Pakistan J. Zool., vol. 49(6), pp 1989-1995, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.6.1989.1995

Comparative Anticoccidial Effect of some Natural Products against *Eimeria* spp. Infection on Performance Traits, Intestinal Lesion and Occyte Number in Broiler

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

[1]*Department of Animal Production, King Saud University, Riyadh, Kingdom of Saudi Arabia*
[2]*ARASCO for Feed, P.O. Box 53845, Riyadh 11593, Kingdom of Saudi Arabia*
[3]*Zoology Department, King Saud University, Riyadh, Kingdom of Saudi Arabia*

Article Information
Received 16 March 2017
Revised 01 May 2017
Accepted 12 May 2017
Available online 13 October 2017

Authors' Contribution
AMA, AHA planned and conducted the growth trials. AMA, AHA, EOS and MQA collected samples and did lab analysis.

Key words
Coccidiosis, Broiler, Drugs, Natural products, Control.

ABSTRACT

This study was performed to find the effect of some natural products (Cozante, Norponin and Organimix) in comparison to standard anticoccidial drug (Elancoban) in experimentally induced coccidiosis in broiler. A total 200 birds (Ross 308) were randomly assigned to 10 treatments on day 15 after introduction of coccidiosis. The results indicated that feed intake and production efficiency factor (PEF) did not differ significantly (P>0.05) between Elancoban and natural products. However, treatment had a significant effect on BWG and FCR (P<0.01 and P<0.001, respectively), chicks which had received Elancoban had the highest BWG (327.9 g) and converted the feed to gain with highest efficiency (1.444 g: g). Challenged birds had longer small intestines and heavier liver as compared to unchallenged birds. Lesion score and oocysts secretion increased significantly in the challenged birds, however, Elancoban had no significant difference with the natural products. It was concluded from the present study that natural products could be effective in comparison with anticoccidial drug to control coccidiosis in broiler chicken.

INTRODUCTION

Coccidiosis is one of the most dangerous intestinal protozoan diseases in broiler resulting in stunt growth and feed efficiency (Tanweer *et al*., 2014; Chand *et al*., 2016). Moreover, the disease makes the bird more vulnerable to other bacterial infections such as *Clostridium perfringens*. The poultry farmers use largely coccidiostats and vaccine to control the disease. It is believed that the use of coccidiostats has resulted in major breakthrough in poultry industry, however, their wide spread and misuse led to the development of drug resistance against Eimeria. Additionally, strong evidence of the presence of the residues of coccidiostats in the meat of treated birds has not been adequately addressed. Therefore, there is a strong desire to use some natural alternative agents to replace the existing coccidiostats. Although the use of vaccine has shown promising results in poultry industry, however, on a commercial scale, their effect is limited largely due to high production cost and ineffectiveness in the case of poor management conditions.

Preventing a disease is much a better option than fighting it. Natural dietary supplements may potentially be used as one of the novel approaches to treat coccidiosis due to their natural origin, wide dose range, and lack of grace period and stimulation of appetite among others (Chand *et al*., 2016; Khan *et al*., 2016; Alzawqari *et al*., 2016; Raza *et al*., 2016; Abudabos *et al*., 2017). A number of plant derived compounds have been successfully used as the natural products to prevent the incidence of coccidiosis (Abudabos *et al*., 2016a, b). The natural products may act as alternative therapeutic agents against coccidiosis since the new chemical compounds have not been reported to possess. Therefore, the objective of the present work was to compare the effect of natural product with anticoccidial drug and growth performance, lesion score of intestines and occyte shedding 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.

* Corresponding author: aabudabos@ksu.edu.sa
0030-9923/2017/0006-1989 $ 9.00/0

Table I.- Dietary ingredients and chemical composition of starter and finisher diets.

	Day 1-14	Day 15-30
Ingredients (%)		
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
VM 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
Total	100	100
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.

