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Research Article

EVALUATION OF POLYETHER IONOPHORES AGAINST COCCIDIOSIS IN BROILER CHICKEN

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ABSTRACT

The efficacy of three polyether ionophores viz. salinomycin, monensin and maduramicin was evaluated in poultry. Day-old chicks (n=350) were randomly divided into seven groups of 50 chicks each. The drugs were administered as feed premix from day one onwards. The chicks at 14-day of age were initially infected with 20,000 sporulated viable oocysts of *Eimeria tenella* and challenged with 40,000 sporulated oocysts of the same batch at 28th day of age. The parameters taken for evaluation of the efficacy of the drugs were mortality rate, weight gain, lesion scores, oocysts per gram of faeces and faecal score during post infection as well as post challenge period. The results revealed that the anticoccidial effect of all the three drugs viz. salinomycin, monensin and maduramicin @ 60, 100 and 5 mg/kg of feed, respectively was almost similar, however, the performance of maduramicin was better in comparison to salinomycin and monensin. It could be concluded that all the three coccidiostats, viz., salinomycin, monensin and maduramicin were equally effective in controlling caecal coccidiosis in poultry birds. However, maduramicin proved to be slightly better in performance and cost effective as it gave better results in checking mortality and development of lesions at comparatively smaller dose rate.

Key words: Ionophores, coccidiosis, *Eimeria* spp., poultry

Coccidiosis is a major parasitic disease of poultry having the greatest economic impact worldwide due to production losses (mortality, reduction in growth rate and feed conversion efficiency) and costs for treatment (Shirley *et al*., 2005). There are several *Eimeria* species causing avian coccidiosis viz. *Eimeria tenella*, *E. necatrix*, *E. brunetti*, *E. praecox*, *E. acervulina*, *E. mitis* and *E. maxima* (Sharman *et al*., 2010). Each species has a particular predilection site in the chicken digestive tract. The broad spectrum anticoccidial drugs were introduced in the 1960s and 1970s. On the basis of specific mode of action they can be classified as either synthetic anticoccidials that are produced by chemical synthesis (often referred to as 'chemicals') or ionophorous antibiotics (ionophores) that are produced by fermentation. About 80 percent of the world broiler population is currently medicated with the ionophores (Ryle, 1981). The ionophores act at ionic levels through formation of complexes with sodium and potassium, increasing the cell permeability and inhibiting the mitochondrial functions of the parasite. Ionophores reduce the lesion advancement, mortality and escalate gain in body weight (McDougald, 1981). The present communication reports the comparative efficacy of different ionophores against *E. tenella* in poultry under tropical environment.

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MATERIALS AND METHODS

Extraction and Sporulation of Oocysts: *E. tenella* oocysts, collected from caeca of dead chickens brought for post-mortem examination, were cultured in Petri dishes containing 2.5% potassium dichromate as described by Richardson and Kendall (1963). After the completion of sporulation the suspension was centrifuged at 1500 rpm. Sediment containing sporulated oocysts was washed six times with distilled water in order to remove the effect of potassium dichromate. After washing sporulated oocysts were concentrated by salt floatation technique and washed four times with distilled water and finally preserved at 4ºC for their further use.

Experimental Design: An experiment was performed on 350, day-old broiler chicks brought from Central Poultry Breeding Farm, Patna. The chicks were reared in a well cleaned shed under standard hygienic conditions at a poultry farm of the College. The chicks were randomly divided into seven groups (groups A, B, C, D, E, F and G) with 50 chicks in each group. All chicks

were vaccinated against Newcastle disease and infectious bursal disease using commercially available vaccines. Feed and water were provided *ad lib* to all chicks. The chicks were given salinomycin @ 60 mg/kg feed in groups A and B, monensin @ 100mg/kg feed in groups C and D and maduramicin @ 5 mg/kg feed in groups E and F, from day one. All the birds of groups A, C and E were infected with 20,000 sporulated oocysts of *E. tenella* on day 14, whereas the birds of groups B, D and F were maintained as non-infected medicated control. The group G was maintained as non-medicated infected control. Ten birds from each group were sacrificed on 7th day post infection (DPI) and 14th DPI for post mortem examination and sample collection. The remaining birds of all the groups were challenged with 40,000 sporulated viable oocysts on 14th DPI. Ionophores were withdrawn two days prior to the challenge infection. On 7th day after challenge all the surviving birds were sacrificed.

