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ANTICOCCIDIAL EFFECT OF SOME NATURAL PRODUCTS IN EXPERIMENTALLY INDUCED *EIMERIA SPP.* INFECTION ON CARCASS QUALITY, INTESTINAL LESION AND ILEAL HISTOLOGY IN BROILERS

Alaeldein M. Abudabos[1*], Abdullah H. Alyemni[2], Elsayed O. S. Hussein[1] and Mu'ath Q. Al-Ghadi [3]

[1]Department of Animal Production, King Saud University, Riyadh, Saudi Arabia; [2]ARASCO for Feed, P.O Box 53845, Riyadh 11593, Kingdom of Saudi Arabia; [3]Zoology Department, King Saud University, Riyadh, Saudi Arabia
Corresponding author's email: aabudabos@ksu.edu.sa

ABSTRACT

We evaluated some natural products to compare their effects against a synthetic anticoccidial drug in an induced Eimeria spp. infection in broiler during the finisher stage. On day 15, a total of 200 birds (Ross 308) were randomly distributed into 10 treatments. Birds were infected with *Eimeria spp.* in the positive control and treatment groups. The results indicated that feed intake, body weight and feed conversion ratio (FCR) did not differ significantly ($P>0.05$) between synthetic anticoccidial drug and natural products. Challenged birds had longer small intestines and heavier liver as compared to unchallenged birds. Lesion score and oocysts secretion increased significantly ($P<0.05$) in the challenged birds, however, synthetic anticoccidial drug had no significant ($P>0.05$) difference with the natural products. It was concluded from the present study natural products are very effective in comparison with anticoccidial drug to control coccidiosis in broiler chicken.

Keywords: coccidiosis, broiler, drugs, natural products, control.

INTRODUCTION

The infection of coccidiosis is caused by intestinal protozoan and causes high mortality, stunt growth and low feed conversion ratio in broiler (Tanweer *et al*., 2014; Chand *et al*., 2016). In addition, the infected birds are easily prone to secondary bacterial infection (Arczewska-Włosek and Świątkiewicz, 2013). Generally, coccdiosis infection is controlled by the use of coccidiostats and vaccine. The use of coccidiostats and vaccines have been used successfully, however, their use are associated with drug resistance and major economic losses for their side effects (Chapman, 1997; Arczewska-Włosek and Świątkiewicz, 2013). In addition, the residues of the drugs in the meat have also been identified which pose another threat for the consumer health (Olejnik *et al*., 2009).

There is a strong urge for the effective natural products to replace the coccidiostatic agents. The use of natural dietary supplement provides a new domain for controlling the coccidiosis possibly due to their origin, wide range doses, absence of grace period and side effects, and stimulation of appetite centre in the brain (Arczewska-Włosek and Świątkiewicz, 2013). Recently, plant-derived natural products have shown effectiveness against Eimeria infection (Abodabos *et al*., 2016). The objective of the present work was to evaluate the effect of some natural products in comparison with standard anticoccidial drug on growth, intestinal lesion score and histology in broiler chicks.

MATERIALS AND METHODS

This experiment was approved by the Departmental board of Studies on Ethics, Methodology and Welfare, King Saud University, Kingdom of Saudi Arabia.

A total of 200 day-old broilers (Ross 308) were randomly distributed into 40 cages. A typical isocaloric and isonitrogenous starter (0 to14 d) and finisher (15 to 35 d) diet was provided according to Ross 308 recommendation guide (Table 1).

At 15 day of age, half birds were infected with live oocysts of Eimeria (*Eimeria acervulina, Eimeria mivati, Eimeria maxima, Eimeria tenella, Eimeria necatrix, Eimeria praecox, Eimeria brunette* and *Eimeria hagani*). Each treatment had 20 birds with four replicates.

