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ORIGINAL ARTICLE

Comparative study of neem leaf (*Azadirachta indica*) suspension and toltrazuril against coccidiosis in Sonali chicken

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Abstract

Background: Coccidiosis is recognized as the parasitic disease with the greatest economic impact on poultry industries worldwide. Sonali chicken are commonly affected by coccidiosis in Bangladesh. Recently, the concerns about possible drug resistance have aroused great caution in the usage of drug in the animal industry. As an alternative herbal extracts are used and Neem leaves (Azadirachta indica) have anti-coccidial property. This study was conducted to evaluate the comparative efficacy of Neem leaves suspension and toltrazuril on coccidial load, blood constituents, growth performance of Sonali chicken infected with *Eimeria tenella*.

Methods: The study was conducted in the laboratory and experimental shed under the department of physiology and pharmacology, HSTU, Dinajpur during, 2nd January to 3rd February, 2019. Eighty (80) Sonali chicken of seven days old were randomly divided into four groups named T_0, T_1 T_2 and T_3 and each group contained 20 birds. All groups were supplied *E. tenella* orally except T_0 group and after 3 days T_2 group was treated with Neem leaves suspension (5%) for 15 days and T_3 groups was treated with toltrazuril (topzuril ®) solution (1 ml/lit) for 2 days.

Results: Results showed that protozoal load decreased significantly ($P<0.01$) in T_2 & T_3 groups whereas increased significantly ($P<0.01$) in T_0 and T_1 group. The total erythrocyte count and hemoglobin in different treatment groups were similar and the differences were non-significant ($P>0.05$) statistically except total leukocyte count (TLC). The body weight of T_0, T_1, T_2 and T_3 group at day 30 were 432.65, 469.81, 548.81, 489.22 gm respectively which is statistically significant ($P<0.01$) and highest body weight gain was recorded from Neem leaves suspension treated group.

Conclusion: Neem leaves suspension have significant effects on protozoal load, body weight and no significant effect on haematological parameters except TLC.

Keywords: Coccidiosis; neem leaf; toltrazuril; Sonali chicken.

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Introduction

Bangladesh is an agriculture based tropical country where more than 80% of the country's 160 million people are living in rural areas and highly dependent on agriculture. Poultry farming is one of the major activities in rural areas as it provides immense employment opportunities to the local communities especially for youth &women that helps in poverty reduction, ensures food security and improves the nutritional status of the people. Poultry industry can produce very high quality proteins for human nutrition as well as a source of income for the community in many countries, therefore poultry production plays very important role in economic development of any country (Tarhyel *et al*., 2012). Poultry is a promising sector in Bangladesh which is increasing day by day. Poultry production is hindered by many problems among which various diseases namely bacterial, viral and parasitic infections are the most important (Ojok, 1993).

The northern part of Bangladesh is considered as a poultry hub especially of Sonali chicken, which is used as meat purpose. Sonali chickens are well adapted in the environmental conditions of Bangladesh, as it requires less care and attention as compared to other breeds. It is also easier for women to rear (Saleque and Saha, 2013). In spite of many positive reasons behind rearing of Sonali chickens, there are some causes that hamper in optimum production of Sonali chicken by smallholder households in Bangladesh (Biswas *et al*. 2006). Poultry diseases are one of the major constraints for developing poultry industry in Bangladesh (Islam and Samad, 2004).

Avian coccidiosis is a parasitic disease of intestinal tract caused by single cell protozoan parasite belonging to genus Eimeria. It causes massive destruction of the epithelial cells, which leads to bloody diarrhea, reduced weight gain and temporary reduction in egg production (Dalloul and Lillehoj, 2005; Razzaq *et al*., 2011).

Coccidiosis has been considered as a very harmful disease affecting growth and performance of poultry (Lin *et al*., 2006; Mujahid *et al*., 2007; Bachaya *et al*., 2012). Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial poultry systems.

In Bangladesh, a number of drugs are available for the treatment and prevention of chicken coccidiosis. Among these sulphaclozine sodium are used extensively throughout the country. In addition, ionophorous antibiotic i.e., maduramycin and lasalocid and some other drugs like amprolium, toltrazuril are also used in the field. It has been found that the efficacy of various anti-coccdial drugs varies considerably against coccidisis (Lee, 1978).

Toltrazuril is a symmetrical triazinetrione compound and 2.5% oral solution has been shown to be effective against all species of Eimeria infecting chickens (Mehlhorn *et al*., 1988). It is active against all intracellular developmental stages.

