EFFICACY OF NEEM DRY LEAVES IN CONTROLLING COCCIDIA AND *E.COLI* INFECTIONS IN BROILER CHICKS

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Abstract

In the present study, we investigated the effect of neem dry leaves powder in controlling coccidia and/or *E. coli* infection. One day old chicks were treated with neem dry leaves powder as 100 mg/kg ration. Control negative or positive coccidia (*Eimeria tenella* 1 X 10^4 sporulated ocysts on 4th day) and/or *E. Coli* (1 X 10^7 and 2 X 10^7 CFU *E. coli* strain (078) on 7^{th} and 14^{th} days, respectively) received non-treated ration by neem. Similar groups were infected and treated simultaneously.

The parasitological examination showed a reduction in the coccidial lesion scores, total oocyst counts of 2 ceci in all groups treated with neem. The bacteriological examination revealed that the severity of post-mortem lesions and the mortality rates were markedly reduced in all *E. coli* infected, neem treated groups. Also, there was a marked reduction in *E. coli* from different internal organs on both 14^{th} and 21^{st}, days old.

Infection of coccidia and/or *E. coli* revealed significantly decreased levels of serum total proteins, albumin, A/G ratio and significantly increased in transferring levels of hemoglobin and iron were significantly decreased, while, total iron binding capacity (UIBC) and unsaturated iron binding capacity (UIBC) were significantly increased.

The supplementation of neem leaves powder minimized the toxic effect of infected microorganism and also helped the immune system to respond against the infection.

INTRODUCTION

Colibacillosis and coccidiosis are responsible for huge economic losses that may threaten poultry industry in many parts of the world (Gross, 1991). Although the intestinal tracts of chickens are well established as reservoirs for pathogenic avian *E. coli* in association with the intestinal micro-flora of healthy birds, but only cause disease in case of secondary environmental and host predisposing factors (Fantinatti *et al.*, 1994 and Dho-Moulin and Fairbrother, 1999).

Eimeria tenella inhabits the ceca and adjacent intestinal tissues causing substantial damage to the caecal mucosal epithelium leading to severe lesions, bleeding, high morbidity, high mortality, loss of weight gain, emaciation, and increases the susceptibility to other disease agents (Conway and Mckenzie 1991),

Azadirachia indica (neem) is one of the most widely tropical trees with almost all its parts being put for a variety of uses. Neem leaves contain a vast array of chemically diverse and biologically active ingredients (Devakumar and Sukti, 1993) who found in low dose to have immunomodulator actions that induce cellular immune reaction, and an inhibitory action on wide spectrum of microorganisms (Talwar *et al.*, 1997). So, it was suggested that neem dry leaves powder could be beneficial in immunosuppressant diseases of poultry (Sadekar *et al.*, 1998).

In the present study, a trial to evaluate the antimicrobial and immunomodulator effect of neem dry leaves powder in controlling coccidia and *E. coli* infections in broiler chicks had been done.

MATERIALS AND METHODS

Chicks: One hundred and sixty, one-day old Cobb broiler chicks were obtained from a local hatchery at the day of hatch. The chicks were subjected to bacteriological and parasitological examinations and proved to be free from pathogenic *E. coli* and coccidia.

Coccidia: The local *Eimeria tenella* strain used in this work was obtained from Department of Poultry Diseases.

E. coli **:** A field strain of *E. coli* was isolated from a case of severe colisepticeamia in chicken submitted to Poultry Diseases Department, and was identified biochemically and serologically to be 0 78 .

Plant materials: Leaves from mature *Azadirachta indica* (Neem) trees were obtained and were washed in sterile distilled water and were shade dried. After complete drying the leaves were ground to obtain dry powder which was added to the neem treated ration at a ratio of 100 mg/kg of the ration.

Experimental design

Upon arrival, the chicks were divided into eight equal groups (20 chicks per group) and caged in battery brooders under hygienic conditions.

The chicks in groups number 1, 3, 5, 7 were provided with unmedicated balanced starter ration, while chicks in groups number 2, 6, 7, 8 were provided with ration containing 100 mg neem per kg ration up to the end of the experiment (Table1).

On the 4th day, the chicks in groups number 3, 4, 7, 8 were inoculated orally with 1 X 10^4 sporulated oocysts of *Eimeria tenella* per chicks. On the 7th day, the chicks in groups number 5, 6, 7, 8 were infected orally with 1 X 10^7 cfu of *E. coli* strain (078) seven days later, the same groups were reinfected orally with 2 X 10^7 cfu of the same *E. coli* strain. The chicks in all groups were observed daily for clinical signs, and mortality throughout the experimental period. On the 14th and 21st days of the experiment, the chicks were sacrificed, and heparinized and non- heparinized blood samples (for serum separation) were collected and were used for hematological and biochemical analysis (Table 2).

Table 1. Experimental design for evaluation of the addition of neem dry leaves powder in coccidian and/or *E.coli* infected broiler chicks.

