*Int.J.Curr.Microbiol.App.Sci* (2018) *7*(4): 1094-1100

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2018.704.119

# Haematological Changes in Broiler Birds with Induced Caecal Coccidiosis following Prophylaxis with Different Coccidiostats

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**Keywords**

Broiler, Caecal coccidiosis, Coccidiostats, Haematology, Maduramicin, Salinomycin

**Article Info**

***Accepted:***
10 March 2018
***Available Online:***
10 April 2018

**A B S T R A C T**

The study was undertaken on Cobb-400 strain of broiler birds (n=300) reared on battery cage system with standard protocols to know the comparative efficacy of 4 commonly used coccidiostats through haematological alterations in experimentally *E. tenella* infected birds. Fifty day-old chicks each of T1, T2, T3 and T4 group were given coccidiostat Diclazuril (0.1%), Salinomycin (12%), Diclazuril + Salinomycin and Maduramicin (1 %) at a dose rate of 100, 50, 100+50 and 50 g/100 kg feed, respectively. Group T5 was kept as infected control and T6 as uninfected control without coccidiostat. Infection of *E. tenella* @ 50,000 oocytes was induced at three week of age in group T1 to T5 to see haematological changes after 1 wk of infection. The study revealed that haemoglobin, packed cell volume and total erythrocytes counts were reduced significantly ($P<0.05$), while total leukocytes counts were increased significantly on account of coccidial infection in all coccidiostat treated and infected non-treated control group of birds 1-week post-exposure. Differential leukocytes count (DLC) revealed significant increase in heterophills, lymphocytes and eosinophills, and decrease in monocytes and basophils in infected birds. Comparative less pathological change was found in Salinomycin treatment group. In comparison with positive control group, all coccidiostat treated groups and Maduramicin in particular showed significant beneficial effect on TEC with less RBCs damage. There was also significant reduction in PCV in post-infection positive control group compared to all coccidiostats treated groups. Better efficacy on PCV was of Salinomycin. TLC count of post infection positive control group was significantly higher than the pre-infection value of same group. Better efficacy on TLC of Salinomycin followed by Maduramicin was observed in *E. tenella* infection. After experimental infection heterophills counts were significantly increased in all treatment groups with highest increase in T1 group followed by T3, T4 and T2 group indicating better efficacy of Salinomycin and Maduramicin. Post-infection positive control group had significant increase in lymphocytes count as compared to pre-infection. Overall, better efficacy of Salinomycin followed by Maduramicin was found in infected birds compared to Diclazuril in terms of haematological alterations in experimentally infected birds.

## Introduction

Broiler production is one of the best ways of supplying good quality animal protein for human consumption. Broilers are the quickest, most economic and the most efficient converter of plant material into food of high biological value (Bootwalla, 2005). Poultry sector is however still confronted with many enteric diseases like coccidiosis which are hindering its progress (Saima *et al.*, 2010), particularly in tropical countries (Chakrabarti, 1989). Coccidiosis is a widely known, greatly studied and yet incompletely understood protozoan disease of poultry. It is accounted for 5-10 % mortality rate of chickens and an unknown loss due to reduced weight gain and feed efficiency, damage to the digestive tract, decreased egg production and lowered resistance of birds to other poultry diseases.

The underlying mechanisms of the host specificity are not well understood but most likely include genetic, nutritional, biochemical and immune factors. In addition to host specificity, a given *Eimeria* parasite only infects particular cell types or tissues in a given host (Lillehoj and Okamura, 2003). *E. tenella* is the ubiquitous and most pathogenic parasite responsible for caecal coccidiosis disturbing nutrient absorption and metabolism with high rate of mortality in poultry (Patra *et al.,* 2010). It produces deviation in the various haematological components of the body (Panda *et al.,* 1997; Patra *et al.,* 2010). In broiler production, numerous anticoccidial drugs are used for prevention and control of coccidiosis. However, development of tolerance to these drugs has led to search for newer molecules and different classes of anticoccidials have been discovered and used from time to time. Hence, a comparative study on efficacy of different coccidiostats in broilers by inducing experimental infection of *E. tenella* was planned with evaluation of haematological alterations, if any.

## Materials and Methods

Total of three hundred Cobb broiler chicks of either sex were procured at day-old age from Venky India Limited, Mogar, Gujarat. They were reared under coccidia-free conditions on battery cage system with standard protocols. The birds were randomly divided into 6 equal groups (T1 to T6) each of 50 birds. The chicks of T1, T2, T3 and T4 group were given Diclazuril (0.1%), