REPORT

In vivo anticoccidial effects of *Azadirachta indica* and *Carica papaya* L. with salinomycin drug as a dietary feed supplement in broiler chicks

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Abstract: A total of thirty suspected broiler chicks were screened for coccidiosis, of them 25 chicks were found to be infected with coccidiosis viz. *Eimeria tenella* (15) *Eimeria maxima* (5) *Eimeria necatrix* (6) and *Eimeria mitis* (4). The anticoccidial efficacy of *Azadirachta indica* and *Carica papaya* with Salinomycin as a dietary feed supplement on the representative *E. tenella* ($25X10^3$ oocyst) infection challenged in broiler chicks was studied in six groups for the period of six weeks. *A. indica* and *C. papaya* leaves were administered in powder form at the concentration of 0.1% and 0.2% respectively. The Oocysts per gram (OPG) count were observed on 8^{th}, 9^{th}, 10^{th}, 11^{th} day of post inoculation (DPI). The challenged experimental chicks revealed haemorrhage, thickening of mucosa, cores of blood and ballooning of caecum. The experimental group T_5 chicks treated with *A. indica* were analyzed to possess the maximum weight gain (2.003), better feed conversion ratio (FCR) (2.32), OPG count (5.87), livability percentage (88) and the lesion score (3.33). Chi-square test analysis revealed no significant differences among the treated groups and the performance parameters. Therefore, this study concludes that plant sources used as a remedial curate for coccidiosis is a perforated growth in the commercial broiler industries.

Keywords: *Eimeria tenella*, Coccidiosis**,** Oocyst, *Azadirachta indica, Carica papaya*, Salinomycin

INTRODUCTION

Avian coccidiosis is one of the major intestinal protozoan diseases caused by the genus *Eimeria*, affecting the poultry industry (Massoud *et al*., 2010; Adnane *et al*., 2011). Chicks are commonly susceptible to species of *Eimeria* that affect the poultry industry including *Eimeria tenella*, *Eimeria maxima*, and *Eimeria acervulina*, of these *E. tenella* causing the caecal coccidiosis is highly pathogenic (Chandrakesan *et al*., 2009; Adnane *et al*., 2011). The symptoms of coccidiosis includes reduced feed conversion efficiency, weakness, drastic physiological damages, diarrhoea, weight loss and aneamia followed by death (Christaki *et al*., 2004; Muazu *et al*., 2008; Patra *et al*., 2009; Adnane *et al*., 2011). In addition, severe intestinal lesions results in fowl deaths between 5 to 20% owing to *Eimeria* colonization affect the commercial production of chicks. The incidence of coccidiosis observed in commercial poultry range from 5 to 70% leading to high rate in morbidity and mortality in the youngest birds (Adhikari *et al*., 2008; Michels *et al*., 2011; Jadhav *et al*., 2011). The continuing issue for the commercial chicks production is due to high expenses to control coccidiosis. Nowadays, the expenses for controlling the coccidiosis in the United states exceeds $90 million, the South African commercial poultry industries spends approximately US $0.02 per bird and more than $300 million worldwide every year (AL Fifi, 2007; Naidoo *et al*., 2008; Liviu *et al*., 2010).

The emergence of drug resistant strains of *Eimeria* and the regulations on using anticoccidial drugs results in searching for the substitute preventive methods. People also seek out for the animal products without any chemical drugs for betterment of livestock production with the help of botanical elements as the sustainable alternatives (Brisibe *et al*., 2008; Giannenas *et al*., 2012). The chemical therapeutic prevention of coccidiosis is either prohibited or not affordable in organic farming; therefore the control measures can be followed by using available plant resources. They have been tested for their anticoccidial dietary additives related with the metabolism of chicks (Adriana *et al*., 2008; Massoud *et al*., 2010; De Almeida *et al*., 2012).

The seeds and leaves of Neem (*Azadirachta indica*) have been used as unconventional feed supplements to control certain diseases in livestock and poultry sectors as they contain nimbin, azadiractin and nimbiodol. Neem and its compounds have been proved in controlling parasitic infestations and possess antiprotozoan, antibacterial and antifungal effects (Patrick and Mgbere, 2010; Tiwary and Pandey, 2010). The leaves of Papaya (*Carica papaya*) as

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the feed supplement was the most ingested plant due to its less bitterness and the presence of carotene. It can enhance the feed palatability and subsequently increase the chick growth performance. The antihelmintic activity of papaya latex, leaves, seeds and roots attributed to possess the proteolytic enzymes such as papain, chymopapain and lysozyme. The anti-inflammatory property of the Papaya helps in caecal epithelial cell protection and prevents the coccidial reproduction (AL Fifi, 2007).