Groups	treatment	Treatment (dose/experimental days)				first slaughter	second slaughter
		Neem (100mg/kg)	Coccidia infection ($1x10^4$ sporulated oocyted/chick)	*E.coli* infection			
				1st infection ($1x10^7$CFU/chick)	2nd infection ($2x10^7$CFU/chick)		
1	Negative control	—	—	—	—	At 14th days old	At 21th days old
2	Neem	1st _ 21d	—	—	—		
3	Coccidia	—	4th day	—	—		
4	Coccidia +neem	1 _21 d	4th day	—	—		
5	E.coli	—	—	7th day	14th day		
6	E.coli + neem	1 _21 d	—	7th day	14th day		
7	Coccidia +E.coli	—	4th day	7th day	14th day		
8	Coccidia + E.coli +neem	1 _21 d	4th day	7th day	14th day		

Postmortem examination of the carcasses were done, samples from internal organs (heart blood, lung, liver, spleen, kidney, intestine) were collected aseptically and subjected to bacteriological and parasitological examinations.

Parasitological examination

The total oocysts output of *Eimeria tenells* was estimated on 14th day` according to Long *et al.* (1976). Cecal lesion scores were determined according to Johnson and Reid (1970), protection percent against lesions and immunity index were calculated by using the formulas described by Singh and Gill (1976).

Bacteriological examination

Re-isolation and identification of the pathogenic *E. coli* (078) strain from different internal organs were done using MacConkey agar and Congo Red agar according to Berkhoff and Vinal (1986).

Hematological examination

Determination of hemoglobin % (Hb%) was estimated according to Schalm (1986). Determinations of leucocytic and differential leucocytic counts were done according to Coles (1986).

Biochemical analysis

The procedures of biochemical parameters allowed in this work were demonstrated in the following (Table 2).

Table 2. Procedures adopted for serum biochemical analysis.

Parameter	Author
Alanine aminotransferase (ALT)	Reitman and Frankel (1957)
Aspartate aminotransferase (AST)	Reitman and Frankel (1957)
Urea	Patton and Crouch (1977)
Creatinine	Husdan and Rapaport (1968)
Total protein (T.P)	Sonnenwirth and Jarett (1980)
Albumin	Doumas *et al.* (1971)
Iron	Tobacco *et al.* (1981)
Total iron binding capacity (TIBC)and unsaturated iron binding capacity (UIBC)	Kunish and Smith (1970)
Transferrin	Tietz (1996)

Statistical analysis

The data obtained from the results of the present study were subjected to statistical analysis according to Petrie and Watson (1999).

RESULTS AND DISCUSSION

In the evaluation of the effect of the addition of 100 mg of neem dry leaves powder per kg ration for controlling coccidia and/or *E. coli* infections in broiler chicks, the following results were obtained:

Concerning coccidia infected groups, the data recorded in Tables 3 and 6 indicated that the addition of neem dry leaves powder to the ration, was efficient in controlling coccidiosis in broiler chicks, which was shown in the reduction of each of the mortality rates (from 25% and 35% to 10% and 15%), cecal score % (from 3.1 and 4 to 2.3 and 2.6) and in total oocysts output /2 ceci (from 3.34 X 10^4 and 3.4 X 10^4, to 2.1 X 10^4 and 1.71 X 10^4), in the infected non-treated groups (3, 7) than the infected treated groups (4, 8), respectively.

Concerning *E. coli* infected groups, the infection with *E. coli* strain O78 was accompanied by pericarditis, periheptitis, airsaculitis, cellulites and severe septicaemia accompanied by death (groups 5,6,7,8). The lesions of septicaemia were observed in all infected chicks.

These results agreed with Harry and Hemsley (1965) who noticed that there is association between the presence of septicaemic *E.coli* in the respiratory and intestinal tracts of chickens and occurrence of colisepticaemia.

It was suggested that, pathogenic *E.coli* isolates that affect poultry commonly belong to certain serotypes which include O78 , which were closely associated with severe septicaemia in chickens (Allan *et al.* 1993 and Gomis *et al.* 2001) .The results obtained from Table 3 revealed that, the highest mortalities were found in the infected non –treated groups (3, 5, 7) .

The highest mortality rate was in group 7 infected with coccidia before *E.coli* (35%) followed by group 3 infected with coccidia alone (25%) and lastly group 4 infected with *E coli* only (20%)

These results explained that the pathogenesis of colibacullosis is affected by many important factors including the dose and route of infection, duration of exposure, exposure to other infectious agents, age and immune status of the bird (Rosenberger *et al.*, 1985).

Tables 4 and 5 showed the rates of reisolation of *E.coli* from infected and challenged broiler chicks on the 14^{th} and 21^{st} day old. It was found that on 14^{th} day (7 days after *E. coli* infection), the highest reisolation rates were from liver and spleen, while on 21^{st} day (7 days after *E. coli* challenge) the highest reisolation rate was from the kidney.