The treatments were as follows:

1) Negative control
2) Coccidial challenge (positive control)
3) Elancoban (0.5 kg/ton monensin sodium), without coccidiosis challenge. Elancoban contains monesin as anticoccidial drug.
4) Elancoban (0.5 kg/ton monensin sodium), with coccidiosis challenge (positive Elancoban)
5) Cozante (0.5 kg/ton), without coccidiosis challenge (negative Cozante). Cozante contains polyphenol compounds from herbal origin.
6) Cozante (0.5 kg/ton) with coccidiosis challenge (positive Cozante)
7) Norponin XO (0.5 kg/ton), without coccidiosis challenge (negative Norponin XO). Norponin is a

natural herbal extract rich in saponin e.g., protodioscine, schidigera saponin B1
8) Norponin XO (0.5 kg/ton), with coccidiosis challenge (positive Norponin XO)
9) Organicox (0.5 kg/ton), without coccidiosis challenge (negative Organicox). Organicox is a blend of organic acid.
10) Organicox (0.5 kg/ton), with coccidiosis challenge (positive Organicox)

Performance measurements: Average feed intake (FI) and body weight gain (BWG) for each group was measured weekly and feed conversion efficiency (FCR) was calculated after adjusting for mortality rate. Production Efficiency Factor (PEF) was weekly calculated by using the formula: PEF= ((Livability×Live weigh (kg)/ (Age in days×FCR)×100.

Carcass measurements: At day 35, eight birds per treatment were selected and slaughtered by the Islamic method. Birds were defeathered and the internal organs were removed. Dressing, breast, thigh, drumstick, breast, abdominal fat and liver weight were determined as percentage of body weight.

Coccidial lesion: Score of the coccidial lesion was carried out in the ileum, jejunal and cecal regions of the intestines by the method described by Johnson and Reid (1970). The lesion score was numbered as 0, 1, 2, or 3, from low to highly severe, respectively.

Histological examination of ileum: On day 35 of age, ileal sections (5 mm) were cut from five birds per group and stained with haematoxylin and eosin. Measurements of height, width and total area of the villi was based on at least 10 well-oriented villi per section of the small intestine per broiler using an IX71 Inverted Olympus Microscope.

Statistical analysis: The data were analyzed using the general linear models procedure of SAS software (SAS Institute, 2004). The data were analyzed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2004) as a 5 × 2 factorial design including five treatments and two levels of challenge and their interactions. Least significant difference (LSD) was applied to compare the treatment means. *P*-value less than 0.05 was considered as statistically significant unless otherwise mentioned.

RESULTS

The Table 2 shows that the challenge had a significant impact on all performance parameters. Unchallenged chicks consumed more feed, gained more weight, converted feed more efficiently and as a result had higher FEF as compared to challenged chicks. Chicks received Elancoban had the highest BWG (327.9 g) and converted the feed to gain with highest efficiency. Chicks on the control diet were intermediate in terms of BWG and FCR but they were better than Cozante, Norponin and Organimix.

Table 1. Dietary ingredients and chemical composition of starter and finisher diets

Ingredients	CONT	CONT
Corn	53.21	60.75
Soybean meal	37.92	25.00
Corn gluten meal	2.00	7.10
Corn oil	2.20	2.80
Dicalcium phosphate	2.30	2.05
Limestone	0.83	0.68
Salt	0.45	0.50
Vitamin-mineral Mix[1]	0.50	0.50
DL-Methionine	0.20	0.10
Lysine-HCL	0.22	0.37
Threonine	0.11	0.10
Choline chloride	0.05	0.05
Chemical composition		
ME, kcal/kg	3000	3150
Crude protein, %	23.5	21.30
Methionine, %	0.55	0.44
Lysine, %	1.42	1.23
Sulfur amino acids, %	0.96	0.80
Threonine, %	0.95	0.85
Calcium, %	1.05	0.90
Phosphorus, %	0.50	0.45

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

The morphometric measurements of the intestinal samples at 25 d of age (10 days post inoculation) are given in Table 2. No significant differences in intestinal length, weight or IRW were observed because of treatment or the interaction between treatment and challenge (P>0.05). However, challenge affected the total length of the small intestine (P<0.01). Challenged birds had longer small intestines as compared to unchallenged birds. Also challenge affected the percentage of cecal length (P<0.05), unchallenged birds had higher cecal length percentage as compared to challenged birds. Challenged birds had higher IRW as compared to unchallenged birds. Liver percentage was affected by treatment and challenge (P<0.05 and P<0.001, respectively). Birds received treatment 2 (Elancoban) had lower liver percentage as compared to those which had received control, Norponin or Organicox

(P<0.05) but similar to those received Cozante. On the other hand, challenged birds had higher liver percentage as compared to unchallenged birds.

Table 5 shows the result of lesion scores in duodenum, jejunum and ceca of birds at 25 of age. Generally, uninfected birds (control, unchallenged) were free of lesions. At day 25, coccidial challenge affected lesion score at duodenum jejunum and ceca (P<0.001). Lesions were higher in challenged group as compared to unchallenged group for all sections measured.

Table 2. Live weight (BW), feed intake, feed conversion ratio (FCR) and production efficiency factor (PEF) of broiler chickens given experimental diets at 25 days.

			Performance			
Treatment	**TRT**	**Challenge**	**FI (g)**	**BWG (g)**	**FC (g: g)**	**PEF**
1	Control	No	524.2	374.5	1.339	298.9
2	Control	Yes	423.4	243.9	1.740	255.1
3	Elancoban	No	460.9	323.2	1.428	291.9
4	Elancoban	Yes	485.4	332.8	1.459	302.5
5	Cozante	No	479.7	333.9	1.440	277.6
6	Cozante	Yes	440.5	251.5	1.764	231.4
7	Norponin	No	479.0	336.1	1.426	297.6
8	Norponin	Yes	436.4	247.9	1.761	280.1
9	Organicox	No	481.9	331.8	1.451	291.2
10	Organicox	Yes	420.9	241.7	1.743	269.3
SEM±			17.31	11.98	0.037	14.70
TRT average						
Control			473.8	309.3	1.569	276.9
Elancoban			473.1	327.9	1.444	297.2
Cozante			460.1	292.7	1.602	254.5
Norponin			457.7	292.0	1.593	288.8
Organicox			451.4	286.8	1.598	280.3
SEM±			12.24	8.47	0.027	10.40
Challenge average						
No			485.1[a]	339.9[a]	1.429[b]	291.4
Yes			441.3[b]	263.6[b]	1.693[a]	267.7
SEM±			7.34	5.08	0.016	6.24
Statistical probabilities						
TRT			NS	**	***	NS
Challenge			***	***	***	**
TRT x Challenge			**	***	***	***

*p<0.05, **p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

Table 3: Effect of different treatments and challenge on small intestinal measurements of broiler chickens at 25 d (10 d post inoculation).