This study mainly focuses on the medicinal plant based treatment for the coccidiosis will be the alternative method for prophylaxis and to avoid the increased resistance on the administration of anticoccidial drug for the chicks.

MATERIALS AND METHODS

Materials

Broiler chicks (*Gallus gallus domesticus*), *E. tenella oocysts*, Salinomycin drug (Health Biotech Limited, India), leaves of *Azadirachta indica* (Neem) and *Carica papaya* (Papaya), commercial feed, McMaster counting chamber and Microscope (De Winter) were used in this study.

Identification of coccidial infection in the broiler chicks

A total of 30 broilers chick's intestine suspected or infected with coccidiosis were removed and examined for the infection.

Experimental chicks

One day old 36 Broiler chicks were used in this study. They were reared under hygienic conditions to prevent infection. The birds were divided into six groups (T_1, T_2, T_3, T_4, T_5 and T_6) based on the treatments and the study began when the chicks became three weeks old. Each group possessed six birds and experimental parasitological study was carried out for six weeks (Lee *et al*., 2012).

Experimental plants

Fresh leaves of *A. indica* and *C. papaya* were collected from Athipuliyur farm, Nagapattinam (Dt), Tamil Nadu, India and dried at room temperature (George and Josephine, 2011).

Anticoccidial drug

The standard anticoccidial Salinomycin drug was supplemented with feed at 0.2% concentration for group T_3.

Methods

Experimental design

The experiment with *E. tenella* infection was carried out in six groups as T_1, T_2, T_3, T_4, T_5 and T_6. All the groups were commonly feed with 5kg of commercial feed. The experimental design have been followed as the group T_1 was infected; the group T_2 was infected and treated 5g of Salinomycin (0.1%); the group T_3 was uninfected; the group T_4 was infected, treated 10g of Papaya leaves powder (0.1%), the group T_5 was infected, treated with 10g Neem leaves powder (0.1%) and the group T_6 was infected and treated by 10g Papaya leaves powder along with 10g Neem leaves powder at the concentration of 0.2% (Gabriel *et al*., 2006).

In-vivo challenge of coccidial infection

The pure oocysts suspension of *E. tenella* was obtained from Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. About 1ml of the sporulated oocyst suspension was prepared using sterile distilled water and inoculated through oral route at a dose of $25X10^3$ per bird of the treatment group except the control group (T_3) (Orengo *et al.*, 2012)**.**

Determination of coccidial infection

Bloody Diarrhoea

The total number of bloody diarrhoea manifested by chicks was investigated during the experimental period following *E. tenella* infection. About 5g of faecal samples were collected from all the infected groups on 8^{th}, 9^{th}, 10^{th}, 11^{th} Days after post inoculation (DPI) (AL Fifi, 2007).

OPG (Oocyst per gram) count

About 5 g of the faecal samples were centrifuged and 0.1 ml of this suspension with saturated salt solution was loaded in McMaster counting chamber and the oocysts were counted. The total number of oocysts counted was multiplied by 100 to achieve the OPG (AL Fifi, 2007).

Lesion score

Intestinal contents of the all experimental chicks were carefully removed and lesion scores from 0 to 4 in ascending order of severity as described by Johnson and Reid (1970) scale were performed. Three birds from each group were sacrificed on 8^{th} DPI after *E. tenella* infection to ascertain the lesion score in the caeca. Lesion score was also noted for the dead birds (Lee *et al*., 2010; AL Fifi, 2007).

Performance parameters of infected birds

Feed consumption

The daily feed consumption (g/bird) and feed conversion ratio (FCR) were obtained by measuring the amount of feed consumed by birds in each group. The average of feed consumption was measured for each bird throughout the experimental study (Brisibe *et al*., 2008).

Weight evaluation

As an index of the physical growth and the weight of the chicks were monitored on weekly basis throughout the experimental study (Brisibe *et al*., 2008).

Mortality or livability percentage

Mortality rate in all the infected groups were noted after *E. tenella* infection on 7th DPI. The dead birds were ascertained for lesion scores (Tipu *et al*., 2002).