Regarded to *E. coli* infected, Neem treated groups (6, 8), it was noticed that the severity of septicaemic lesions were greatly reduced accompanied with a reduction in the mortality rates from 20% and 35% in the infected non-treated groups (5, 7, respectively), to 10% and 15% in the infected treated ones (7, 8, respectively) as shown Table 3. on 14^{th} day old the rates of reisolation of *E.coli* from different organs were greatly reduced from 70 and 100% in non-treated infected group (5, 7) to 50 and 80% in the infected treated ones accompanied by 20 and 30% reduction in the number of *E. coli* positive chicks in infected treated groups 6, 8, respectively (Table4).

On 21^{st} day, the reisolation rates were reduced from 66.6 and 100% in infected non-treated groups to 25 and 57% in infected treated ones (Table 5). Also, there was a decline in the number of positive chicks from 100% in infected non-treated groups to 37.5 and 57.14 in the infected treated groups (6, 8).

The above mentioned results revealed that, addition of neem dry leaves powder in the ration was effective in controlling *E.coli* infection in broiler chicks.

These results were in agreement with those of Talwar *et al.* (1997) who reported the inhibitory action of neem leaves and seeds extract on a wide spectrum of microorganisms including urinary tract *E.coli.* The results also agreed with those of Das *et al.* (1999) who recorded the antibacterial effect of neem against *E. coli* infection in fishes.

Concerning hematological examinations, the results obtained in Table 7 demonstrated that there is a significant increase in total leucocytic heterophils, monocytes and eosinophil counts accompanied with a marked decrease in lymphocyte counts These were observed during the infection of coccidia and/or *E. coli* on the 14^{th} and 21^{st} day. The stress and hypovitaminosis caused by infection increased the heterophils and decreased the lymphocytes counts (Coles, 1986). The alterations in leucocytic picture may be attributed to the irritation of the bone marrow by the infection by-products and toxins.

The biochemical analysis of serum for the present study revealed that, infection with coccidia and/or *E.coli* were often accompanied by a significant decrease in serum total proteins albumin and A/G ratio (Albumin / Globulin ratio) on both 14^{th} and 21^{st} day Conversely, there was a non significant decease of total globulins on 14^{th} day compared with the control non infected groups. (Table 8). The previous findings were confirmed by Liao *et. al.* (1986). who found that infection with coccidia and/or *E. coli* cause a decrease in nutrient absorption from the intestine, and these lead to serum protein loss and hypoimmune syndrome. The increase in total globulins on 21^{st} day well agreed with Peace and Kaplan (1984).

Supplementation with neem leaves at a small dose in the ration (100 mg/kg) of broiler chicks led to reduce the harmful effects of coccidia and/or *E. coli* as shown in Tables 7 and 8. Neem enhanced the increase in leucocytic counts and lymphocyte counts (Garg *et. al.*, 1994). Neem has immunostimulant effect that activates the cell mediated immune response and therefore creates an enhanced response to any future challenges occurred by disease organisms. So, the feeding neem leaves to immunosuppressed birds increase their humoral and cell mediate immune responses (Sadekar *et al.*, 1998).

Table 8 showed that transferrin was increased significantly with infection by coccidia and/or *E. coli.* Similar findings were recorded by Klasing (1991).

Infection of broiler chicks with coccidia and/or *E.coli* has resulted in significant decrease of serum iron, significant increase in total iron binding capacity, unsaturated iron binding capacity and significant decrease in hemoglobin (Table 9). These changes in iron metabolism along with decrease in the intestinal hemorrhage and absorption, suggested that anemia in infected chicks may reflect reduced availability of iron in the body (Turk, 1986 and Linda-Tufft *et al.*, 1988).

Mcknight *et al.* (1980) showed increased transferrin synthesis in iron of infected chicks which showed results in elevated total iron binding capacity (TIBC).

Coccidiosis had a little effect on serum iron concentration on 21st day, which called the recovery phase of coccidia. Following neem supplementation, Hb, iron concentration, TIBC and UIBC controlled the toxic effect of coccidia and/or *E. coli.*

Neem produced an increase in red blood cells, and so, there was an increase in hemoglobin and iron concentrations (Garg *et al.*, 1994).

The data obtained in Table 10 indicated that, during infection with coccidia and/or *E. coli* there was a significant increase in serum AST and ALT.

Infection with coccidia and/or *E. coli* produced a general increase in the relative size of the liver (Huff and Ruff, 1982) and the inoculation with *E. coli* caused inflammatory cell infiltration and cellular debris on the serosal surfaces of the liver and spleen (Sayed *et al.*, 1997). These attribution explain the elevation of enzyme serum activities of liver. Dietary use of neem leaves provided protection against the in flammation in the liver cells caused by infection with coccidia and/or *E. coli* and decrease the serum enzyme activities AST and ALT (Okpanyi and Ezeutwa, 1981). The data of the present investigation indicated that there were no significant changes in urea or creatinine levels among treated and non-treated groups.