During the starter period, a total of 200, day old broiler chicks (Ross 308) were allotted to 40 cages. A typical isocaloric and isonitrogenous starter (0 to14 d) and finisher (15 to 35 d) diets were formulated in mashed form based on Ross 308 recommendation guide (Table I). Birds received one of the following six treatments: At day 15 of age, half birds received coccidial challenge while the other half had the treatment without challenge which yielded 10 treatments as follow: 1) Negative control, 2) Coccidial challenge (positive control), 3) Elancoban (0.5 kg/ton monensin sodium), without coccidiosis challenge, 4) With Elancoban (0.5 kg/ton monensin sodium), with coccidiosis challenge (positive Elancoban), 5) With Cozante (0.5 kg/ton), without coccidiosis challenge (negative Cozante). Cozante is a polyphenol compound which is highly effective against Eimeria species, 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 and 10) Organicox (0.5 kg/ton) with coccidiosis challenge (positive Organicox).

The challenge was achieved on day 15 by a mixture of sporulated oocysts of pathogenic strains of *Eimeria* by live oocysts Coccivac®-D (contains live oocysts of *Eimeria acervulina, Eimeria mivati, Eimeria maxima, Eimeria tenella, Eimeria necatrix, Eimeria praecox, Eimeria brunette* and *Eimeria hagani.*

Performance measurements

Average body weight gain (BWG) and feed intake (FI) for each pen was recorded every 5 days and average feed conversion efficiency (FCR) were adjusted for mortality and computed. Production Efficiency Factor (PEF) was calculated weekly by using the formula:

$$PEF = \frac{\text{Livability} \times \text{Live weigh (kg)}}{\text{Age in days} \times \text{FCR}} \times 100$$

Small intestine and carcass measurements

At day 25 eight birds per treatment were selected for small intestinal measurements, the entire gastrointestinal tract was removed aseptically after slaughtering and defeathering. Small intestine and ceca were weighed and the total length was measured then the small intestine was separated into duodenum, jejunum (proximal to Meckel's diverticulum) and ileum (proximal to ileo-caecal junction) and for each part measurements of length and weight. Intestine relative weight was calculated as a ratio between total intestinal weight (g) and dressed weight (g).

Coccidial lesion

Coccidial lesion scoring in the ileum, jejunal and cecal regions of the intestine was carried out using the method of Conway *et al*. (1999). Lesion scores were recorded as 0, 1, 2, or 3, from least to most severe.

Oocyst counting

The oocysts number per gram of excreta (OPG) was determined in pooled excreta samples collected on days 25 post infections. A modified McMaster counting chamber technique was used. Excreta suspension in a salt solution was prepared (10% w/v). Then 1 mL of the suspension was mixed with 9 mL of a salt solution. McMaster chamber was used to count the number of oocysts.

Statistical analysis

The collected data were evaluated with ANOVA for a complete randomized block design, using the general linear models procedure of SAS software (SAS, 2003). The data were analyzed using the General Linear Model

procedure of the Statistical Analysis System (SAS, 2003) as a 5 × 2 factorial arrangement of treatments that included 5 dietary treatments (5 levels) and challenge (2 levels), and their respective interactions. LSD test was applied for mean to compare the treatment means when the treatment effect was significant at $P < 0.05$.

RESULTS

Table II 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 (1.444 g: g). Chicks on the control diet were intermediate in terms of BWG and FCR but they were better than Cozante, Norponin and Organimix.

The morphometric measurements of the intestinal samples at 25 d of age (10 days post inoculation) are given in Table III. No significant differences in intestinal length, weight or (intestine relative weight) 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; 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 II.- Live weight (BW), feed intake (FI), feed conversion ratio (FCR) and production efficiency factor (PEF) of broiler chickens given experimental diets at 25 days.

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

*, p<0.05, **p<0.01; ***, p<0.001; NS, not significant; SEM, Standard error of the mean.

Table III.- 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. Average														
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 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, 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 IV.- Effect of experimental treatments and challenge on lesion score (0-4) at 25 d challenged by coccidia (10 day post inoculation).

Treatment	Trt	Challenge	Duodenum (points)	Jejunum (points)	Ceca (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 x Challenge			NS	NS	NS

*, p<0.05; **, p<0.01; ***, p<0.001, NS, not significant; SEM, standard error of the mean.

Table IV 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 V indicates the effects of different feed additives and challenge on the oocyte output. The results indicated that the natural feed additives performed better than and no significant difference was noticed between Elancoban and other feed additives.

DISCUSSION

Search for the alternatives to anticoccidial drugs to control coccidiosis is an important field of study in poultry production. 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 miroflora modulation, reduce oocysts shedding, decrease intestinal inflammation, enhance immunity and improve antioxidant status (Chand *et al*., 2014; 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.

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

Treatment	Trt	Challenge	PI-10 (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<0.05; **, p<0.01; ***, p<0.001, NS, not significant; SEM, standard error of the mean.

In the present 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*., 2012; 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 could be effective in comparison with anticoccidial drug to control coccidiosis in broiler chicken.

ACKNOWLEDGEMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-273.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCE

Abbas, R.Z., Colwell, D.D. and Gilleard, J., 2012. Botanicals: an alternative approach for the control of avian coccidiosis. *World's Poult. Sci. J.*, **68**: 203-215. https://doi.org/10.1017/S0043933912000268

Abudabos, A.M., Alyemni, A.H., Dafalla, Y.M. and Khan, R.U., 2016a. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase. *J. appl. Anim. Res.*, **45**: 538-542. https://doi.org/10.1080/09712119.2016.1219665

Abudabos, A.M., Alyemni, A.H., Dafallah, Y.M. and Khan, R.U., 2016b. The effect of phytogenic feed additives to substitute in-feed antibiotics on growth traits and blood biochemical parameters in broiler chicks challenged with *Salmonella typhimurium*. *Environ. Sci. Pollut. Res.*, **23**: 24151-24157. https://doi.org/10.1007/s11356-016-7665-2

Abudabos, A.M., Alyemni, A.H., Dafalla, Y.M. and Khan, R.U., 2017. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase. *J. appl. Anim. Res.*, **45**: 538-542. https://doi.org/10.1080/09712119.2016.1219665

Almeida, G.F.D., Stig, M.T., Madeira, A.M.B.N., Ferreira, J.F.S., Magalhaes, P.M., Arczewska-Włosek, A. and Świątkiewicz, S., 2013. Improved

performance due to dietary supplementation with selected herbal extracts of broiler chickens infected with *Eimeria* spp. *J. Anim. Feed Sci*., **22**: 257–263. https://doi.org/10.22358/jafs/66141/2013

Alzawqari, M.H., Al-Baddany, A.A., Al-Baadani, H.H., Alhidary, I.A., Khan, R.U., Aqil, G.M. and Abdurab, A., 2016. Effect of feeding dried sweet orange (*Citrus sinensis*) peel and lemon grass (*Cymbopogon citratus*) leaves on growth performance, carcass traits, serum metabolites and antioxidant status in broiler during the finisher phase. *Environ. Sci. Pollut. Res.,* **23**: 17077-17082. https://doi.org/10.1007/s11356-016-6879-7

Bozkurt, M., Aysul, N., Küçükyilmaz, K., Aypak, S., Egeg Çatli, A.U., Akşit, H., Çöven F., Seyrek, K. and Çinar, M., 2014. Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp. infected broilers. *Poult. Sci.*, **93**: 389-399. https://doi.org/10.3382/ps.2013-03368

Bozkurt, M., Selek, N., Küçükyilmaz, K., Eren, H., Güven, E., Çatli, A.U. and Çinar, M., 2012. Effects of dietary supplementation with a herbal extract on the performance of broilers infected with a mixture of *Eimeria* species. *Br. Poult. Sci.*, **53**: 325-332. https://doi.org/10.1080/00071668.2012.699673

