

## Evaluation of herbal coccidiostat 'Coxynil' in broiler

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Anticoccidial efficacy of "Coxynil" a polyherbal preparation was tested against *Eimeria tenella* in broilers. Body weight of birds challenged with *E. tenella* in Coxynil treated groups was higher as compared to Coxynil untreated. Oocyst out put, lesion score, HI titres against New Castle disease virus were significantly higher in Coxynil supplemented groups in comparison to Coxynil un-supplemented groups. Examination of caeca of the birds, revealed that the Coxynil interfered with life cycle of coccidia. The typical second generation schizonts were absent in caecal section of Coxynil treated groups. The results indicate that Coxynil is effective herbal coccidiostat.

**Keywords:** Broilers, Coxynil, Herbal coccidiostat, Immunology.

In recent years, intensive livestock and poultry farming have led to an increase in stress and incidence of diseases. Intestinal infections such as coccidiosis and salmonellosis are becoming increasingly prevalent in commercially reared broilers. Coccidiosis is associated with mortality, reduced growth rate and impaired feed conversion leading to poor performance of commercial birds. Many compounds have been introduced for the control of coccidiosis, including synthetic drugs or ionophores antibiotics. Still the information available on selective mode of action of some of these compounds is poorly understood<sup>1</sup>. Currently chemotherapy is used extensively to control coccidiosis, however, development of drug resistance in field strains of parasites and withdrawal period for these drug prior to slaughter mandates development of alternative methods to control coccidiosis.

Acquired immunity is important in protection against coccidiosis. Attenuated live vaccines comprising oocysts have been attempted to achieve immunization in chicks<sup>2</sup>. In order to acquire protective immunity several cycles of parasite development are necessary in chicks<sup>3</sup>. In natural host, immunity to coccidia is species specific. Chicks immune to one species of *Eimeria* are nevertheless susceptible to others<sup>4</sup>.

Youn and Noh<sup>5</sup> tested 15 herbal extracts for anticoccidial activity in chicks with varying degrees of results. Herbs like *Valeriana wallichii* DC,

*Cinnamomum camphora* Nees, *Elephantopus scaber* Linn and *Eberum*, are used traditionally to treat dysentery cases in human beings<sup>6</sup>. Polyherbal preparation (Coxynil; M/s Growell India, Pune) from these herbs was tested in present study for its efficacy during experimental coccidiosis in broilers.

### Materials and Methods

*Composition of Coxynil*—*Allium sativum* Linn 15%, *Cinnamomum camphora* Nees & *Eberum* 15%, *Elephantopus scaber* Linn 15%, *Valeriana wallichii* DC 15%, Sulphur dioxide (Gandak) 25% and NaCl 15%.

*Birds*—One day old broiler chicks (240) were procured from a commercial hatchery. The chicks were divided into 6 equal treatment groups A, C, D, E, F and G (of 40 chicks each) and maintained on rice husk litter under standard managerial practices for 45 days of age. Feed without coccidiostat was procured and used as basal feed in the experiment. Chicks from all groups were vaccinated against New Castle disease virus (NCDV) on 7<sup>th</sup> day with Lasota strain and booster vaccination was performed with Lasota strain on 28<sup>th</sup> day of age. All birds were immunized against Infectious Bursal Disease virus on 14<sup>th</sup> day with intermediate strain of IBD virus.

*Sporulation of oocyst*—Oocysts were collected from field outbreaks of coccidiosis in broilers. Oocysts were separated from faeces by floatation technique on saturated magnesium sulphate solution. These oocysts were subjected to sporulation in 2.5%

potassium dichromate solution for 48 hr at room temperature. Oocysts were examined microscopically and oocysts collected were identified as *Eimeria tenella*<sup>7</sup>. These sporulated oocysts of *E. tenella* were used to challenge the birds. Each experimental bird was challenged with  $5 \times 10^4$  oocysts as a challenged dose on 30<sup>th</sup> day of age.

**Treatment**—The details of treatments were as follows:

Group	Coxynil supplementation in feed from 1 <sup>st</sup> to 45 <sup>th</sup> day of age	Challenge with <i>E. tenella</i> oocyst
A. Infected control	—	at 30 <sup>th</sup> day of age
C. Control	—	—
D. Medicated infected	@ 200 mg/kg feed	at 30 <sup>th</sup> day of age
E. Medicated control	@ 200 mg/kg feed	—
F. Medicated higher dose	@ 300 mg/kg feed	—
G. Medicated higher dose infected	@ 300 mg/kg feed	at 30 <sup>th</sup> day of age

**Growth and clinical parameters**—Fifteen chicks were selected from each treatment group and weighed individually at weekly intervals. Experimental birds were observed for clinical symptoms and mortality.