In conclusion, the addition of neem dry leaves powder in ration in small doses (100 mg/kg ration) was efficient in controlling colibacillosis and/or coccidiosis in infected broiler chicks. As it has antibacterial, antiparasitic and immunostimulant

activities against coccidia and/or *E. coli* infections in broiler chicks, neem could be beneficial in controlling immunosuppressed conditions in poultry.

Table 3. Influence of Neem on mortality rates of coccidia and/or *E. coli* infection in broiler chicks.

Groups	Treatment	No. of Dead chicks / total (%)					
		Up to 14 day old		Up to 21 day old		Total	
		No.	%	No.	%	No.	%
1	Control negative	0/20	0%	0/10	0%	0/20	0%
2	Neem	0/20	0%	0/10	0%	0/20	0%
3	Coccidia	4/20	20%	1/6	16.66%	5/20	25%
4	Coccidia + Neem	2/20	10%	0/7	0%	2/20	10%
5	*E. coli*	3/20	15%	1/7	14.28%	4/20	20%
6	*E. coli* + Neem	2/20	10%	0/8	0%	2/20	10%
7	Coccidia + *E. coli*	4/20	20%	3/6	50%	7/20	35%
8	Coccidia + *E. coli* + Neem	2/20	10%	1/8	12.5%	3/20	15%

Table 4. Effect of Neem on *E. coli* reisolation from coccidia and/or *E. coli* infected broiler chicks on the 14th day old (n=10).

Groups	Treatment	Liver		Spleen		Heart		Lung		Kidney	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Control negative	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
2	Neem	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
3	Coccidia	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
4	Coccidia + Neem	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
5	*E. coli*	9/10	90	9/10	90	8/10	80	8/10	80	7/10	70
6	*E. coli* + Neem	7/10	70	7/10	70	6/10	60	6/10	60	5/10	50
7	Coccidia + *E. coli*	10/10	100	10/10	100	9/10	90	9/10	90	8/10	80
8	Coccidia + *E. coli* + Neem	8/10	80	8/10	80	7/10	70	6/10	60	6/10	60

Table 5. Effect of Neem on *E. coli* reisolation from coccidia and/or *E. coli* infected broiler chicks on the 21st day old (n=10).

Groups	Treatment	Liver		Spleen		Heart		Lung		Kidney		Positive chicks	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	Control negative	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
2	Neem	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0	0/10	0
3	Coccidia	0/5	0	0/5	0	0/5	0	0/5	0	0/5	0	0/5	0
4	Coccidia + Neem	0/8	0	0/8	0	0/8	0	0/8	0	0/8	0	0/8	0
5	*E. coli*	4/6	66.6	4/6	66.6	4/6	66.6	4/6	66.6	5/6	83.3	6/6	100
6	*E. coli* + Neem	2/8	25	2/8	25	2/8	25	2/8	25	3/8	37.5	3/8	37.5
7	Coccidia + *E. coli*	3/3	100	3/3	100	3/3	100	3/3	100	3/3	100	3/3	100
8	Coccidia + *E. coli* + Neem	3/7	42.86	3/7	42.86	3/7	42.86	2/7	28.57	4/7	57.14	4/7	57.14

Table 6. Effect of Neem on cecal score, total oocyst output, protection against lesions and immunity index in cocedia and/or *E. coli* infected broiler chicks on the 14th day old (n=10).

Groups	Treatment	Cecal score (%)	Total oocysts Output/2 ceci (X 10^4)	Protection against lesions (%)	Immunity index
1	Control negative	0	0	100	300
2	Neem	-	-	-	-
3	Coccidia	3.1	3.34	72	127
4	Coccidia + Neem	2.3	2.10	85	251
5	*E. coli*	-	-	-	-
6	*E. coli* + Neem	-	-	-	-
7	Coccidia + *E. coli*	4	3.40	62	107
8	Coccidia + *E. coli* + Neem	2.6	1.71	82	248

Table 7. Effect of Neem on total and differential leucocytic count in broiler chicks infected with coccidia and/or *E. coli* on the 14th and 21st day old (Mean ± S.E).

