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THE EFFICACY OF SELECTED FEED ADDITIVES IN THE PREVENTION OF BROILER CHICKEN COCCIDIOSIS UNDER NATURAL EXPOSURE TO *EIMERIA* SPP.*

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Abstract

The aim of this study was to investigate, under conditions similar to commercial broiler production, the effect of the herbal extract blend (HE) at a quantity of 1 g per kg feed (200 mg of each herbal extract, *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare*), used individually or in combination with mannan oligosaccharide (MOS; 1 g per kg feed) or chitosan (3 ml containing 2% deacetylated chitin per kg feed) on the performance parameters of broiler chickens, the results of the slaughter analysis, litter moisture and the number of oocysts excreted in feces. The experiment was conducted on 4,500 broiler chickens of both sexes kept in straw-bedded pens. Chickens were randomly assigned to 5 experimental treatments with 5 replicates (pens) of 180 birds. The experimental design included negative and positive (diclazuril, 1 mg per kg feed) control groups. The examined herbal extract blend used individually during natural exposure to the coccidia improved, compared to the negative control diet, the performance parameters to a greater extent than coccidiostat, lowered the litter moisture content and reduced the oocyst output. Combined dietary supplementation with a herbal extract blend of chitosan or mannan oligosaccharide did not result in further improvement.

Key words: broiler chickens, performance, feed additives, *Eimeria* spp.

Coccidiosis, which is caused by protozoa of the genus *Eimeria*, still remains one of the most important and common parasitic threats to the broiler industry which causes global economic losses estimated at over \$3 billion annually (Dalloul and Lillehoj, 2006; Shivaramaiah et al., 2014). Coccidiostats are the main form of coccidiosis prevention but their widespread use and misuse have led to the occurrence of strains of *Eimeria* spp. which are resistant to most of the available anticoccidials

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(Williams, 2002). Nutritional methods, mainly based on natural feed additives are considered to be one of the most promising alternatives to anticoccidials (Abbas et al., 2012; Bozkurt et al., 2013, 2014; Habibi et al., 2014; Masood et al., 2013).

This study is at the final stage of determining, under conditions similar to commercial broiler production, the efficacy of the herbal extract blend (HE) used individually or simultaneously with other feed additives as a possible alternative to coccidiostat in broiler chickens facing natural exposure to *Eimeria* spp. The composition of the studied herbal extract blend has been developed on the basis of screening the particular extracts with regards to their effect over the course of clinical coccidiosis (Arczewska-Włosek and Świątkiewicz, 2013) and comprises extracts derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare*. In the next trials on broilers challenged with clinical coccidiosis, the efficacy of HE used individually (Arczewska-Włosek and Świątkiewicz, 2012) or in combination with other feed additives such as synbiotic, acidifier, chitosan or mannan oligosaccharide (Arczewska-Włosek and Świątkiewicz, 2014) was determined. The most effective dietary treatments were chosen for the next experiment. Consequently the aim of this study was to investigate, under conditions similar to commercial broiler production, the influence of the herbal extract blend, used individually or in combination with chitosan and mannan oligosaccharide, on the performance parameters of broiler chickens, the results of slaughter analysis, litter moisture and the number of oocysts excreted in feces.

Material and methods

All of the experimental procedures involving animals were approved by the Local Ethical Committee on Animal Testing. Forty-five hundred one-day-old Ross 308 broiler chickens of both sexes obtained from a commercial hatchery were randomly allocated to 5 groups with 5 replicates (pens) of 180 birds. The chickens were reared to the age of 42 days in straw-bedded pens with an equal stocking density of 14 birds/m² and with free access to water and feed. The pens were separated from each other by 1.5 m high wooden walls. All birds were fed with a starter (1 to 21 days) and grower-finisher (22 to 42 days) maize-soya bean basal diet, free of antibiotic growth promoters and coccidiostats and formulated to satisfy the nutrient requirements of broilers (Smulikowska and Rutkowski, 2005). The basal diets contained, calculated on the basis of a chemical analysis: 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 MJ (grower-finisher).