Treatment	Trt	Challenge	Total weight (g)	Total length (cm)	Weight (%)¥				Length (%)¥				IRW[1] (%)	Liver[§] (%)
					Duodenum	Jejunum	Ileum	Ceca	Duodenum	Jejunum	Ileum	Ceca		
1	Control	No	46.97	129.33	23.03	36.27	40.70	18.00	19.60	40.63	39.77	20.80	7.73	3.70
2	Control	Yes	52.00	147.53	22.67	36.57	40.77	11.93	17.67	39.30	43.07	16.97	11.00	5.20
3	Elancoban	No	46.03	135.77	20.63	40.77	38.60	21.20	19.30	40.53	40.17	19.87	7.47	3.27
4	Elancoban	Yes	48.13	156.30	22.63	41.70	35.70	12.47	16.97	43.97	39.03	17.80	9.43	3.87
5	Cozante	No	37.67	129.23	24.63	40.77	34.57	18.17	19.00	41.90	39.10	18.80	6.70	3.47
6	Cozante	Yes	44.63	142.00	26.07	42.77	31.13	19.87	18.97	39.13	41.87	18.83	9.77	4.57
7	Norponin	No	45.30	131.10	21.70	39.73	38.53	12.07	20.07	37.60	42.40	19.50	8.03	4.07
8	Norponin	Yes	48.30	156.50	22.37	41.13	36.47	17.43	18.10	41.77	40.13	16.67	11.27	4.97
9	Organimix	No	38.40	139.13	22.83	41.00	36.17	27.57	16.73	42.20	41.07	18.13	7.97	3.53
10	Organimix	Yes	40.40	145.97	26.10	43.67	30.20	18.43	17.53	40.70	41.77	17.90	11.10	5.00
SEM±			3.787	6.991	1.852	2.582	2.859	3.649	1.054	2.090	1.623	1.054	0.670	0.304
Trt. Aver1age														
Control		49.48	138.43	22.85	36.42	40.73	14.97	18.63	39.97	41.42	18.88	9.37	4.45[a]	
Elancoban		47.08	146.03	21.63	41.23	37.15	16.83	18.13	42.25	39.60	18.83	8.45	3.57[b]	
Cozante		41.15	135.62	25.35	41.77	32.85	19.02	18.98	40.52	40.48	18.82	8.23	4.02[ab]	
Norponin		46.80	143.80	22.03	40.43	37.50	14.75	19.08	39.68	41.27	18.08	9.65	4.52[a]	
Organimix		39.40	142.55	24.47	42.33	33.18	23.00	17.13	41.45	41.42	18.02	9.53	4.27[a]	
SEM±		2.678	4.943	1.310	1.826	2.021	2.580	0.745	1.478	1.148	0.745	0.474	0.215	
Challenge average														
NO		42.87	132.91[b]	22.57	39.71	37.71	19.40	18.94	40.57	40.50	19.42[a]	7.58[b]	3.61[b]	
YES		46.69	149.66[a]	23.97	41.17	34.85	16.03	17.85	40.97	41.17	17.63[b]	10.51[a]	4.72[a]	
SEM±		1.694	3.127	0.828	1.155	1.278	1.632	0.471	0.935	0.726	0.471	0.300	0.136	
Statistical probabilities														
Trt		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	
Challenge		NS	**	NS	NS	NS	NS	NS	NS	NS	*	***	***	
Trt*Challenge		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

[abc]Means in the column with different superscripts differ significantly (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N.S: Not significant)

SEM: Standard error of the mean

[1]IRW: intestine relative weight = total intestinal weight/ dressed weight

¥Measurements of weight and length percentages of each part were calculated based on total weight and length of the small intestine. §liver percentage was calculated based on dressed carcass weight

Table 4. Effect of experimental treatments and challenge on lesion score (0-4) at 25 d challenged by coccidia (10 d post inoculation).

			Duodenum	**Jejunum**	**Ceca**
Treatment	Trt	Challenge	(points)	(points)	(points)
1	Control	No	0.00	0.00	0.00
2	Control	Yes	3.00	2.33	3.00
3	Elancoban	No	0.00	0.00	0.00
4	Elancoban	Yes	1.67	1.33	1.00
5	Cozante	No	0.00	0.33	0.00
6	Cozante	Yes	2.00	1.33	2.33
7	Norponin	No	0.00	0.33	0.00
8	Norponin	Yes	2.33	2.33	2.67
9	Organimix	No	0.00	0.33	0.00
10	Organimix	Yes	2.67	3.00	3.00
SEM±			0.316	0.333	0.350
Trt. Average					
Control			1.50	1.17	1.50
Elancoban			0.83	0.67	0.50
Cozante			1.00	0.83	1.17
Norponin			1.17	1.33	1.33
Organimix			1.33	1.67	1.50
SEM±			0.224	0.236	0.247
Challenge average					
NO			0.00^{b}	0.20^{b}	0.00^{b}
YES			2.33^{a}	2.07^{a}	2.40^{a}
SEM±			0.141	0.149	0.156
Statistical probabilities					
Trt			NS	NS	NS
Challenge			***	***	***
Trt*Challenge			NS	NS	NS

*p<0.05, **p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

Table 5: Effect of different treatments and challenge on oocyst output of broilers (10 days post-inoculation).