	Leucocytes ($X 10^3/mm^3$)		Heterophil %		Lymphocyte %		Monocyte %		Eosinophil %		Basophil %	
	14	21	14	21	14	21	14	21	14	21	14	21
Control	14.00 ± 0.56	14.80 ± 0.82	24.11 ± 0.84	24.84 ± 0.99	67.32 ± 1.11	67.45 ± 0.94	4.81 ± 0.11	4.54 ± 0.22	2.87 ± 0.06	2.60 ± 0.04	0.89 ± 0.01	0.57 ± 0.02
Neem	15.83 ± 0.96	16.11 ± 1.84	23.87 ± 0.61	25.55 ± 0.58	69.52 ± 0.94	68.00 ± 0.86	4.56 ± 0.06	4.83 ± 0.10	2.05 ± 0.01	1.62 ± 0.01	0	0
Coccidia	17.95* ± 1.25	16.25 ± 1.58	28.23* ± 1.05	27.31* ± 0.89	62.39* ± 0.67	64.26* ± 0.81	6.33* ± 0.09	6.01* ± 0.07	3.05* ± 0.02	2.42 ± 0.01	0	0
Coccidia + Neem	17.01* ± 1.99	16.71 ± 1.88	26.94* ± 0.79	25.83 ± 0.73	64.68 ± 0.71	66.52 ± 0.98	5.40* ± 0.10	5.17 ± 0.14	2.98 ± 0.02	2.48 ± 0.03	0	0
E. coli	19.90* ± 1.01	20.79* ± 0.73	29.10* ± 1.11	28.03* ± 0.97	60.82* ± 1.02	62.07* ± 0.95	6.10* ± 0.08	6.85* ± 0.12	3.98* ± 0.02	3.05* ± 0.03	0	0
E. coli+ Neem	18.16* ± 1.62	18.83* ± 1.32	26.71* ± 0.76	25.75 ± 0.85	65.26 ± 0.89	66.14 ± 0.94	5.22*± 0.07	5.13 ± 0.13	2.81 ± 0.03	2.98 ± 0.07	0	0
Coccidia + *E. coli*	21.45* ± 2.13	22.72* ± 1.13	29.27* ± 1.21	28.91* ± 1.01	58.36* ± 0.57	62.12* ± 0.88	7.68* ± 0.09	5.80* ± 0.19	4.69* ± 0.03	3.17* ± 0.04	0	0
Coccidia + *E. coli* + Neem	18.19* ± 2.88	18.79* ± 1.76	26.91* ± 0.87	26.84 ± 0.69	63.31* ± 0.96	64.9 ± 0.82	5.77* ± 0.10	5.10 ± 0.16	4.01* ± 0.01	3.16* ± 0.03	0	0

- n = 5
- * Significant at $P < 0.05$

Table 8. Effect of Neem on serum total protein as well as its fractionation in broiler chicks infected with coccidia and/or *E. coli* on the 14^{th} and 21^{st} day old (Mean ± S.E)

	T.P. (gm/dl)		Albumin (gm/dl)		T. globulin (gm/dl)		A/G ratio		Transferren (mg/dl)	
	14	21	14	21	14	21	14	21	14	21
Control	3.78 ± 0.05	3.95 ± 0.05	1.17 ± 0.04	1.28 ± 0.05	2.61 ± 0.05	2.67 ± 0.04	0.44 ± 0.01	0.48 ± 0.01	93.72 ± 5.00	97.13 ± 7.98
Neem	3.95 ± 0.08	4.11 ± 0.08	1.24 ± 0.06	1.33 ± 0.13	2.71 ± 0.11	2.78 ± 0.08	0.46 ± 0.01	0.48 ± 0.02	91.90 ± 4.41	96.56 ± 5.22
Coccidia	3.50* ± 0.05	3.86 ± 0.04	0.96* 0.07	1.10 ± 0.11	2.54 ± 0.03	2.69 ± 0.06	0.38* ± 0.02	0.41* ± 0.01	110.96* ± 3.61	110.91 ± 6.88
Coccidia + Neem	3.63 ± 0.10	3.93 ± 0.08	1.07* ± 0.03	1.22 ± 0.07	2.56 ± 0.10	2.71 ± 0.03	0.42 ± 0.01	0.45 ± 0.02	104.26 ± 3.31	106.70 ± 7.42
E. coli	3.42* ± 0.08	3.72* ± 0.09	0.90* ± 0.03	0.96* ± 0.06	2.52 ± 0.05	2.76 ± 0.10	0.35* ± 0.02	0.34* ± 0.01	119.19* ± 5.40	121.92* ± 6.11
E. coli+ Neem	3.57* ± 0.06	3.80 ± 0.09	1.01* ± 0.05	1.08 ± 0.09	2.56 ± 0.08	2.72 ± 0.11	0.39* ± 0.02	0.40* ± 0.02	102.40 ± 4.92	100.26 ± 6.55
Coccidia + *E. coli*	3.34* ± 0.11	3.74 ± 0.11	0.86* ± 0.08	0.98* ± 0.04	2.48 ± 0.08	2.76 ± 0.09	0.35* ± 0.03	0.36* ± 0.01	131.00* ± 5.63	126.27* ± 6.13
Coccidia + *E. coli* +Neem	3.69 ± 0.12	3.89 ± 0.07	0.95* ± 0.06	1.09 ± 0.08	2.74 ± 0.10	2.80 ± 0.06	0.35* ± 0.02	0.40* ± 0.02	115.91* ± 6.51	113.00 ± 7.33

- n = 5* Significant at $P < 0.05$

Table 9. Effect of Neem on blood Hb%, ion and serum TIBC and UIBC in broiler chicks infected with coccidia and/or *E. coli* on the 14th and 21st day old (Mean ± S.E).