The design of the experiment included two control groups: a negative one (group 1) fed basal, non-supplemented diets and a positive one (group 2) fed the diets with the chemical coccidiostat diclazuril added at a quantity of 1 mg/kg feed. The experimental dietary treatments in groups 3–5 were formulated by supplementing the basal diets with herbal extract blend (HE), administered individually (group 3), in combination with chitosan (group 4; 3 ml containing 2% deacetylated

chitin/kg feed; Chimet Pasz; Gunitex Poli-Farm, Łowicz, Poland) or in combination with mannan oligosaccharide (group 5: 1 g/kg feed; SAF-Mannan, LeSaffre, Cedex, France). The blend contained an equal amount of dried extracts, with maltodextrin as the carrier, derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* at a quantity of 1 g/kg feed (200 mg of each herbal extract/kg; Naturex S.C., Katowice, Poland). The total content of phenolic compounds, expressed as caffeic acid, in particular the extracts before application to the maltodextrin carrier, was respectively ($\text{mg}/100 \text{ g}^{-1} \pm$ standard deviation): 380.42 ± 3.14 ; 660.66 ± 4.21 ; 256.52 ± 6.98 ; 486.56 ± 8.79 ; 984.43 ± 44.72 .

The birds were weighed at the age of 1, 21, and 42 days. The body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and mortality were calculated for days 1–21, 22–42 and 1–42. FCR was calculated as kg feed/kg BWG and data were corrected for mortality. The Production Index (PI) was calculated for the entire feeding period as described previously by Koreleski et al. (2011).

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 at 14, 21, 28, 35 and 42 days old. To obtain the pooled excreta sample the several fecal samples from different locations in the pen were collected and homogenized for analysis of OPG from each pen (replicate) separately.

From 14 days of age, at intervals of several days, the litter moisture content was determined using a digital moisture tester HMM (Dramiński S.A., Olsztyn, Poland). The value of the litter moisture content for every replicate was the average of five individual measurements performed in the different areas of pen.

At the end of the experiment and after 12 h of feed withdrawal, all of the chickens were weighed and 4 representative male and 4 female chickens with live body weights close to the group average were chosen from each group, marked with numbers and decapitated. The chickens were plucked, the intestines and crop were removed and the carcasses were stored overnight at 4°C. The mass of the cooled carcass with the edible giblets (gizzard, liver and heart) was estimated and the carcass yield calculated. The breast muscles, abdominal fat, livers, gizzards and spleens were excised and weighed. The breast muscle and abdominal fat contents were expressed as a percentage of the cold carcass. The weights of the liver, gizzard and spleen were expressed as a percentage of the live weight (Ziołocki and Doruchowski, 1989).

The data were analysed by means of a one-way ANOVA and the significance of the differences between the mean values was assessed using Duncan's test (SAS Enterprise Guide 4.3). Differences were considered significant at a probability level of $P < 0.05$.

Results

Performance indices

The performance data obtained in the experiment are presented in Table 1. In the first period of feeding the lowest BWG was observed in the negative control group (1). The administration of HE, individually (group 3) and in combination with chi-

tosan (group 4) or MOS (group 5), resulted in a significant increase of BWG to the level recorded in the group which received coccidiostat supplement (2). The BWG found in groups 3, 4 and 5 was 6.1%, 4.3%, and 3.8% ($P < 0.05$) more favorable, respectively, in comparison to the negative control group. The BWG of the chickens fed with a diet supplemented with synthetic coccidiostat (group 2) did not statistically differ from those receiving the non-supplemented diet (group 1). No significant differences in FI and FCR were observed and the mortality rates noted in all groups were low and did not exceed 1.44%.