The experiment was carried out for five weeks. Ten out of 50 chicks from each group were randomly chosen to record body weight at weekly intervals. Clinical symptoms were observed after inoculation of infection. Faecal sample of the birds were collected randomly from day 0 to 8th day after inoculation/ challenge to determine oocysts per gram of faeces (OPG). Mortality in each group was recorded and post mortem examination was carried out. Faecal scoring was done on the basis of the presence of blood and mucous in the faeces as per the method used by Morehouse and Baron (1970). Lesion score was recorded on 7th DPI, 14th DPI and at the end of experiment as per Johnson and Reid (1970).

Statistical Analysis: The data collected was subjected to statistical analysis by one way analysis of variance (ANOVA) and least significant differences LSD.

RESULTS AND DISCUSSION

The efficacy of three ionophore anticoccidials viz. salinomycin, monensin and maduramicin was evaluated on the basis of certain parameters e.g. weight gain, faecal score before death, OPG and lesion score after sacrifice or natural death as well as the rate of mortality. The results of the average body weight gain in chicks of all the groups revealed that the maximum increase in weight was attained in birds fed maduramicin (group E) and minimum weight gain was observed in birds fed monensin (group C). The weight gain was markedly affected in the birds of non-medicated infected control (group G), which eventually suffered from clinical coccidiosis. However, slightly higher weight gain was observed in all the non-infected medicated group birds (groups B, D and F) in comparison to their respective medicated infected group. There was no significant difference in weight gains between all treatment groups (Table 1).

The results of the post-infection mortality rate, faecal score, OPG and lesion score in chicks have been

Table 1
Effects of anticoccidial agents on body weight gain (g) of birds infected with *Eimeria tenella*

Group	1st week	2nd week[1]	3rd week	4th week[2]	5th week
A	49.24^{Aa}±0.40 (50)	91.96^{Ba}±0.34 (50)	130.42^{Ca}±0.54 (40)	207.70^{Da}±1.08 (30)	248.40^{Ea}±0.53 (30)
B	49.92^{Aa}±0.36 (50)	92.14^{Ba}±0.31 (50)	130.82^{Ca}±1.41 (40)	208.96^{Da}±1.09 (30)	248.16^{Ea}±0.44 (30)
C	48.24^{Aa}±1.55 (50)	91.60^{Ba}±0.36 (50)	128.92^{Ca}±0.86 (40)	206.03^{Da}±1.20 (30)	247.80^{Ea}±0.80 (30)
D	48.98^{Aa}±0.48 (50)	91.80^{Ba}±1.28 (50)	129.42^{Ca}±1.30 (40)	206.73^{Da}±1.08 (30)	247.13^{Ea}±0.60 (29)
E	50.58^{Aa}±0.44 (50)	92.72^{Ba}±0.42 (50)	131.07^{Ca}±1.32 (40)	209.36^{Da}±1.08 (30)	250.03^{Ea}±1.39 (30)
F	50.68^{Aa}±0.40 (50)	92.32^{Ba}±0.42 (50)	131.62^{Ca}±0.72 (40)	209.53^{Da}±0.80 (30)	249.90^{Ea}±1.30 (30)
G	51.02^{Aa}±0.49 (50)	92.86^{Ba}±0.29 (50)	127.85^{Ca}±1.33 (27)	167.64^{Db}±3.36 (17)	201.11^{Eb}±2.75 (17)

[1]Infective inoculum administered on day 14; [2]Challenge inoculum on day 28.

Means with different superscripts (capital letters for row and small letters for column) differ significantly ($P<0.01$).

Figure in parenthesis indicates number of birds (observation).

Group A= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with salinomycin @60mg/kg of feed; group B= Uninfected and treated with salinomycin @ 60 mg/kg of feed; group C= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with monensin @100mg/ kg feed; group D= Uninfected and treated with monensin @100mg/ kg feed; group E= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with maduramicin @5 mg/kg of feed; group F= Uninfected and treated with maduramicin @5 mg/kg of feed; group G= Infected with 20000 sporulated oocyst of *E. tenella* but untreated

Table 2
Effects of anticoccidial agents on chicks infected with sporulated oocysts of *Eimeria tenella*