Chand, N., Faheem, H., Khan, R.U., Qureshi, M.S. and Abudabos, A.M., 2016. Anticoccidial effect of mananoligosacharide against experimentally induced coccidiosis in broiler. *Environ. Sci. Pollut. Res.*, **23**: 23930-23935. https://doi.org/10.1007/s11356-016-7604-2

Chand, N., Naz, S., Shah, Z., Khan, S., Shah, A.S. and Khan, R.U., 2014. Growth performance and immune status of broilers fed graded levels of *Albizia lebbeck* seeds. *Pakistan J. Zool.,* **46**: 574-577.

Christaki, E., Florou-Paneri, P., Giannenas, I., Papazahariadou, M., Botsoglou, N.A. and Spais, A.B., 2004. Effect of mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. *Anim. Res.,* **53**: 137-144. https://doi.org/10.1051/animres:2004006

Conway, D.P., Dayton, A.D. and Mckenzie, M.E., 1999. Comparative testing of anticoccidials in broiler chickens: The role of coccidial lesion scores. *Poult. Sci*., **78**: 529-535. https://doi.org/10.1093/ps/78.4.529

Dalloul, R.A. and Lillehoj, H.S., 2005. Poultry coccidiosis: Recent advancements in control measures and vaccine development. *Exp. Rev. Vaccc*., **5**: 143-163. https://doi.org/10.1586/14760584.5.1.143

Garcia, C.C. and Bolis, D., 2005. Nutrition is a key building block of successful drug-free. *World Poult*., **21**: 6-7.

Ibrir, F., Greathead, H.M.R. and Forbes, J.M., 2009. Effect of dietary thymol/carvacrol mixture at a range of concentrations on coccidial infection in broiler chickens. *Proceedings of the 2nd Mediterranean Summit of WPSA*, Antalya-Turkey, pp. 309-317.

Khan, R.U., Chand, N. and Ali, A., 2016. Effect of organic acids on the performance of Japanese quails. *Pakistan J. Zool.*, **48**: 1799-1803.

Khan, R.U., Nikosefat, Z., Tufarelli, V., Naz, S., Javdani, M. and Laudadio, V., 2012. Garlic (*Allium sativa*) supplementation in poultry diet: effect on production and physiology. *World's Poult. Sci. J*., **68**: 417-424. https://doi.org/10.1017/S0043933912000530

Küçükyilmaz, K., Bozku, M., Selek, N., Güven, E., Eren, H., Atasever, A., Bintaş, E., Peek, H.W. and Landman, W.J.M., 2012. Resistance to anticoccidial drugs of Dutch avian *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. *Avian Pathol.*, **32**: 391-401.

Raza, T., Chand, N., Khan, R.U., Shahid, M.S. and Abudabos, A.M. 2016. Improving the fatty acid profile in egg yolk through the use of hempseed (*Cannabis sativa*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*) in the diet of Hy-Line White Leghorns. *Arch. Anim. Breed.,* **68**: 183-190. https://doi.org/10.5194/aab-59-183-2016

SAS, 2002-2003. *SAS users guide: Statistics, Version 9.1.3*. SAS Institute, Cary, NC.

Tanweer, A.J., Chand, N., Saddique, U., Bailey, C.A. and Khan, R.U., 2014. Antiparasitic effect of wild rue (*Peganum harmala* L.) against experimentally induced coccidiosis in broiler chicks. *Parasitol. Res.,* **113**: 2951-2960. https://doi.org/10.1007/s00436-014-3957-y

Tehseen, M., Tahir, M., Khan, R.U., Jabbar, A., Ahmad, B., Ahsan, T., Khan, S. and Abudabos, A.M., 2016. Additive effect of *Nigella sativa* and *Zingiber officinale* herbal mixture on performance and cholesterol profile in broiler. *Philipp. agric. Sci.,* **99**: 408-413.