kg); 3. infected birds and an unsupplemented diet; 4. infected birds and a diet with the addition of coccidiostat diclazuril at a level of 1 mg/kg feed; and 5. infected birds and a diet supplemented with the herbal extract blend at a level of 1 g/kg feed (200 mg of each herbal extract/kg). Throughout the 42 days of the experimental period, performance parameters, mortality, lesion score, oocyst output, and serum carotenoid concentration were recorded. Dietary supplementation with the herbal extract blend increased growth performance to the level found in the group fed the coccidiostat and in the non-challenged groups. The number of oocysts per gram of excreta did not differ significantly between the groups fed the herbal extract blend and the group receiving the coccidiostatic supplement. The herbal extract blend had no effect on mortality, lesion score, with the exception of the duodenum, or blood carotenoid concentration.

KEY WORDS: broiler chickens, coccidiosis, herbal extracts, performance, diclazuril, *Eimeria*

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INTRODUCTION

Coccidiosis, which is caused by protozoa of the genus *Eimeria*, is an intestinal parasitosis common in poultry. Infection results from ingestion of sporulated oocysts, which are universal contaminants of poultry environments. Global annual losses to the industry as a result of coccidiosis are estimated at over $3 billion (Dalloul and Lillehoj, 2006). According to Williams (1999), coccidiosis-related losses in the United Kingdom, estimated at thirty million pounds sterling, consisted of 80.6% lower production rates, i.e. higher mortality, reduced weight gain and higher feed conversion ratio, and 17.5% prevention and treatment costs.

In modern, intensive poultry production, the control of coccidiosis is based on chemoprophylaxis, which relies on the routine supplementation of feed with coccidiostats, taking into account the grace period and, on a lesser scale, immunoprophylaxis (vaccination). The improper use of coccidiostats has, however, led to the emergence of drug-resistant strains of *Eimeria* spp. (Ruff and Danforth, 1996). Moreover, there is strong evidence that residues of some coccidiostats may be present in the meat and the consumer is not being given adequate protection against this (Olejnik et al., 2009). The increasing drug resistance of coccidia, the risk of accumulation of these chemotherapeutic residues in meat and their potential negative impact on human health, consumer pressure in respect to food safety, and the risk of potential cross-resistance of microorganisms to the antimicrobials used to treat humans or animals gave rise to Regulation (EC) No 1831/2003 of the European Parliament and Council which provides for a ban on the prophylactic use of coccidiostats that will go into effect in 2013 (Anonymous, 2003). Consequently, there is great interest in the development of alternative methods for the prevention of coccidiosis. In this context, a number of plants and herbal products have been tested for their anticoccidial properties (Youn and Noh, 2001; Chandrakesan et al., 2009; Abbas et al., 2010; Lee et al., 2010; Haq et al., 2011).

On 1st January 2008, The European Commission submitted a report (Anonymous, 2008) on the withdrawal of coccidiostats and histomonostats from use in feed additives. According to the EC, there is currently no comparably effective alternative to coccidiostats, although the search for their natural replacements is the subject of many studies. There is thus an urgent need to continue the studies in order to develop efficient coccidiostat substitutes. An optional method, or one complementary to vaccination, is the use of herbal plants that exhibit antimicrobial, antioxidant and anti-stress properties, exert effects on gut microflora, and show nutrigenomic effects and immune enhancement properties (Hashemi and Davoodi, 2010).

The aim of this study was to determine the effect of a dry herbal extract blend derived from garlic (*Allium sativum*), sage (*Salvia officinalis*), echinacea

(*Echinacea purpurea*), thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) on the course of coccidiosis in the gastrointestinal tract of broiler chickens.

MATERIAL AND METHODS

All of the experimental procedures involving animals were approved by the Local Animal Care and Use Committee.

In total, 280 one-day-old Ross 308 chicks of both sexes were randomly assigned to 5 experimental treatments. Each treatment was divided into 7 replicates (cages) of 8 birds (4 male and 4 female chicks). The chickens were reared to 42 days of age in wire-floored cages with free access to water and feed. All birds were fed with a starter (1 to 21 d) and grower-finisher (22 to 42 d) maize-soyabean meal basal diet, free of antibiotic growth promoters and coccidiostats and formulated to satisfy the nutrient requirements of broilers (Smulikowska and Rutkowski, 2005). Basal diets contained, %: crude protein 22, Lys 1.23, Met 0.56, Ca 0.95 and available P 0.44, ME/kg 12.4 MJ (starter), or, crude protein 20, Lys 1.18, Met 0.51, Ca 0.93 and available P 0.41, ME/kg 13.1 MJ (grower-finisher). The experimental dietary treatments were formulated by supplementing the basal diet. The experimental design allocated the groups as follows: 1. uninfected birds and an unsupplemented diet; 2. uninfected birds and the diet supplemented with the herbal extract blend derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* at a level of 1 g/kg feed (200 mg of each herbal extract/kg); 3. infected birds and an unsupplemented diet; 4. infected birds and a diet with the addition of a coccidiostat, diclazuril, at a level of 1 mg/kg feed; and 5. infected birds and a diet supplemented with the herbal extract blend at a level of 1 g/kg feed (200 mg of each herbal extract/kg). From 1 to 11 d of age, all of the chickens were fed experimental diets, at 12 d of age groups 1 and 2 were orally inoculated with water, while birds from groups 3-5 were inoculated *per os* with 170,000 sporulated oocysts of *E. acervulina, E. tenella, E. maxima* and *E. necatrix*.