Group	Mortality (%)	Mean faecal score	OPG	Lesion score		Protection against lesions (%)	
				7th DPI	14th DPI	7th DPI	14th DPI
A	2	0.33	2,425	1.22	1.00	69.50	75.00
B	0	0.00	0	0	0	100	100
C	2	0.50	10,250	1.40	1.11	65.00	72.50
D	0	0.00	0	0	0	100	100
E	0	0.16	1,000	1.20	1.00	70.00	75.00
F	0	0.00	0	0	0	100	100
G	46	2.16	62,000	4.00	2.60	0	35

Group A= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with salinomycin @60mg/kg of feed; group B= Uninfected and treated with salinomycin @ 60 mg/kg of feed; group C= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with monensin @100mg/ kg feed; group D= Uninfected and treated with monensin @100mg/ kg feed; group E= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with maduramicin @5 mg/kg of feed; group F= Uninfected and treated with maduramicin @5 mg/kg of feed; group G= Infected with 20000 sporulated oocyst of *E. tenella* but untreated

summarized in Table 2. The lowest mortality (0%) was observed in group E and 2% mortality was recorded in groups A and C. However, 46% chicks succumbed post infection in group G. It was further observed that at the given dose, the efficacy in respect of arresting mortality was the highest with maduramicin in comparison to salinomycin and monensin. On the other hand, no mortality was observed in birds of all the three non-infected medicated control groups (groups B, D and F).

Among the infected groups, group E had the lowest average faecal score (0.16) followed by group A (0.33) and group C (0.50). Further, no faecal score was observed in birds of non-infected medicated groups (groups B, D and F), while very high score (2.16) was observed in birds of group G.

The lowest average OPG was recorded in birds of group E, while average OPG in group G was the highest. The faeces of the birds from the groups B, D and F, which were not infected, remained negative for any coccidia oocyst.

In respect to the lesion score, the lesions observed in the caeca during post-mortem examination of dead birds and also in sacrificed birds were scored in the units of 0 to 4. In salinomycin medicated birds (group A) the lowest lesion score of 1.00 was observed in 14th DPI but a lesion score of 1.46 was recorded on 7th days post challenge. In birds of monensin medicated group (group C), where a comparatively higher lesion score was observed after infection (1.40 and 1.10 score on 7th and 14th DPI) and a lower score of 1.16 during post-challenge period. Birds medicated with maduramicin (group E) which showed lower mean lesion score i.e. 1.20 and 1.00 on 7th and 14th DPI respectively, showed the highest score of 1.50 after challenge. On 7th DPI, group E showed the lowest lesion score among all the three medicated infected groups. Whereas, on 14th DPI similar lesion score was recorded for groups A and E. Further, it was noted that the group C had higher lesion score than the other two medicated groups (groups A and E). The lesion score suggested that the maduramicin gave higher protection in comparison to other two drugs i.e. monensin and salinomycin upto 7th DPI.

The result of the challenge infection of both medicated and non-medicated groups are presented in Table 3. Bird's survival rate was good in all the groups except group D. During the post challenge infection period, the highest mean faecal score of 1.33 was observed in group F, and the lowest (0.83) in group B among all the medicated groups. However, no faecal score was observed in group G. Among all the medicated groups the lowest mean OPG was recorded for group C and the highest in group F during post challenge period. On 7th DPC the lowest lesion score was observed in groups C and F whereas the highest score was noticed in group G. It indicated that the maduramicin and monensin gave higher protection after challenge infection.

In poultry industry the ionophores are being used to increase the production efficiency through the control

Table 3
Effects of anticoccidial agents on coccidia exposed chicks challenged with sporulated oocysts of *Eimeria tenella*

Group	No. of birds per group	Mortality (%)	Mean faecal score	Mean OPG	Lesion score	Protection %
A	30	0.00	1.16	800	1.46	63.50
B	30	0.00	0.83	7,625	1.20	70.00
C	30	0.00	1.0	733	1.16	71.00
D	30	3.33	1.00	8,000	1.51	62.25
E	30	0.00	1.00	1,000	1.50	62.50
F	30	0.00	1.33	8,125	1.16	71.00
G	17	0.00	0.00	333	1.88	53.00