	Hb%		Iron µg/dl		TIBC µg/dl		UIBC µg/dl	
	14	21	14	21	14	21	14	21
Control	8.21 ± 0.35	8.56 ± 0.31	93.00 ± 8.46	97.66 ± 7.93	168.18 ± 8.05	174.48 ± 8.30	75.18 ± 5.45	76.82 ± 6.93
Neem	8.71 ± 0.16	8.85 ± 0.74	98.99 ± 7.75	95.27 ± 8.49	159.86 ± 6.27	166.52 ± 7.51	60.87 ± 5.11	71.25 ± 5.49
Coccidia	6.81* ± 0.46	7.94 ± 0.39	70.66* ± 4.42	86.24 ± 6.99	173.23 ± 8.88	185.02± 7.69	104.57* ± 7.96	98.78* ± 6.48
Coccidia + Neem	7.21 ± 0.44	8.66 ± 0.38	73.01 ± 5.27	90.00 ± 8.68	177.97 ± 6.39	181.00 ± 8.66	101.96 ± 6.58	90.27 ± 6.99
E. coli	6.73* ± 0.55	6.05* ± 0.38	65.65* ± 8.22	56.91* ± 5.10	191.78* ± 8.60	215.60* ± 10.17	120.13* ± 6.11	150.69* ± 8.73
E. coli+ Neem	7.30* ± 0.20	7.11 ± 0.59	79.52 ± 6.89	67.39* ± 5.57	174.86 ± 6.26	187.52* ± 8.51	95.34± 6.17	120.13* ± 8.58
Coccidia + *E. coli*	6.13* ± 0.22	6.15* ± 0.64	58.43* ± 3.41	49.20* ± 4.16	201.39* ± 9.11	227.54* ± 8.02	142.57* ± 8.17	178.34* ± 8.70
Coccidia + *E. coli* + Neem	6.55 ± 0.43	6.98* ± 0.65	67.38* ± 5.58	61.31* ± 5.05	187.20 ± 8.37	190.13 ± 7.00	117.28 ± 7.54	128.82 ± 9.12

- n = 5

TIBC = total iron binding capacity UIBC = unsaturated iron binding capacity

* Significant at P < 0.05

Table 10. Effect of Neem on some serum enzymatic activities, urea and creatinine in broiler chicks infected with coccidia and/or *E. coli* on the 14th and 21st day old (Mean ± S.E).

	AST (u/l)		ALT (u/l)		Urea (mg/dl)		Craetinine (mg/dl)	
	14	21	14	21	14	21	14	21
Control	37.67 ± 2.95	39.00 ± 3.87	16.67 ± 1.23	17.28 ± 1.25	11.04 ± 0.93	11.21 ± 0.82	0.98 ± 0.09	1.90 ± 0.10
Neem	32.33 ± 2.46	35.33 ± 3.58	15.00 ± 0.87	14.67 ± 1.44	9.26 ± 0.49	9.00 ± 0.73	0.90 ± 0.03	0.92 ± 0.10
Coccidia	44.67* ± 1.36	39.67 ± 1.25	24.33*± 2.11	20.26 ± 1.26	9.47 ± 0.66	10.60 ± 0.54	0.89 ± 0.09	0.96 ± 0.21
Coccidia + Neem	40.67 ± 1.62	37.0 ± 5.03	20.67 ± 1.02	20.00 ± 0.51	10.11 ± 0.69	10.46 ± 0.30	0.90 ± 0.07	1.01 ± 0.08
E. coli	49.33**±1.26	51.00*± 0.57	21.17*± 0.96	23.67* ±0.93	9.60 ± 0.40	9.20 ± 0.62	0.81 ± 0.13	0.86 ± 0.03
E. coli+ Neem	40.00 ± 1.68	41.33 ± 3.67	19.21 ± 1.37	22.71 ± 1.25	10.42 ± 0.70	9.96 ± 0.40	0.93 ± 0.010	0.95 ± 0.7
Coccidia + *E. coli*	46.00* ± 1.70	50.33* ±3.72	26.81* ±1.24	27.42*± 1.81	9.10 ± 0.74	9.37 ± 0.85	0.79 ± 0.08	0.86 ± 0.08
Coccidia + *E. coli* + Neem	41.33 ± 1.25	42.00 ± 3.44	21.18 ± 1.54	25.87 ± 1.25	9.97 ± 0.61	9.96 ± 0.40	0.86 ± 0.07	0.90 ± 0.09

- n = 5

* Significant at P < 0.05

REFERENCES

1. Allan B.J., J.V. Van den Hurk and A.A.Potter. 1993. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. Can. J. Vet. Res., 57: 146-151.

2. Augustine P. and H.D. Danforth. 1990. Development of protective immunity against *Eimeria tenella* and *E. acervulinai* in white Leghorn chickens inoculated repeatedly with high doses of turkey coccidia. Avian Dis., 39: 707-717.

3. Berkhoff H.A. and A.C. Vinal. 1986. Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. Avian Dis., 30: 117-121.

4. Coles E.H. 1986. Veterinary Clinical Pathology. 4th Ed., W.B. Saunders Company, Philadelphia, London, Toronto.

5. Conway D.P. and M.E. Mckenzie.1991. Poultry coccidiosis, diagnostic and testing procedures. 2nd Ed., Pfizer Inc., New York.