The birds were weighed at 1, 21 and 42 d of age. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and mortality were calculated for 1-21, 22-42 and 1-42 d of age. The European broiler index (EBI) was calculated for the entire feeding period according to the equation (Koreleski et al., 2010):

$$EBI = [\text{body weight (kg)} \times \text{survival rate (\%)} / \text{age (days)} \times \text{FCR (kg feed/kg BWG)}] \times 100$$

The number of oocysts per gram of excreta (OPG) was determined using a McMaster chamber (Mazurkiewicz, 2005) in pooled excreta samples taken from

each replicate on post-inoculation days 6, 7, 8, 9, 14 and 21. Lesion scoring was performed on day 6 post-inoculation, and graded from 0 to 4 on the scale described by Conway and McKenzie (2007). Blood samples were collected from 6 birds in each group (one bird from replicates 1-6) on post-inoculation day 6 in order to analyse the carotenoid concentrations in serum filtrates (Negro et al., 1998).

At the end of the experiment and after 12 h feed withdrawal all chickens were weighed and 4 representative cockerels and 4 hens were chosen from each group (one male and one female from replicates 1-4) with live body weights close to the group average, marked with number signs and decapitated. Chickens were plucked, the intestines and crop were removed and carcasses stored overnight in 4^{o}C. The mass of the cooled carcass with edible giblets (gizzard, liver, heart) was estimated and carcass yield calculated. The breast muscles, abdominal fat, livers and gizzards were excised and weighed. The breast muscle and abdominal fat contents were expressed as % of cold carcass. The weight of the liver and gizzard were expressed as % of liveweight (Ziołecki and Doruchowski, 1989).

The data were analysed by means of one-way ANOVA and the significance of differences between mean values was assessed using Duncan's test (STATISTICA 5.0 software package; StatSoft, 1997). Differences were considered significant at $P<0.05$.

RESULTS

The performance data obtained from the trial are given in Table 1. The production results in the first period of feeding confirmed the high efficiency of the experimental infection of the chickens with viable oocysts. The mixed *Eimeria* infection negatively affected BWG, FCR and mortality at 21 d of age, which is post-inoculation day 9 (groups 3-5 vs 1 and 2). The BWG of the infected chickens fed the unsupplemented diet were more than 40% lower compared with the group of uninfected ones (Table 1). The addition of the herbal extract blend to the diet (group 5) alleviated the negative effects of the infection, thus BWG and FCR were superior by 25.0 and 19.5% over the infected and unsupplemented group (group 5). The highest mortality rate was noted in group 5, however.

In the second feeding phase (22-42 day of age), the trend towards deterioration in BWG and FCR in the infected group of chickens fed the unsupplemented diet was statistically confirmed ($P<0.05$). The performance indices in the group of infected chickens (group 5) receiving the herbal extract blend did not statistically differ from those chickens (group 4) fed the diet supplemented with the coccidiostat (Table 1).

Table 1. Effect of experimental treatments on performance of broiler chickens

Group number	Infection with *Eimeria* oocysts	Dietary treatment	Body weight gain (BWG) g	Feed intake g	Feed conversion ratio g feed/g BWG	Mortality %	European broiler index points
Days 1-21							
1	-	-	518[a]	896[a]	1.73[a]	0	
2	-	Herbal extracts	501[a]	858[ab]	1.71[a]	0	
3	+	-	307[c]	790[b]	2.66[c]	19.6	
4	+	Diclazuril	418[b]	889[a]	2.14[b]	19.6	
5	+	Herbal extracts	384[b]	839[ab]	2.19[b]	25.0	
SEM			14.6	14.1	0.078		
Days 22-42							
1	-	-	1663[ab]	3199	1.93	0.0	
2	-	Herbal extracts	1686[a]	3176	1.88	0.0	
3	+	-	1585[b]	3076	1.94	1.8	
4	+	Diclazuril	1723[a]	3339	1.94	1.8	
5	+	Herbal extracts	1701[a]	3164	1.86	0.0	
SEM			15.9	40.7	0.023		
Days 1-42							
1	-	-	2181[a]	4095[ab]	1.88[a]	0.0	282[a]
2	-	Herbal extracts	2186[a]	4030[ab]	1.84[a]	0.0	288[a]
3	+	-	1892[b]	3904[b]	2.06[b]	21.4	177[b]
4	+	Diclazuril	2141[a]	4238[a]	1.99[ab]	21.4	212[b]
5	+	Herbal extracts	2085[a]	4026[ab]	1.93[ab]	25.0	197[b]
SEM			24.8	75.7	0.04		18.4