STATISTICAL ANALYSIS

Mean ± SE of body weight gain, cumulative feed consumption, feed conversion ratio, livability percentage, gross lesion score and their comparative evaluation were subjected to statistical analysis of one-way analysis of variance (ANOVA), complete randomized design (CRD), Kruskal Wallis and nonparametric test (Akhtar *et al*., 2012).

RESULTS

Identification of the coccidal infection in the broiler chicks

Among the 30 coccidiosis infected chicks, the lesion were found in caecum of 15 birds indicates *E. tenella* infection, anterior and mid intestine lesion indicates *E. maxima* infection in 5 chicks, mid intestine lesion indicates *E. necatrix* infection in 6 chicks and the mucous lesions indicates *E. mitis* infection in 4 chicks. As a representative study with the respect to the pathogenicity of these parasites, *E. tenella* was found to be more pathogenic. Therefore, anticoccidial herbal efficacy for *E. tenella* infection in chicks was observed in this study (fig.1 a-h).

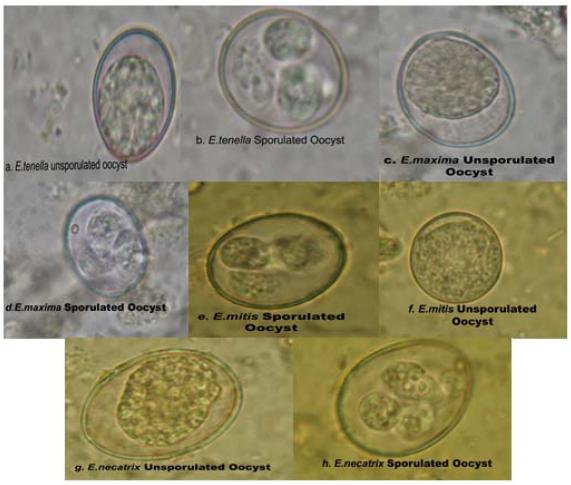

Fig. 1: Micrograph of Sporulated and Unsporulated oocyst of *Eimeria* sp

Clinical observation of the experimental coccidial infection

Blood tinges were visualized in fecal droppings in the group T_4 and T_5 on 6th DPI after *E. tenella* infection. Sickness and severe bloody diarrhoea were observed in T_4 leading to death on 8th DPI (fig. 2 a, b).

Parasitological analysis of E. tenella infected birds

Mortality was found in group T_4, T_5 and T_6 on the 8th DPI. The postmortem analysis of these dead and the sacrificed birds revealed hemorrhages (or) thickening of mucosa, cores of blood and ballooning of the caecum due to *E. tenella* infection (fig. 2 c, d).

Lesion score

The experimental chicks group such as T_4, T_5 and T_6 was susceptible with caecal congestion to which the lesion score was given as 4+, 3+ and 2+ indicating the infection rates (table 1). Lesions were not found in group T_2 and T_3. Among all the treated groups, lesion score was better in T_5 as 3.33±0.094 (table 2; fig. 2 e, f).

Fig. 2: Clinical observations and Parasitological infections of *E.tenella* challenged birds

Performance parameters of the coccidial infected birds

Mean cumulative feed consumption, feed conversion ratio (g/bird), body weight gain (kg), mean livability percentage and mean OPG of broilers in different treatment groups from 3 to 6 weeks of age were noted in this study period as the indication of performance.

Table 1: Lesion score of dead and sacrificed broiler chicks

Groups	1	2	3
T_1	++++4+Dead	+++3+	+++3+
T_2	0	0	0
T_3	0	0	0
T_4	++++4+Dead	++++4+Dead	+++3+
T_5	++++4+Dead	++++4+Dead	++2+
T_6	++++4+Dead	++2+	++2+

0 =No Lesions; +1=Mild lesions; +2=Moderate lesions; +3=Severe lesions;
+4=Extremely severe lesion (or) Death.

Feed consumption and conversion ratio

With regard to the feed intake in all the groups, the feed consumption was noted better in T_5- 4651.33 compared to other treatment groups T_2, T_4 and T_6 and the feed conversion ratio was better at the 6th week in T_5 as 2.32 compared to other treatment groups T_2, T_4 and T_6 (table 3).