6. Das B.K., S.C. Mukherjee, B.B. Sahu and G. Murjani.1999. "Neem (Azadiracta indica) extract as an antibacterial agent against fish pathogenic bacteria. Indian J. Exp. Biol., Nov.

7. Devakumar C. and D.V. Sukтt.1993. Chemistry, in: RANDhawa, N.S. & Parmar, B.S. (Eds), Neem Research and Development, Publication No. 3, pp. 63-96 (India, Society of Pesticide Science).

8. Dho-Moulin M. and J.M. Fairbrother.1999. Avian pathogenic *Escherichia coli* (APEC). Vet. Res., 30: 299-316.

9. Doumas B.T., W.A. Walson and H.G.Biggs.1971. Albumin standards and the measurement of serum albumin with bromocrysol green. Clin. Chem. Acta, 31-37.

10. Fantinatti F., W.D. Sliveira and A. F. Castro.1994. Characteristics associated with pathogenicity of avian septicemic *Escherichia coli* strains. Vet. Microbiol., 41: 75-86.

11. Fukota T., K. Komnba, E. Sasai, E. Baba and A. Arakaw. 1997."Evaluation of plasma chemistry and haematological studies on chickens infected with *Eimeria tenella* and *E. acervulina*. Vet. Rec., 141: 4-46.

12. Garg H.S., G.P. Talwar and S. Upadhyay. 1994. Comparison of extraction process the immunocontraceptive activity of neem seed extracts. J. Ethnopharmacol., 87-92.

13. Gomis S.M., C. Riddell, A.A. Potter and B.J. Allan. 2001. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulites and other colibacillosis lesions. Canadian J. Vet. Res., 65 (1): 1-6.

14. Gross W.B. 1991. Colibacillosis. In: Disaeses Calnek, B.W.; Barnes, H.J.; Beard, C.W.; Reid, W.M.; Yoder, Jr. H.W. (Eds). of Poultry 9th Ed., Iowa State Univ. Press Ames, Iowa, pp. 138-144.

15. Harry E.G. and L.A. Hemsley. 1965. The association between the presence of septicemic strains of pathogenic *Escherichia coli*

16. Huff W.E. and M.D. Ruff. 1982 . Eimeria acervulina and *Eimeria tenella* infections in ochratoxin A- Compromised Broiler chicken. Poult Sci., 61: 685-692.

17. Husdan H. and Rapaport. 1968. Chemical determination of creatinine with deproteinization. Clin. Chem., 14: 222-238.

18. Johnson J. and W.M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasit., 28: 30-36.

19. Klasing K.C. 1991. Avian inflammatory response: Mediation by macrophages. Poult Sci., 70: 1176-1186.

20. Kunish J.P. and L.L. Smith. 1970. determination of serum total iron binding capacity by precipitation method. Clin. Chem., 16: 148-150.

21. Liao W.S., L.S. Jafferson and J.M. Taylor. 1986. Changes in plasma albumin concentration synthesis rate and mRNA level during acute inflammation. Am. J. Physiol., 251: 928-934.

22. Linda S. Tufft, F. Chercyl Nokels and M.J. Fettman. 1988. Effects of *Escherichia coli* on iron, copper and zinc metabolism in chicks. Avian Dis., 32: 779-786.

23. Long P., L. Joyner, B. Millard and C. Norton. 1976. A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Veterinaria Latina, 6: 201-217.

24. Mcknight G.S., D.C. Lee and R.D. Pslmiter. 1980. Transferrin gene expression regulation of mRNA transcription in chick liver by steroid hormones and iron defiance. J. Biol. Chem., 255:148-153.

25. Okpanyi S.N. and G.C. Ezeutwa.1981. Anti-inflammatory and antipyretic of Azadiracta indica. Plant Med., 41: 34-39.

26. Patton G.J. and S.R. Crouch. 1977. Determination of urea (urease modified Berthelot reaction). Anal. Chem., 49: 464-469.

27. Pesce A.J. and L.A. Kaplan. 1987. Methods in Clinical Chemistry. The C.V. Mosby Co., St. Louis, Washington, D.C., Toronto.

28. Petrie A. and P. Watson. 1999. Statistics for Veterinary and Animal Science. 1st Ed., pp. 90-99, The Blackwell Science Ltd, United Kingdom.

29. Reitman S. and S. Frankel. 1957. A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Path., 28:56-58.

30. Rosenberger J.K., P.A. Fries, S.S. Cloud and R.A. Wilson. 1985. In vitro and in vivo characterization of avian *Escherichia coli*. II. Factors associated with pathoegnicity. Avian Dis., 29: 1094-1107.

31. Sadekar R.D., A.Y. Kolte, B.S. Barmase and V.F. Desai. 1998. Immunopotentating effects of Azadiracta indica (neem) dry leaves powder in broilers, naturally infected with IBD virus. Indian J. Exp. Biol., 36 (11): 1151-1153.