[a, b, c] - within periods means in columns with different superscripts differ significantly at $P<0.05$

Analysis of the results for the entire experimental period showed a statistically significant decrease in BWG, EBI and poor FCR in the infected chickens that received no additive in the feed, in comparison with the non-infected groups. The growth performance indices obtained in the infected chickens fed the diet supplemented with the herbal extract blend were comparable to those recorded in the group given the coccidiostat-supplemented diet and both uninfected groups. The exception was the EBI value, which did not differ among any of the infected groups and was influenced by the high mortality rate, which reached 21.4% in groups 3 and 4 and 25% in group 5 (Table 1).

The results of the slaughter analysis showed no differences between the experimental groups in respect to the analysed parameters, with the exception of the abdominal fat content in the carcass, which was the lowest in the infected group fed the diet supplemented with the herbal extract blend (Table 2).

Table 2. Effect of experimental treatments on results of slaughter analysis (n=8)

Group number	Infection with *Eimeria* oocysts	Dietary treatment	Carcass yield % LBW[1]	Breast meat yield % carcass	Abdominal fat, % carcass	Liver % LBW	Gizzard % LBW
1	-	-	76.5	23.3	2.27^{b}	1.83	1.26
2	-	Herbal extracts	76.6	23.1	1.83^{ab}	1.91	1.29
3	+	-	76.6	23.4	1.81^{ab}	1.98	1.20
4	+	Diclazuril	76.9	23.4	1.87^{ab}	1.81	1.19
5	+	Herbal extracts	76.2	23.4	1.51^{a}	1.86	1.21
SEM			0.136	0.243	0.088	0.03	0.017

[a, b] - means in columns with different superscripts differ significantly at $P<0.05$

[1] LBW - live body weight before slaughter

The concentration of OPG confirms the high effectiveness of the experimental infection of the chickens with sporulated *Eimeria* oocysts (Table 3). The highest OPG value was recorded in the excreta collected from group 3 on post-inoculation

Table 3. Effect of experimental treatments on oocyst counts, thous./1 g of excreta

Group number	Infection with *Eimeria* oocysts	Dietary treatment	Days post infection					
			6	7	8	9	14	21
1	-	-	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
2	-	Herbal extracts	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
3	+	-	2788^{c}	950^{b}	419^{c}	99.8^{c}	64.5^{c}	1.12^{ab}
4	+	Diclazuril	1931^{b}	814^{b}	103^{ab}	10.5^{a}	34.5^{b}	0.90^{ab}
5	+	Herbal extracts	1587^{b}	700^{b}	179^{b}	71.1^{b}	27.0^{b}	1.74^{b}
SEM			249	91.6	35.2	8.69	5.68	0.241

[a, b, c] - means in columns with different superscripts differ significantly at $P<0.05$

day 6 and was statistically higher in comparison with those obtained from the remaining infected groups. A similar profile of differences in OPG value was also recorded on post-inoculation days 8, 9 and 14. The addition of the herbal extract blend significantly lowered the OPG value to the level recorded for the group given the coccidiostat. Oocysts were not found in the excreta samples from the uninfected birds. Moreover, the uninfected birds were free of lesions; cross contamination had thus not occurred (Table 4). In all of the infected groups, macroscopic lesions were highest in the caeca, which is the site of invasion for the strongly pathogenic *E. tenella*. There were no differences in lesions among the infected groups, with the exception of the lesions observed in the duodenum of the birds, which were most severe in group 3, i.e. in birds that were infected and fed the unsupplemented diet.

The statistically significant reduction in serum carotenoid concentration was found in all of the groups of infected chickens, as compared with the uninfected groups. Nonetheless, supplementing the diet with the coccidiostat or herb extract

blend had no statistically confirmed effect on the serum carotenoid concentration in the infected birds (Table 4).