Table 2: Treatment means and percentage distribution of gross lesion score for *E. tenella* on 9 DPI of 21 days period of infection

Treatment	Mean lesion score (±S.E)	Lesion score distribution (%)				
		0	1+	2+	3+	4+
T_1	3.33±0.94	0	34	0	66	34
T_2	0.333±0.47	66	0	0	0	0
T_3	0	100	0	0	0	0
T_4	3.66±0.47	0	0	0	34	66
T_5	3.33±0.094	0	0	34	0	66
T_6	3.33±0.81	0	0	0	66	34

Weight gain

The maximum weight gain of the birds at 6th week was better in T_5 group as 2.003±0.1. CRD analysis showed that the treatment groups do not differ significantly with the mean body weight gain (table 3).

Livability percentage

With the effect of the treatment to all the infected groups the livability percentage was better in T_5 as 88±0.0 compared to other infected groups T_1, T_2, T_4 and T_6 (table 4).

OPG count

On 9th DPI (6874×10^5) oocysts count was better in T_5 as 5.87 (table 5). Kruskal-Wallis test analysis revealed that there was a significant difference in the mean OPG and the DPI.

Comparative evaluation of performance parameters

Chi-square test analysis of all the performance parameters showed that there was no significant difference in the treatment groups and the evaluation parameters at 5% level of significance (table 6).

DISCUSSION

Coccidiosis is an important disease in poultry production industries and its infection leads to secondary intestinal bacterial infection thereby resulting in reduced growth (Gabriel *et al*., 2006). Chicks treated with *V. amigdalina* and *A. indica* showed significant growth performance with $3X10^4$ *E. tenella* oocysts infection when compared to the other treated groups with *C. papaya*, since the addition of *V. amigdalina* and *A. indica* leaves powder influenced the elimination of sporozoites recorded by AL Fifi (2007). In this study, chicks challenged with *E. tenella* oocysts infection showed the maximum weight gain when treated with Neem. As proportionate to this obtained results, the infected broiler chicks had a higher feed gain ratio than the control group during the post inoculation period as 2.10 vs.1.71g This performance was associated with the lower average daily gain ($-13.2\pm3.3 g d^{-1}$) and increase in feed intake ($+8.0 \pm 2.7 g\ d^{-1}$) as reported by Orengo *et al*. (2012).

Treatment of *A. indica* ground fruit with *Eimeria* sp infected chicks showed better feed intake (4185g), since *A. indica* powder is recognized for its toxicity inducing the repellent action against the parasites (Tipu *et al*., 2002). Feed conversion ratio of this study group T_5 (infected, treated with Neem (0.1%)) showed better Feed conversion ratio as 2.32 than other treated groups. Observations from Chandrasekaran *et al*. (2009) stated that the Feed conversion ratio with herbal complex (15% *Aloe vera*, 15% *Mentha arvensis*, 35% *Moringa indica*, 35% *Solanum nigram*) treated groups (1.77±0.43) was higher with $3X10^4$ sporulated oocysts of *E. tenella* infection, since the herbal treatment diminishing the damage to the intestinal cells rather than interference with multiplication of parasite.

The challenging of *E. tenella* infection, OPG count was noted better in T_5 (infected, treated with Neem (0.1%)) as 5.87 on 9th DPI than other treated groups. Kruskal-Wallis test analysis showed that there is a significant difference in the mean OPG and the DPI. AL Fifi (2007) observed that the *C. papaya* leaves treatment induced OPG reduction was 53% in the infected group, since the vitamin A supplementation shows huge reduction of oocysts. Experimental reports of Tipu *et al*. (2002) stated that the OPG count was noted better in Neem fruit ground powder, as the compound margosate influenced the breakdown of *Eimeria* life cycle.

The lesion scores of dead and sacrificed birds was observed better in T5 (infected, treated with Neem (0.1%)) as 3.33±0.094 in this study. Similarly, 5% herbal complex treated chicks with (15% *Aloe vera*, 15% *Mentha arvensis*, 35% *Moringa indica*, 35% *Solanum nigram*) showed caecal lesion grade as (++), since the herbal complex had some palliative effect against coccidiosis (Chandrakesan *et al*., 2009).

Mortality (or) livability ratio was found better in T_5 (infected, treated with Neem (0.1%)) as 88±0.00 when compared to other groups. Mortality studies of herbally treated chicks by Massoud *et al*. (2010) revealed the mortality rate reached only 10% in the infected groups treated with Myrrh as 3.33% than the other treated groups, since the usage of Myrrh reducing *Eimeria* oocysts.