32. Sayed A.P., M., Boulinne, M. Martineu, C.M. Dozois, C. Desautals and J.M. Fairbrothex.1997. Dynamics of *Escherichia coli* infection in experimentally inoculated chickens. Avian Dis., 41: 221-233.

33. Schalm O.W. 1986. Veterinary Haematology. 4th Ed., Lea and Febiger, Philadelphia, USA.

34. Singh J. and B.S. Gill. 1976. Activity of Amprolium against different levels of *Eimeria necartix* infection and effect of medication on development of immunity. Riv. Parsitol., 37: 63.

35. Sonnenwirth A. and L. Jarett. 1980. Gardwhols Clinical Laboratory Methods and Diagnosis. Vol. 1, 8th Ed., Mosby.

36. Tobacco A., E. Moda and P. Tarli. 1981. Colorimetric method for determination of iron in serum. Clin. Chem. Acta, 114: 287-290.

37. Talwar G.P., P. Raguvanshi, R. Misra, S. Mukherjee and S. Shah. 1997. Plant immunomodulators for termination of unwanted pregnancy and for contraception and reproductive health. Immunol. Cell Biol., 75 (2): 190-192.

38. Tietz N.W. 1996. Fundamentals of clinical chemistry. 4th Ed., Carl, A. and Esdward, R, Saunders Company, Philadelphia, USA.

39. Turk D.E. 1986. Microelements in the circulation of coccidiosis-infected chicks. Poult. Sci., 65: 2098-2103.

كفاءة أوراق النيم الجافة فى التحكم فى عدوى الكوكسيديا والميكروب القولونى فى بدارى التسمين

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تمت دراسة تأثير مسحوق أوراق النيم الجافة فى التحكم فى عدوى الكوكسيديا والميكروب القولونى فى كتاكيت التسمين بإضافة مسحوق أوراق النيم الجافة بنسبة ١٠٠ مجم / كجم إلى العليقة المقدمة للمجموعات العلاجية المختلفة.

تم عمل ثلاث مجموعات ضابطة لكل من النيم والعدوى بالكوكسيديا والعدوى بالميكروب القولونى بالإضافة إلى مجموعة ضابطة غير معالجة وغير معدية وكذلك أربع مجموعات معالجة ومعدية بكل من الكوكسيديا أوالميكروب القولونى أو كليهما. تم إجراء العدوى للمجموعات المعدية بالكوكسيديا بجرعة مقدارها ١ X ١٠^{٤} حويصلة من الابميريا تينيلا فى اليوم الرابع من التجربة. تم إجراء العدوى بالميكروب القولونى (العترة ٧٨:٥) بجرعة مقدارها ١ X ١٠^{٧} ٢ X ١٠^{٧} فى اليوم السابع والرابع عشر على التوالى. وأظهرت الإختبارات الطفيلية نقص درجة الإصابة بالكوكسيديا ونقص عدد الحويصلات وزيادة درجة الحماية ضد الإصابة بالمرض فى جميع المجموعات التى عولجت بالنيم. أثبتت الفحوصات البكتيريولجية انخفاضا كبيرا فى شدة الامراض ودرجة الإصابة فى التشريح المعملى وانخفاضا فى نسبة الوفيات بدرجة كبيرة فى المجموعات المعدية بالميكروب القولونى والمعالجة بالنيم ، بالإضافة إلى انخفاض نسبة عزل الميكروب القولونى من الأعضاء الداخلية للطيور عند ١٤ ، ٢١ يوم مع انخفاض عدد الكتاكيت المعدية بالميكروب القولونى.

العدوى بكل من الكوكسيديا والميكروب القولونى أوضحت أنخفاضاً ملحوظاً فى تركيز البروتين الكلى والألبيومين ونسبة الألبيومين إلى الجلوبيولين وأيضا ارتفاعاً ملحوظاً فى الترانسفرين. بينما القدرة الكلية على الارتباط بعنصر الحديد وعدم المقدرة على الارتباط بعنصر الحديد كانت له زيادة ملحوظة.

إن إضافة مسحوق أوراق النيم ساعد على تقليل التأثير المرضى للعدوى بالكوكسيديا والميكروب القولونى ويساعد على رفع قدرة الجهاز المناعى للتصدى لها.

التوصية:

وفى المجمل ، وجد أن إضافة مسحوق أوراق النيم الجافة بنسبة ١٠٠ مجم / كجم إلى علائق كتاكيت التسمين قد ساهم بشكل كبير فى التحكم وخفض درجةالإصابة بكل من الكوكسيديا والميكروب القولونى فى كتاكيت التسمين كما ثبتت كفاءته فى تقليل التأثير المرضى للعدوى بالميكروبات المختلفة وفى رفع كفاءة الجهاز المناعى للطائر للتصدى للعدوى بالكوكسيديا والميكروب القولونى.