Table 4. Effect of experimental treatments on lesion score (0-4) and serum carotenoids concentration at 6 d post-inoculation (n=6)

Group number	Infection with *Eimeria* oocysts	Dietary treatment	Duodenum points	Jejunum, ileum, points	Caeca points	Carotenoids mg/l
1	-	-	0.0^{a}	0.0^{a}	0.0^{a}	1.419^{a}
2	-	Herbal extracts	0.0^{a}	0.0^{a}	0.0^{a}	1.406^{a}
3	+	-	2.33^{c}	1.58^{b}	3.08^{b}	0.327^{b}
4	+	Diclazuril	1.83^{b}	1.67^{b}	2.92^{b}	0.370^{b}
5	+	Herbal extracts	1.92^{b}	1.33^{b}	3.25^{b}	0.420^{b}
SEM			0.195	0.181	0.302	0.109

^{a,b,c} - means in columns with different superscripts differ significantly at $P<0.05$

DISCUSSION

The data presented in this paper confirms the detrimental effect of coccidiosis on performance parameters, mortality, lesion scoring, and blood carotenoid concentration. The high mortality recorded in all of the infected groups, primarily during the first days after inoculation, indicates the high pathogenicity of the field strain inoculant used in the experiment.

The worst growth performance data were found in the group of infected chickens fed the unsupplemented diet. The significantly lower BWG in this group during all feeding periods was a consequence of reduced feed intake and the disruption of intestinal integrity, which affected the absorption of nutrients and the efficiency of feed utilization, these being common effects of coccidiosis (Peek and Landman, 2003; Walk et al., 2011). The detrimental effect of infection was also reflected in the highest OPG recorded in the excreta collected on post-infection days 6, 8, 9 and 14 and in the lesion score of the duodenum on post-infection day 6. The duodenum is the site of invasion for *E. acervulina*, which does not cause a drastic increase in mortality, but has a negative impact on the digestion, absorption and assimilation of nutrients (Adams et al., 1996).

The tested herbal extract blend was shown to alleviate the deleterious effect of the experimental infection and was as efficient in the improvement of BWG, FI, FCR, and lowering of OPG as the coccidiostat used; however, it did not exert a beneficial effect on the mortality rate in first period of feeding. The efficacy of the herbs tested in the present trial has previously been evaluated in other experiments; however, these herbs have never been used together as an herbal extract blend. Giannenas et al. (2003) demonstrated a significant improvement of performance, survival rate, lesion score and oocyst numbers in chickens infected with *E. tenella*

and fed diets supplemented with oregano essential oil (300 mg/kg), in comparison with an infected group fed an unsupplemented diet, but the improvements were lower than in chickens fed a diet with the coccidiostat, lasalocid. Oregano extract used to supplement vaccination for the prevention of coccidiosis in organically reared chickens has also been shown to have a positive effect on weight gain in uninfected birds (Waldenstedt, 2003). Feed supplementation with 0.1 or 0.5% immunopotential *Echinacea purpurea* during the first 2 weeks of life has been demonstrated to significantly improve BWG and reduce total lesion scores in vaccinated broilers, as compared with those given a live vaccination alone (Allen, 2003). Similarly, the anticoccidial effect exerted by sage, thyme and their phenols, such as cineol, borneol, thymol and carvacrol has been established (Ibrir et al., 2001; Szaboova et al., 2008).

In the current study data from uninfected birds receiving the herbal extract blend suggest that these additives have no effect on growth parameters and carotenoid concentration. It can thus be assumed that the beneficial effect of the extracts obtained in the group of infected chickens was not a consequence of its stimulating influence on the birds' appetite, but a result of its anticoccidial properties. The lower OPG recorded in the infected group given the herbal extract was comparable with that obtained in the group being administered the coccidiostat and was probably the effect of the phenolic compounds in the herbal extracts. Phenols can interact with cytoplasmic membranes and change their cation permeability, leading to impairment of crucial processes in the coccidia cells and, finally, their death (Sikkema et al., 1995).

In the face of the ban on the prophylactic use of coccidiostats in the EU that will go into effect in 2013, the search among substances of natural origin for alternative methods of controlling coccidiosis seems to be highly justified. In addition to the advantages previously mentioned, herbal extracts do not carry the risk of contaminating meat with harmful substances. To date, the emergence of resistance to herbal products among coccidia species has not been reported.

CONCLUSIONS

In conclusion, the results of the present study suggest that treatment with a herbal extract blend comprising *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* at a dose of 1 g/kg partly alleviates the negative impact of *Eimeria* infection in broiler chickens. The beneficial effect of the herbal extract blend on the growth performance and number of oocytes per gram of excreta of the infected chickens was comparable to that exhibited by diclazuril.

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