Table 1. Effect of experimental treatments on the performance data of broiler chickens (n=5)

Dietary treatment (Group No.)	– (1)	D ¹ (2)	HE ² (3)	HE + Ch ³ (4)	HE + MOS ⁴ (5)	SEM ⁵
Days 1–21						
BWG (g)	624 b	643 ab	662 a	651 a	648 a	3.71
FI (g)	903	922	930	921	918	2.99
FCR (kg·kg ⁻¹)	1.45	1.43	1.40	1.41	1.42	0.006
Mortality (%)	0.89	0.78	1.33	1.44	1.33	
Days 22–42						
BWG (g)	1411 c	1517 b	1585 a	1507 b	1582 a	15.4
FI (g)	2928 c	2975 abc	3062 a	2958 bc	3032 ab	15.9
FCR (kg·kg ⁻¹)	2.08 a	1.96 b	1.93 b	1.96 b	1.92 b	0.014
Mortality (%)	1.11	0.89	0.78	1.22	1.00	
Days 1–42						
BWG (g)	2036 c	2160 b	2247 a	2158 b	2230 ab	18
FI (g)	3831 c	3897 abc	3992 a	3879 bc	3950 ab	17.1
FCR (kg·kg ⁻¹)	1.88 a	1.80 b	1.78 b	1.80 b	1.77 b	0.009
PI	257 c	286 ab	300 a	284 b	298 a	3.66
Mortality (%)	2.00	1.67	2.11	2.67	2.33	

a, b, c – means in rows with different letters differ significantly at $P < 0.05$; ¹D-diclazuril administered at a dose of 1 mg/kg feed; ²HE – herbal extract blend derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* administered at a dose of 1000 mg/kg feed (200 mg of each herbal extract/kg feed); ³Ch – chitosan administered at a dose of 3 ml/kg feed (2% deacetylated chitin); ⁴MOS – mannan oligosaccharide at a dose of 1 g/kg feed; ⁵SEM – standard error of the mean.

In the second feeding phase (22–42 days) statistically the lowest BWG was found in the negative control group ($P < 0.05$). The addition of all the experimental additives resulted in an improvement in FCR and led to a significant increase in BWG. The largest increase of BWG was found in groups fed a diet supplemented with HE, individually or in combination with MOS, and the difference in respect to the non-supplemented group (1) was 12.3% and 12.1%, respectively.

An analysis of the results for the entire period of the experiment (1–42 days) showed a statistically significant decrease in the performance parameters in the control group not receiving any dietary supplement. The incorporation of any of dietary additives resulted in an improvement in all of examined performance indices ($P < 0.05$) to a level comparable (group 4 and 5) to that observed in the group receiv-

ing coccidiostat supplement or even better (group 3). Generally, adding HE to the diet (group 3) led to the most favorable BWG, FI, FCR and PI results in all of the experiment groups and the difference compared to the negative control group was 10.4%, 4.2%, 5.3% and 16.7%, respectively. The adding of chitosan or MOS to a HE supplemented diet did not further improve the performance indices (group 4 or 5 vs group 3). The mortality rates noted up to 42 days of age in all experimental groups were relatively low and ranged from 1.67% (group 2) to 2.67% (group 4).

Slaughter analysis

The results of the slaughter analysis (Table 2) showed no differences among the experimental groups regarding the analysed parameters ($P>0.05$).

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

Dietary treatment (Group No.)	– (1)	D ¹ (2)	HE ² (3)	HE + Ch ³ (4)	HE + MOS ⁴ (5)	SEM ⁵
Carcass yield (% LBW ⁶)	75.0	75.1	75.7	75.7	74.1	0.233
Breast meat yield (% carcass)	21.3	21.6	23.0	22.3	22.0	0.225
Abdominal fat (% carcass)	2.44	2.46	2.38	2.33	2.5	0.078
Liver (% LBW ⁶)	2.51	2.45	2.33	2.35	2.38	0.032
Gizzard (% LBW ⁶)	1.31	1.31	1.33	1.32	1.3	0.019
Spleen (LBW ⁶)	0.120	0.128	0.118	0.116	0.127	0.004

$P>0.05$; ¹D – diclazuril administered at a dose of 1 mg/kg feed; ²HE – herbal extract blend derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* administered at a dose of 1000 mg/kg feed (200 mg of each herbal extract/kg feed); ³Ch – chitosan administered at a dose of 3 ml/kg feed (2% deacetylated chitin); ⁴MOS – mannan oligosaccharide at a dose of 1g/kg feed; ⁵SEM – standard error of the mean; ⁶LBW – live body weight.

Oocyst count

Throughout the period of the experiment the concentration of oocysts remained at a relatively low level (Table 3). The largest increase in oocyst excretion was observed on the 35th day. Despite the lack of statistically significant differences ($P>0.05$) a trend towards higher concentrations of oocysts in the control group was observed.

Table 3. Oocysts count (thous./1 g excreta; n=5)

Dietary treatment (Group No.) Days of age	– (1)	D ¹ (2)	HE ² (3)	HE + Ch ³ (4)	HE + MOS ⁴ (5)	SEM ⁵
14	0	0.02	0.06	0.02	0	0.013
21	0.57	0.40	0.40	0.57	0.57	0.069
28	2.69	2.24	3.51	1.78	7.75	0.903
35	35.5	21.9	17.4	16.7	24.6	3.32
42	8.46	6.88	5.59	7.83	1.76	1.54

$P>0.05$; ¹D – diclazuril administered at a dose of 1 mg/kg feed; ²HE – herbal extract blend derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* administered at a dose of 1000 mg/kg feed (200 mg of each herbal extract/kg feed); ³Ch – chitosan administered at a dose of 3 ml/kg feed (2% deacetylated chitin); ⁴MOS – mannan oligosaccharide at a dose of 1g/kg feed; ⁵SEM – standard error of the mean.

Litter moisture content

No significant differences were shown between the experimental groups in the results of litter moisture from 14 to 38 days of age (Table 4). The measurements carried out on days 38 and 42 demonstrated that the administration of coccidiostat (group 2) and the herbal extract blend (group 3) significantly ($P<0.5$) lowered the litter moisture and the results obtained in those groups did not differ ($P>0.5$).

Table 4. Litter moisture content (%; n=5)

Dietary treatment	–	D ¹	HE ²	HE + Ch ³	HE + MOS ⁴	SEM ⁵
(Group No.) Days of age	(1)	(2)	(3)	(4)	(5)	
14	29.2	29.2	27.8	27.2	28.2	0.373
17	40.0	40.8	39.7	38.8	40.0	0.566
21	45.2	45.5	46.4	47.2	47.1	0.579
25	49.5	48.6	49.4	52.0	51.2	0.598
28	52.3	51.6	53.4	54.9	54.1	0.537
31	55.8	56.0	56.1	59.1	58.5	0.485
35	65.2	60.1	61.6	62.9	64.3	0.668
38	69.1 a	66.1 b	68.6 ab	69.8 a	70.3 a	0.486
42	74.9 a	69.6 c	71.9 bc	73.6 ab	72.7 ab	0.498

a, b, c – means in rows with different letters differ significantly at $P<0.05$; ¹D – diclazuril administered at a dose of 1 mg/kg feed; ²HE – herbal extract blend derived from *Allium sativum*, *Salvia officinalis*, *Echinacea purpurea*, *Thymus vulgaris* and *Origanum vulgare* administered at a dose of 1000 mg/kg feed (200 mg of each herbal extract/kg feed); ³Ch – chitosan administered at a dose of 3 ml/kg feed (2% deacetylated chitin); ⁴MOS – mannan oligosaccharide at a dose of 1 g/kg feed; ⁵SEM – standard error of the mean

Discussion

The high density of animals reared on litter floors, which is characteristic for modern production and the high reproductive capacity of *Eimeria*, as well as the rising drug resistance of *Eimeria* field strains, are favorable for the high transmission, replication and accumulation of *Eimeria* spp. and make birds more susceptible to the risk of a coccidiosis outbreak (Shirley et al., 2005). The development of a unique method to control coccidiosis is crucial to maintaining productivity and economic efficiency in the poultry industry. According to Chapman et al. (2002) live vaccines are the only practical alternative to coccidiostats, but there has been a widespread reluctance to use them in broilers mainly because of their high production costs (Gómez-Verduzco et al., 2009) as well as reports of poorer performance, measured by reduced weight gain and feed efficiency, in comparison to prophylactically medicated birds (Danforth, 1998; Williams, 2002; Arczewska-Włosek and Świątkiewicz, 2014). Another promising alternative was shown by the results of a study by Ziomko et al. (2005) indicating the protective efficacy of inactivated subunit vaccine Cox-Abic[®] used in breeding hens on the course of coccidiosis in their progeny. Nutritional strategies based on natural substances such as herbs, probiotics, prebiotics with coc-