Chemoprophylaxis with Toltrazuril enhances immunity against poultry coccidiosis (Chapman, 1987). It has been proved that therapeutic medication with Toltrazuril protects the birds from clinical coccidiosis (Ramadan *et al*., 1997).

Drug resistance is very much alarming matter nowadays. New drug resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. So, we need to quickly replace of anticoccidial with other options. These new options should be inexpensive and available in everywhere as well as healthy for human society. It is thought that medicinal plants are a good alternative for this purpose. Since ancient times medicinal plants play important role in health management of traditional poultry production (Eevuri and Putturu, 2013). In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries.

Secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus it is anticipated that phytochemicals with adequate anticoccidial efficacy will be used

for the treatment of the protozoal infections . Also, in contrast to anticoccidial, most active components of medicinal plants are readily absorbed in intestine and have short half life.

Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of Neem.

The tender leaves along with piper nigrum Linn are found to be effective in intestinal helminthiasis. Since Neem leaf increases immunity against some common diseases which assists to reduce mortality , it is likely that the growth pattern of sonali chicks will proceed uninterrupted.

So, this study was undertaken to evaluate the comparative efficacy of Neem leaves suspension and toltrazuril on coccidial load, blood constituents, growth performance of sonali chicken infected with *Eimeria tenella.*

Materials and Method

The experiment was conducted at the experimental shed under the department of Physiology and Pharmacology, for a period of 65 days from 2nd January to 3rd February, 2018.

Preparation of house

First the room as well as the wire cages were washed by sweeping with tap water using hose pipe connected with the tap. The room was properly ventilated and disinfected with a phenolic disinfectant and allowed to dry leaving the room unused with the electric fan and the bulb switched on overnight. All the utensils required for the experiment such as feeder, water pot, beakers, pestle and mortar, syringe, needle etc. were set in the experimental shed.

Grouping of birds

Eighty (80) Sonali chicken were used to evaluate the dietary effect of Neem leaf suspensionon *Eimeria tenella* infection, growth performance. The Sonali chicken were assigned into four groups containing 20 birds in each groups. 20 birds kept in each group were considered as an experimental unit. Sonali chicken were randomly distributed in every groups.

Group T_0: The Sonalis were fed normal diet and given water ad-libitum and their body weight was recorded at every 15 days interval. Body weights, blood parameter and *Eimeria tenella* protozoal load were measured at the times when that of other groups were measured. This group was served as "Negative control" group.

Group T_1: The Sonali chicken were supplied with protozoa after acclimatization to induce *Eimeria tenella* infection in this group. No antiprotozoal treatment was given against *Eimeria tenella* in T_1 group. Adequate feed and drinking water was given. This group served as "Positive control" group.

Group T_2: The Sonali chicken were supplied with *Eimeria tenella* protozoa after acclimatization to induce *Eimeria tenella* infection as like as T_1 group. This group was left untreated for 24 hours to establish *Eimeria tenella* infection. After 24 hours, this group was treated with Neem leaves suspension as herbal antiprotozoal agent at a dose rate of 0.25 ml per 100 ml of drinking water. This group served as "Neem leaf suspension" group.

Group T_3: After acclimatization Sonali chicken of this group were supplied with *Eimeria tenella* protozoa as like as T_1& T_2 group. This group was left untreated for 24 hours to establish *Eimeria tenella* infection. After 24 hours, this group was treated with antiprotozoal drug (Topzuril liquid @ 1 ml per 2lit water). This group served as "Topzuril liquid" group.

Collection of feed

Polli mash commercial feed were collected from Griholokkhi Poultry Feed, Kalitola, Dinajpur from a reputed Sonali feed exporter. Mash and water were provided *ad-libitum* during the whole experimental period.

The ingredients that were used for the composition of mash feed are described in Table 1.

Table 1. Feed formulation

Name of ingredients	Percentage of ingredients	Required Amount of ingredients (kg)	Rate of ingredients/kg (TK)	Cost of ration (TK)
Maize	59	4.956	23	113.988
Soybean meal	22	1.848	58	107.184
Protein conc.	5	0.42	80	33.60
Rice polish	3	0.252	20	5.04
Saw dust	8	0.672	05	3.36
Oil	2	0.168	110	18.48
Lime stone	1	0.084	08	0.672
Enzyme	Trace amount			
Total	100	8.4		282.324

Metabolizable Energy (Kcal/100kg) was 3009.11 and Crude Protein was 18.95%
Feed cost per kg 33.61 Taka

Acclimatization of Sonali chicken

Immediately after reaching the destination the sonali were shifted to wire cages. They were fed with Griholokkhi Poultry Feed, Kalitola, Dinajpur and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for first three days to overcome the transportation stress. Sonalis were allowed to acclimatize in their new environment for 13 days before the commencement of the experiment.