**Estimation of oocyst per g of faeces**—Faeces (3 g) were collected from six birds of each treatment group on 30<sup>th</sup> day of age (before challenge) and 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day post challenge with oocyst of *E. tenella* for estimation of oocyst per gram of faeces (OPG) with Stoll's method<sup>7</sup>.

**Pathological study**—The experimental birds from groups A, D and G were challenged on 30<sup>th</sup> day of age orally with  $5 \times 10^4$  oocyst of *E. tenella*. Six birds from all groups were sacrificed on 4<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> day of post challenge to ascertain the lesion score in the caeca in scale of 0 to 4 (Ref. 8). For histopathological observation representative tissue of intestine was collected in 10% neutral formal saline. Tissues were processed by routine methods and 5  $\mu$ m thickness

sections were cut and stained by Haematoxylin and Eosin.

**Humoral immune response**—Blood was collected from jugular vein at weekly intervals from chicks of all groups. Serum was separated and stored at 4°C for studying haemagglutination inhibition (HI) titre against NCDV. Haemagglutination inhibition test was carried out as per the standard procedure<sup>9</sup> at weekly interval against NCDV. Haemagglutination titre was expressed in log<sub>2</sub> value.

**Cell mediated immune response**—A delayed type hypersensitivity response against 2'-4' dinitrochlorobenzene was observed on 30<sup>th</sup> and 45<sup>th</sup> day of age in all the groups to study cell mediated immune response<sup>10</sup>.

**Statistical analysis**—The data were analyzed using ANOVA by Completely Randomized Design on the day of observation<sup>11</sup>.

## Results

The mean body weights of chicks from different treatment groups showed numerically lower body weight in group A at the end of experiment and were higher in group F. There was no statistical difference between mean body weights of chicks of different treatment groups. The cumulative feed conversion ratio of chicks was 2.22, 2.14, 2.16, 2.11, 1.98 and 2.06 for different treatment groups A, C, D, E, F and G, respectively.

The birds from un-medicated and challenge groups showed mild diarrhoea on 4<sup>th</sup> day post challenge. Droppings were blood tinged on 6<sup>th</sup> day post challenge indicating establishment of coccidial infection. Birds from group D and G receiving coxynil and challenged respectively also showed mild diarrhoea on 4<sup>th</sup> and 6<sup>th</sup> day post challenge. The birds from group E and F however, did not show any change in consistency of faeces confirming coccidiostatic action of coxynil. Oocysts per gram (OPG) of faeces of birds from different treatment groups are presented in Table 1.

Table 1—Oocyst per gram (thousands) of faeces of bird from different treatment groups

Age in day	Group					
	A	C	D	E	F	G
30 <sup>th</sup> day	2.48 <sup>B</sup> ±0.26	2.51 <sup>B</sup> ±0.24	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0
4 <sup>th</sup> Post Infection	2.76 <sup>B</sup> ±0.25	2.66 <sup>B</sup> ±0.21	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0
6 <sup>th</sup> Post Infection	2.71 <sup>B</sup> ±0.35	2.90 <sup>B</sup> ±0.31	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0
7 <sup>th</sup> Post Infection	8.36 <sup>D</sup> ±1.20	3.03 <sup>B</sup> ±0.35	0.51 <sup>A</sup> ±0.13	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0
8 <sup>th</sup> Post Infection	77.60 <sup>E</sup> ±3.97	4.08 <sup>B</sup> ±0.56	8.41 <sup>C</sup> ±0.86	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.16±0.01
10 <sup>th</sup> Post Infection	7.10 <sup>D</sup> ±0.90	5.86 <sup>C</sup> ±0.94	2.44 <sup>B</sup> ±0.34	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.05 <sup>B</sup> ±0.02

*P*<0.01 Figures with common superscript did not differ significantly within a row.

Average lesion score recorded in experimental chicks is presented in Table 2. The results indicated that the unchallenged birds receiving coxynil @ 200 mg and 300 mg/kg of feed (Group E and F respectively) did not show any lesion at the various periods of observation. However, in the control chicks (Group C) ceecal congestion was observed and average lesion score was to the tune of 1.12 per bird. In group D and G, on 30<sup>th</sup> day of age no lesion was recorded.