Group A= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with salinomycin @60mg/kg of feed; group B= Uninfected and treated with salinomycin @ 60 mg/kg of feed; group C= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with monensin @100mg/ kg feed; group D= Uninfected and treated with monensin @100mg/ kg feed; group E= Infected with 20,000 sporulated oocysts of *E. tenella* and treated with maduramicin @5 mg/kg of feed; group F= Uninfected and treated with maduramicin @5 mg/kg of feed; group G= Infected with 20000 sporulated oocyst of *E. tenella* but untreated

of *Eimeria* spp. Ionophores act by interrupting transmembrane movement and intracellular equilibrium of ions in certain classes of bacteria and protozoa that inhabit the gastrointestinal tract. The action of ionophores provides nutritional and metabolic advantages to the ionophore-supplemented animal over a non-supplemented animal. Economic benefits derived from feeding ionophores include improved feed efficiency, increased weight gain and a reduction in morbidity and mortality (McGuffey *et al*., 2001).

The effectiveness of ionophore coccidiostats lies in the fact that whilst they kill the majority of the invading parasites, they permit a small leakage of coccidia enabling a degree of host immunity to develop (Hafez, 2008). In the present study the maximum body weight gain was attained in birds fed with maduramicin and minimum body weight gain was observed in birds fed with monensin, whereas the body weight gain was markedly affected in birds of non-medicated infected birds. An increase in body weight in birds receiving ionophores as feed premix has been reported by Greuel *et al.* (1992) and Costa *et al.* (2000). Hence the findings suggest that medication with anticoccidials should commence as early as two weeks to achieve the dual benefit of higher weight gain and prevention from coccidiosis.

The chemoprophylactic efficacy of maduramicin @ 5 mg/kg of feed as feed premix was most effective in checking mortality and it provided 100% protection against coccidiosis. Salinomycin and monensin @ 60 and 100 mg/kg of feed, respectively as feed premix showed almost equal performance. Similar observations have also been recorded by other investigators (Tschirch, 1988; Munoz *et al.,* 1988).

The effect of challenge infection further suggested that the ability of the drug to induce immunity was greater in birds of all the medicated groups. However, 3.33% mortality was observed in birds kept on monensin medicated uninfected control group. This may be because of lack of development of immunity as this group did not have any prior exposure to coccidia infection. The present observations are in close agreement with the results of Munoz *et al.* (1988) and Guangming *et al.* (2003).

In the present investigation the mean faecal score was the least in maduramicin medicated group (group F) than the other two groups after infection with 20,000 viable sporulated oocysts, whereas, these chicks showed highest faecal score when challenged with 40,000 sporulated viable oocysts. However, no score was observed in birds of untreated infected control groups after challenge dose. The oocyst count plays an important role in assessing the efficacy of an anticoccidial (Peeters *et al.,* 1994). The results of the present investigation showed that maduramicin medicated group shed least oocysts in faeces followed by salinomycin. The mean OPG for monensin group was higher than the other two medicated groups, however, it was very less than untreated infected group. This finding is suggestive of

higher efficacy of the maduramicin over the salinomycin and monensin. Similarly in challenge infection the birds of groups A, C and E which received medicated feed and had prior exposure to coccidia infection shed very less oocysts in faeces in comparison to the groups that received the infection for the first time during challenge.

Lesion score is considered as the visible effect of the drug on coccidiosis. The results of the present investigation on the lesion score were in agreement with the findings of Jo and Jang (1987) and Bing (2002) who also observed lesser lesions score in birds having prior exposure to coccidiosis. Thus it was observed that the drugs which performed better did not allow the *Eimeria* parasite to multiply in the host intestines and the host reaction to the infection was least and showed lower lesion score after challenge infection. Due to lesser host reaction, the host immune system did not have enough immunological stimuli for the development of immunity; hence these birds when challenged, reacted severely and showed much higher lesion score. Contrary to these, both salinomycin and monensin which were not as effective as maduramicin in checking the lesion formation by *E. tenella* generated host reaction, helped in the formation of second generation schizonts and provided enough stimulus for the development of immunity. Hence the birds of these groups had higher degree of protection against lesion formation during subsequent challenge infection. The present results are in conformity with the results reported by Al Taee *et al.* (1993).

On the basis of the results obtained, it could be inferred that salinomycin, monensin and maduramicin are all effective against caecal coccidiosis caused by *Eimeria tenella* and may also act as growth promoters. Maduramicin appears to have an edge over other two compounds as far as efficacy is concerned and is also cost effective. Hence, it can be more effective in controlling coccidiosis in broiler chicks.

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