cidistatic properties have also been the subject of many studies, with highly diverse results. Due to the lack of highly effective, and repeatable effectiveness of coccidiostats substitutes there is currently no comparably efficient alternative and anticoccidials remain the main form of coccidiosis prophylaxis according to the report on the withdrawal of coccidiostats and histomonostats from use in feed additives, submitted by The European Commission (Anonymous, 2008). There is therefore an urgent need to continue studies.

The data presented in this study confirms the high effectiveness of the herbal extract blend on the performance of broiler chickens reared under conditions similar to those in the field. The performance results obtained in the HE-supplemented groups in all experimental periods improved significantly compared to the non-supplemented group and were comparable or even better to those recorded in the coccidiostat-supplemented group. The positive effect of HE supplements was also reflected in the lower litter moisture content at 38 and 42 days of age compared to the level recorded in the coccidiostat-supplemented group. Maintaining the moisture levels in poultry litter within the proper range is an important factor that influences the health status of birds, and an excessive increase may indicate an intestinal environment imbalance (Doyle and Erickson, 2006). The maximum OPG was observed in all experimental groups at 35 days of age. The decrease in OPG at 42 days of age recorded in all groups may be the result of immunity to coccidia developed in conditions of continuous and low natural exposure to coccidia. Although the statistical analysis did not indicate a significant difference in OPG at 35 and 42 days of age, the birds which received a diet with HE supplements excreted less oocysts in comparison with non-supplemented groups. Those results were also numerically lower than those recorded in the coccidiostat-supplemented group. It should be noted, however, that all the OPG levels recorded during the whole rearing period were low and indicate good hygiene conditions in the poultry house used for the experiment. The positive effect of HE-supplementation agrees with the previous results about its addition to the diet of broilers suffering from clinical coccidiosis (Arczewska-Włosek and Świątkiewicz, 2012). Dietary HE-supplementation increased the growth performance to the level found in the group fed with coccidiostat, or in uninfected groups, and diminished *E. acerulina*-related lesions, with no significant reduction of oocyst output or the mortality rate. The efficacy of plant-derived anticoccidials in controlling avian coccidiosis is well documented and has been thoroughly reviewed and discussed by Abbas et al. (2012) or Bozkurt et al. (2014). In these reviews the phytochemicals were found beneficial for coccidiosis due to the wide range of effects including the improvement of performance, decline in oocyst shedding, modulation of microflora composition, antioxidant activity reducing the intestinal epithelium inflammation and the enhancement of intestinal structure and immunity.

The expected positive synergistic effect of the simultaneous administration of diets with HE and other additives, i.e. chitosan or MOS, on improving performance and lowering the oocyst output was not observed. HE and MOS supplementation led to a performance level comparable with HE used individually, whereas the combined use of HE and chitosan slightly worsened the effectiveness of HE, to the level observed in the group supplemented with coccidiostat, although it was still signifi-