Average HI titer of experimental chicks is presented in Table 3. The overall highest mean HI titre against NCDV was observed in group F followed by E and G. Whereas, the least titre was recorded in group A and C, respectively. In chicks maintained on feed supplemented with coxynil at the rate 300 and 200 mg/kg of feed, HI titre was higher in comparison to control chicks (Group C) at various periods of observation. Among the experimental birds challenged with oocyst significantly lower HI antibody titre was observed in coxynil untreated (Group A).

In chicks treated with coxynil @ 200 mg/kg of feed (Group D) and 300 mg/kg of feed (Group G), HI antibody titre were significantly higher in contrast to

chicks maintained on feed without coxynil (Group A). Amongst coxynil supplemented groups significantly higher levels of antibody was recorded in chicks supplemented with higher dietary levels of coxynil in comparison to lower level.

Mean increase in skin thickness in the chicks of various experimental groups is studied. Though differences are non-significant statistically, the birds receiving coxynil showed dose dependent numerically higher increase in skin thickness.

Histopathological observations of ceecal section from groups D and G showed vacuolar degeneration in mucosal epithelium of caeca. However, in groups A different developmental stages of schizogony were observed (Fig. 1). The birds from groups A and C showed all developing stages of schizont indicating progression of life cycle. The group G wherein chicks were treated revealed developing stages of merozoites, however, typical second schizogony was not observed in this group (Fig. 2).

### Discussion

Before and after challenge with *E. tenella* and treatment with coxynil the growth and performance, clinical symptoms, oocyst excreted per gram of

Table 2—Ceecal lesion score of birds from different treatment groups

Age in day	Group					
	A	C	D	E	F	G
30 <sup>th</sup> day	1.16 <sup>B</sup> ±0.13	1.16 <sup>B</sup> ±0.16	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0
4 <sup>th</sup> Post Infection	1.33 <sup>C</sup> ±0.21	1.16 <sup>C</sup> ±0.16	0.50 <sup>B</sup> ±0.22	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.165 <sup>A</sup> ±0.16
6 <sup>th</sup> Post Infection	2.83 <sup>E</sup> ±0.30	1.16 <sup>C</sup> ±0.16	1.33 <sup>C</sup> ±0.21	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.66 <sup>B</sup> ±0.21
7 <sup>th</sup> Post Infection	2.60 <sup>D</sup> ±0.16	1.33 <sup>C</sup> ±0.21	0.66 <sup>B</sup> ±0.21	0.00 <sup>A</sup> ±0.0	0.00 <sup>A</sup> ±0.0	0.50 <sup>B</sup> ±0.22

*P*<0.01 Figures with common superscript did not differ significantly within a row.

Table 3—Mean HI titre (log<sub>2</sub>) against NCDV in the birds from different treatment groups

Age in day	Group					
	A	C	D	E	F	G
14 <sup>th</sup>	2.83±0.16	2.66±0.21	3.00±0.25	2.83±1.16	3.16±0.16	2.83±0.16
21 <sup>st</sup>	3.83 <sup>A</sup> ±0.30	3.83 <sup>A</sup> ±0.30	5.16 <sup>B</sup> ±0.30	5.00 <sup>B</sup> ±0.25	5.83 <sup>C</sup> ±0.40	5.83 <sup>C</sup> ±0.30
28 <sup>th</sup>	2.33 <sup>AB</sup> ±0.42	2.16 <sup>AB</sup> ±0.47	2.33 <sup>A</sup> ±0.42	2.50 <sup>B</sup> ±0.22	2.50 <sup>B</sup> ±0.42	2.83 <sup>C</sup> ±0.16
35 <sup>th</sup>	4.50 <sup>A</sup> ±0.22	5.33 <sup>B</sup> ±0.21	5.33 <sup>B</sup> ±0.21	7.00 <sup>D</sup> ±0.25	7.33 <sup>D</sup> ±0.33	6.50 <sup>C</sup> ±0.42
42 <sup>nd</sup>	3.16 <sup>A</sup> ±0.30	4.66 <sup>B</sup> ±0.33	4.83 <sup>B</sup> ±0.13	6.41 <sup>D</sup> ±0.36	6.16 <sup>D</sup> ±0.30	5.50 <sup>C</sup> ±0.34

*P*<0.01 Figures with common superscript did not differ significantly within a row.