Lighting

During the whole experimental period, all Sonali chicken were exposed to a 16 hours continuous photoperiod (natural light plus artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

Collection and processing of neem leaves

Neem leaves were collected from the HSTU, Dinajpur. Young Neem leaves were collected and washed with fresh water. Before chopping it into small pieces, it was soaked with cotton for removing the adhesive water. Then the leaf was chopped into small pieces and was mashed with the help of pestle and mortar. Leaf juice was collected by squeezing mashed leaf. Then it had been produced 0.25% of suspension of grinded Neem leaf with distilled water.

Collection, preservation and administration of toltrazuril

Topzuril liquid was collected from Square pharmaceuticals Ltd. It was preserved in a dry place at room temperature and was administered at a dose rate of 1ml per 2 litre of drinking water for 3-7 days.

Recording of body weight

The effect of the Neem leaves extract and toltrazuril on body weight was recorded before and during administration of drugs.

Chickens under trial and control groups were weighed with Electric weighing machine. The weight of each chicken was taken before feeding in the morning, in noon and afternoon. The average of these three weights was calculated and recorded.

Collection and preparation of test organism

The test organism (*Eimeria tenella*) was collected from the Gopalgonj local farm area, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

Collection and examination of feces for parasitic oocyst investigation

Feces were examined by two different types of qualitative tests; namely direct smear and flotation techniques were used to examine the fecal samples to identify the morphological features of oocysts (Hendrin and Robinson, 2006; Soulsby, 1982).

Hematological parameters

Blood samples were collected from the wing vein of chicken of both control and treated groups at pre-feeding and during feeding (28 days) period at 7 days interval to study the effect of the Neem

leaves extract and Toltrazuril. The following parameters were observed:

Total Erythrocyte Count (TEC)
Hemoglobin Estimation (Hb)
Packed Cell Volume (PCV)
Erythrocyte Sedimentation Rate (ESR)
Total Leukocyte Count (TLC)

Total Erythrocytes Count (TEC), Erythroctes Sedimentation Rate (ESR), Packed Cell Volume (PCV) and Total Leukocyte Count (TLC) were performed as per methods described by Schalm (1967). Hemoglobin estimation was perfomed as per method described by Coffin (1955).

Statistical analysis

The data were analyzed statistically between control and treated groups of chicken by using SPSS version 22 and Microsoft Excel. Statistically significant differences between group means were determined by analysis of variance (ANOVA).

Results and Discussion

Anticoccidial effect of Neem leaves suspension and toltrazuril liquid

Coccidial load in feces of *E. tenella* infected Sonali chicken were presented in Table 1. This study showed that, coccidial load in feces varied significantly among the treated groups. Coccidial load significantly ($P<0.01$) increased in T_0 (negative control) and T_1 (positive control) group. Coccidial load significantly ($P<0.01$) decreased in T_2 group supplied with 1% Neem leaves suspension and toltrazuril liquid suspension. Karim *et al.* (2016) also observed similar observation.

Total Erythrocyte Count

Total Erythrocyte Count (TEC) is presented in Table 2. The values of TEC in all treated groups and control group were similar and within the normal range at15th and 30th day of experimental period.

Table 2. Estimation of oocyst per gram (OPG) during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
1 day	0.00 ± 0.00^a	319.31 ± 1.67^b	412.48 ± 0.92^c	431.48 ± 1.25^d	*
15 day	0.00 ± 0.00^a	390.89 ± 1.81^c	190.36 ± 1.56^b	0.00 ± 0.00^a	*
30 day	0.00 ± 0.00^a	387.28 ± 1.62^c	55.68 ± 1.09^b	0.00 ± 0.00^a	*

Values are expressed as mean ± standard error of means.
a, b, c Means in each row with different superscript are significantly different at (0.05).
* = Significant at 1% level of significance.
T_0 = Control (Basal diet),
T_1 = (Basal diet plus *E. tenella*.),
T_2 = (Basal diet plus *E. tenella.* plus 0.5% neem leaves suspension*),*
T_3 = (Basal diet plus *E. tenella.* plus toltrazuril @ 1 ml per 1 litre drinking water)

Table 3. TEC in different groups of Sonali chicken

Days	T_0	T_1	T_2	T_3	Significant level
15 day	2.97±.18	3.19±.21	3.14±.16	3.28±.06	NS
30 day	3.15±.22	3.47±.09	3.35±.07	3.34±.06	NS

NS= Non significant
Values are expressed as mean ± standard error of means. Means within column and between column are not statistically significant ($P>0.05$). NS=Not significant.