cantly more favorable than in the non-supplemented control group. The underlying mechanism for why there was no further improvement in the studied parameters is unclear. The adverse effect of the combined use of HE and MOS, at the same dose as in the current study, was observed in previous trial on broilers with a high pathogenic mixture of *Eimeria* spp. inducing the clinical form of coccidiosis (Arczewska-Włosek and Świątkiewicz, 2014). In this experiment the chickens infected with a mixture of 170,000 *Eimeria* oocysts and fed a diet supplemented with HE and MOS, were characterized by a significantly lower BWG on days 1–21, days 22–42 and during the entire period of the experiment in comparison with the HE supplementation where performance parameters were comparable to coccidiostat supplementation. The combined administration of HE and MOS, however, did lower the mortality rate in comparison to the use of added HE or coccidiostat in the diet of infected chickens (17.9% vs 25 or 21.4%, respectively). Mannan oligosaccharides are carbohydrate derivatives from the outer layer of the cell wall of the yeast *Saccharomyces cerevisiae* and have been reported to bind and reduce intestinal pathogen counts, improve intestinal morphology and enzymatic activity, support the increase of *Bifidobacterium* spp. and *Lactobacillus* spp. in the intestinal tract and also modulate gut and systemic immunity (Spring et al., 2000; McCann et al., 2006; Elmusharaf et al., 2006). The literature about the efficacy of MOS, with regards to coccidiosis prophylaxis is, however, inconsistent. Gómez-Verduzco et al. (2009) reported that chickens naturally infected with coccidia and fed a diet containing MOS in the form of a yeast cell wall at a dose of 0.05% demonstrated improved BWG, FCR, reduced oocyst excretion in feces and increased local mucosal IgA secretion, humoral and cell-mediated immune responses. In the study by Elmusharaf et al. (2006), enhanced immunity was observed in chickens infected with *Eimeria tenella* and was reflected in the decreased number of schizonts in the lamina propria of the cecum, but had no effect on the growth performance or reduction of cecal lesions. Another study by Elmusharaf et al. (2007) reported reduced oocyst excretion and diminished severity of *E. acervulina* related lesions with no positive effect on the performance or lesions induced by *E. tenella* and *E. maxima*. Strong evidence of the immune-stimulating properties of the *Saccharomyces cerevisiae* fermentation product in chickens challenged with *E. tenella* is given in the paper by Gao et al. (2009), where its supplementation improved cell immunity (increased CD3+, CD4+ and CD8+ T-lymphocyte counts, and ratio CD4+:CD8+ in blood and spleen, ileum intraepithelial lymphocyte count), humoral immunity (cecal tonsil secretory IgA) and non-specific immunity (increased serum lysozyme content). By contrast, McCann et al. (2006) found that MOS supplementation did not reduce the negative impact of coccidiosis and is not able to improve performance to the level achieved with the use of antibiotic growth promoters in broilers infected with a mixture of *E. acervulina*, *E. tenella* and *E. maxima*.

The simultaneous dietary supplementation with HE and chitosan has worsened the beneficial effect of HE on performance indices in the second and entire experiment period ($P < 0.05$). The BWG, FCR and PI in this group were, however, found to be significantly improved in comparison to the negative control group and were comparable to those obtained in the coccidiostat-supplemented group. Chitosan, a linear polymer composed of β -(1 \rightarrow 4)-2-acetamido-D-glucose and β -(1 \rightarrow 4)-2-amino-D-

-glucose units is a product of the partial hydrolysis and deacetylation of chitin, one of the most common natural constituents of the exoskeletons of shellfish and insects. Chitosan and its derivatives are reported to exhibit many beneficial properties such as biodegradability, non-toxicity, relatively cheap cost of production and anti-microbial, anti-inflammatory, anti-oxidative, anti-tumor, immunostimulatory, hypocholesterolemic and growth promoting effect and its efficacy in poultry nutrition has been thoroughly reviewed and discussed by Świątkiewicz et al. (2014). The results of a previous trial with chickens suffering from clinical coccidiosis showed (Arczewska-Włosek and Świątkiewicz, 2012) lowered BWG to below the level observed even in an infected and non-supplemented group of chickens. The negative effect of chitosan supplementation on the performance indices may probably be the result of decreased lipase activity and consequently decreased fat digestion (Kobayashi et al., 2002). Due to the impaired digestion, nutrients and the energy-deficiency characteristics of coccidiosis (Williams, 2005), this hypothetical chitosan mode of action could enhance the negative effect of infection, although further research seems to be necessary to explain the observed effects.

Conclusions

The examined herbal extract blend used individually during natural exposure to coccidia improves the performance parameters and could be an effective alternative to coccidiostats. The combined administration of the herbal extract blend with chitosan or mannan oligosaccharide to the diet, did not result in further improvement.

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