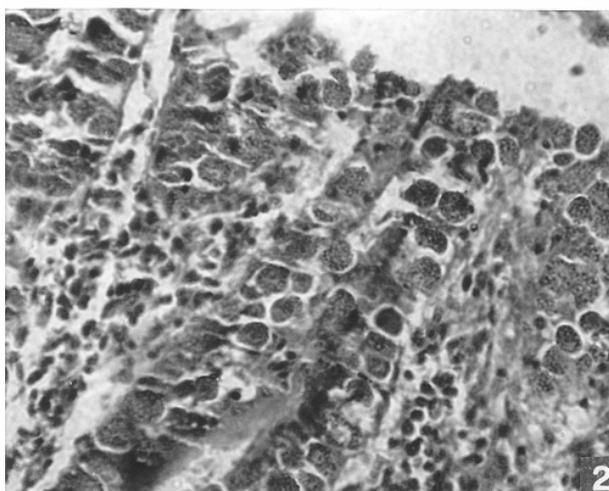
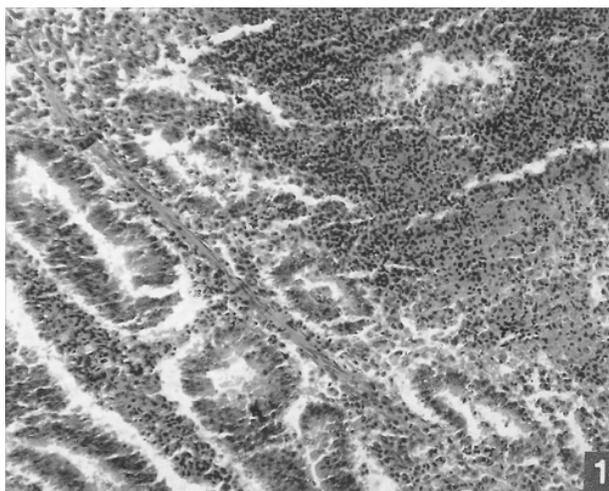


Fig. 1—Microphotograph showing haemorrhages in the intestinal mucosa due to development of various stages of *E. tenella* in group A (H & E, 200 $\times$ )

Fig. 2— Microphotograph showing arrest of developmental stages of *E. tenella* in the intestinal epithelium from group G (H & E, 400 $\times$ )

faeces, lesion score, humoral immune response, cell mediated immune response and histopathological observations were investigated. Coccidial challenge was seen to adversely affect body weight and feed conversion ratio of chicks. Treatment with coxynil reduced the effect of coccidiosis on body weight and feed conversion ratio of chicks. After challenge, blood tinged diarrhoea was noticed in un-supplemented groups while in supplemented group only mild diarrhoea was noticed. This indicates anticoccidial activity of coxynil against *E. tenella* in broilers. Reduction in bloody diarrhoea and better performance of chicks by herbal extracts after *E. tenella* challenge

were also noticed by Youn and Noh<sup>5</sup>. The estimation of oocysts per gram of faeces, lesion score of birds revealed significant reduction of these parameters due to coxynil treatment indicating effect on reproductive potential of *E. tenella*. Similar reduction in oocyst output and lesion score of birds challenged with *E. tenella* by herbal extracts of *Sophora flavescens*, *P. koreana* Nakai, *Ulmus macrocappa*, *Artemisia asiatica* Nakai, *Gleditisa japonica* Miquel and *Artemisia annua* Linn was reported by Youn and Noh<sup>5</sup>. Oh *et al.*<sup>12,13</sup> observed that extracts of *Artemisia annua* Linn were shown to improve body weight gain, lesion score and feed conversion ratio in chicks infected with *E. tenella*. Akthar and Riffat<sup>14</sup> also reported anticoccidial, activities of fruit of *Melia azedarach* Linn. in naturally infected chickens.

Histologically, birds from challenge and un-supplemented with coxynil showed developmental stages of coccidia. However, in coxynil treated groups the typical second generation schizogony was lacking in the sections indicating the interference of herbal preparation on schizogony arresting the coccidial life cycle. The effect was seen more pronounced in higher dose of 300 mg/kg of feed. The humoral immune response was reduced in chicks challenged with *E. tenella*. The supplementation of coxynil has reduced the effect of coccidia on humoral immune response of chicks; however, there was no significant difference on cell mediated immune response of experimental chicks.

Results of present study indicate anticoccidial action of coxynil, however, further study is needed to know its exact mechanism of action on different developmental stages of coccidia.

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