			Oocytes output, PI-10
Treatment	Trt	Challenge	(Log_{10} per g excreta)
1	Control	No	0.00
2	Control	Yes	3.71
3	Elancoban	No	0.00
4	Elancoban	Yes	2.92
5	Cozante	No	0.00
6	Cozante	Yes	3.05
7	Norponin	No	0.00
8	Norponin	Yes	2.97
9	Organimix	No	0.00
10	Organimix	Yes	3.18
SEM±			0.104
Trt. Average			
Control			1.86^{a}
Elancoban			1.46^{b}
Cozante			1.53^{b}
Norponin			1.48^{b}
Organimix			1.59^{b}
SEM±			0.073
Challenge average			

NO	0.00[b]
YES	3.17[a]
SEM±	0.046
Statistical probabilities	
Trt	**
Challenge	***
Trt*Challenge	**

**p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

DISCUSSION

Search for the alternatives to anticoccidial drugs to control coccidiosis is an important field of study in poultry production (Arczewska-Włosek and Swiatkiewicz, 2015). Intestinal integrity of broiler may be compromised during exposure to pathogens of coccidiosis. This disease impacts both the performance and health of the birds leading to significant economic losses.

This study was designed to find the success of feed additives from natural sources in comparison with a standard anticoccidial drug against *Eimeria spp*. As expected, the performance was most negatively affected by the Eimeria challenge in the positive control group. Dietary supplementation of natural additives improved the feed efficiency and weight gain almost similar to the birds treated with anticoccidial drug. The efficient role of anticoccidials derived from plants for the controlling of coccidiosis has been previously documented (Abbas *et al*., 2012; Bozkurt *et al*., 2014). The positive effects of the use of botanicals on the performance has been linked to the active compounds which alter microflora modulation, reduce oocysts shedding, decrease intestinal inflammation, enhance immunity and improve antioxidant status (Arczewska-Włosek and Swiatkiewicz, 2015; Abudabos *et al*., 2017; Abudabos *et al*., 2016; Raza *et al*., 2016; Tehseen *et al*., 2016). The effectiveness of herbal additives alleviates the destructive effect of coccidial infection and maintains growth. Confirming our report, previous studies have also documented improved performance in birds in response to anticoccidials (Garcia and Bolis, 2005; Küçükyilmaz *et al*., 2012). In the current study, the increased liver and intestine relative weight increased significantly in the infected birds showing the damaging effect of the infection.

In the current study, the lesion score was significantly reduced in the duodenum, jejunum and caeca of the infected birds in response to treatments showing the effectiveness of the natural additives. The positive effect of herbal mixture may be due to the presence of flavonoids, tannins, alkaloids and saponins, which exhibit anti-inflammatory, antioxidant and anti-parasitic properties (Khan *et al*., 2012c&d; Tanweer *et al*., 2014).

In the current study, the oocysts shedding after 10 days of the infection were similar in the anticoccidial drug and natural products. confirming our work, several earlier studies have reported efficient reduction in the fecal oocysts in birds challenged with experimental coccidial infection in response to natural products (Christaki *et al*., 2004; Ibrir *et al*., 2009; Bozkurt *et al*., 2012; Almeida *et al*., 2014; Tanweer *et al*., 2014).

It was concluded from the present study natural products are very effective in comparison with anticoccidial drug to control coccidiosis in broiler chicken.

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