Total Leukocyte count (TLC)

The values of TLC are presented in Table 4. The values of TLC of T_0 (Basal diet) and T_3 (*E. tenella* plus topzuril liquid) group were similar. But in case of T_1 (Basal diet plus *E. tenella*) and T_2 (*E. tenella* plus Neem leaves suspension) groups the values differed.

Hemoglobin

Hemoglobin (Hb) is presented in Table 5. The values in all treated groups and control group were similar and the values were within normal range.

Table 4. TLC in different groups of Sonali chicken

Days	T_0	T_1	T_2	T_3	Significant level
15 day	$6.39\pm.05^b$	$6.27\pm.05^d$	$6.83\pm.14^c$	$6.58\pm.21^a$	*
30 day	$6.35\pm.07^d$	$6.09\pm.28^b$	$6.75\pm.12^a$	$6.66\pm.07^c$	*

Values are expressed as mean ± standard error of means.
a, b, c Means in each row with different superscript are significantly different at (0.05).
* = Significant at 1% level of significance.
T_0 = Control (Basal diet),
T_1 = (Basal diet plus *E. tenella*.),
T_2 = (Basal diet plus *E. tenella.* plus 0.5% neem leaves suspension*),*
T_3 = (Basal diet plus *E. tenella.* plus toltrazuril @ 1 ml per 1 litre drinking water)

Table 5. Estimation of Hemoglobin during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
15 day	7.67±.44	7.54±.16	7.90±.02	8.04±.06	NS
30 day	8.05±.05	7.53±.25	8.25±.07	8.41±.03	NS

NS= Non significant
Values are expressed as mean ± standard error of means. Means within column and between column are not statistically significant (P>0.05). NS=Not significant.

Body Weight

Table 6 indicated that there were a significant (P<0.01) increase in body weight in T_2 (Neem leaf suspension) and T_3 (topzuril liquid) group than the T_0 (Basal diet) and T_1 (*E. tenella*). Birds treated with Neem leaves suspension (T_2 group) had the highest body weight gain compared to other groups. Lakkundi *et al.* (2002) also observed the same effect of toltrazuril and amprolium on body weight and feed efficiency of broiler chicken experimentally infected with *Eimeria tenella*.

Table 6. Estimation of Body Weight during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
1 day	180.84±.51	182.01±0.93	152.43±0.94	182.41±0.87	NS
7 day	$223.47\pm.05^d$	210.54 ± 1.43^a	250.31 ± 0.71^b	261.92 ± 1.42^c	*
14 day	253.50 ± 1.18^b	264.23 ± 0.84^c	343.72 ± 1.55^d	314.11±0.43a	*
21 day	375.70 ± 0.4^d	351.33 ± 1.49^c	497.37 ± 1.89^b	362.42 ± 1.64^a	*
30 day	432.65 ± 0.71^b	469.81 ± 1.23^c	548.86 ± 1.29^d	489.22 ± 1.33^a	*

Values are expressed as mean ± standard error of means.
a, b, c Means in each row with different superscript are significantly different at (0.05).
* = Significant at 1% level of significance,
T_0 = Control (Basal diet) ,
T_1 = (Basal diet plus *E. tenella*.) ,
T_2 = (Basal diet plus *E. tenella.* plus 0.5% neem leaves suspension*),*
T_3 = (Basal diet plus *E. tenella.* plus toltrazuril @ 1 ml per 1 litre drinking water)

Conclusions

Nneem leaves (*Azadirachta indica*) suspension and toltrazural have anticoccidial effect and effects on growth performance of sonali chicken with *E. tenella.* It can be used for the control of coccidiosis and as a good growth promoter, leukocyte enhancer in Sonali chicken. It may be stated that the neem leaf (*Azadirachta indica*) may provide a new therapeutic avenue against *E. tenella* because of its availability in our country and effectiveness.

Conflict of interest

None to declare.

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