Table 3: The effect of the plant powders on all the groups and their Mean Cumulative Feed Consumption, Feed Conversion Ratio (g/bird) and weight gain in Broilers on the 6 week of age

Treatment Groups	Mean Cumulative Feed Consumption in Broilers	Mean Cumulative Feed Conversion Ratio in Broilers	Weight gain
T_1	4583.00	2.45	1.854±0.04
T_2	4709.33	2.22	2.118±0.10
T_3	4775.33	2.19	2.173±0.006
T_4	4533.67	2.34	1.897±1.2
T_5	4651.33	2.32	2.003±0.1
T_6	4639.33	2.63	1.763±0.08

Table 4: Mortality determination and the Mean (± S.E.) livability percentage of broilers in the treatment groups from 1 to 6 weeks of age

Treatment groups	Livability percentage from 1 to 3-Weeks	Livability percentage from 4 to 6-Weeks	Livability percentage from 1 to 6-Weeks
T_1	100± 0.0	100± 0.0	100± 0.0
T_2	100±0.0	100±0.0	100± 0.0
T_3	100± 0.0	100± 0.0	100± 0.0
T_{4-}	100± 0.0	88 ± 0.0	88 ± 0.0
T_5	100± 0.0	88 ± 0.0	88 ± 0.0
T_6	100± 0.0	94 ± 0.0	94 ± 0.0

Table 5: Oocyst depletion from 8 t to 11 days post inoculation (DPI) and mean oocyst per gram of faeces (OPG) of broilers challenged with $25x10^3$ *E. tenella* oocysts in the treatment groups

Treatment groups	Oocysts depletion			
	8^{th} DPI	9^{th} DPI	10^{th} DPI	11^{th} DPI
T_1	0.342	5.82	1.47	0.514
T_2	0.003	3.59	1.7	0.453
T_3	0	0	0	0
T_4	1.286	7.23	4.349	2.13
T_{5-}	0.89	5.87	4.113	2.29
T_6	2.11	6.1	3.87	3.243

*Significant difference in the mean OPG and the DPI at 5% level of Significance

Table 6: Comparative evaluation of performance parameters among all the treatment groups

Treatment groups	Mean lesion score (± S.E) Caeca	Faecal oocyst output on 9^{th} DPI (Nx 10^5)	Mean body weight gain in 6^{th} week (kg)	Mean cumulative feed consumption in 6^{th} week (g)	Mean feed conversion ratio in 6^{th} week	Mean ivability in 6^{th} week
T_1	3.33±0.94	5.82	1.854±0.04	4583.0	2.45	100±0.0
T_2	0.333±0.47	3.59	2.118±0.10	4709.33	2.22	100±0.0
T_3	0	0	2.173±0.06	4775.33	2.19	100±0.0
T_4	3.66±0.47	7.23	1.897±1.2	4533.67	2.34	88±0.0
T_5	3.33±0.094	5.87	2.003±0.1	4651.33	2.32	88±0.0
T_6	3.33±0.81	6.1	1.763±0.08	4639.33	2.63	94±0.0

*No significant differences among the treatment groups and the evaluation parameters at 5% level of significance

Avian coccidiosis is accounted as a drastic disease in poultry production leading to disruption in intestines. Current antimicrobial agents or coccidiostats incorporated as a medicated remedial way in curing, although the consequences of resistant nature to these coccidiostat customed the mode of using herbal sources in the diets (Michels *et al*., 2011). The drugs such as antibiotics or chemicals employed for the treatment of birds, their sheds and yards possess hazards due to occurrence of the drug residues in eggs and in the meat. These chemical residues may persist for some time in the birds, thereby it requires holding period before their eggs or meat are free of

residue and safe for human consumption (Janmaat and Morton, 2010).

CONCLUSION

As per the parasitological protocols, *Eimeria* sp could not be cultured *in vitro*, however the artificially induced *in-vivo* infection helps to evaluate the potentiality of the disease. Equipping systematic drugs on the medication could enhance some undesirable effects that affect the bird's growth. Subsequently, as an alternative the medication of herbal sources were analyzed for anticoccidial effects to be a boon for the poultry sectors. This study also emphasized the efficacy of these herbal sources in apprehending the coccidial infection diminishing activity of chymopapain, papain, lysozyme of *C. papaya* and azadirachtin of *A. indica*. Accordingly, the reduced mortality and better growth found to be assisting the poultry industries to elevate their economic value.

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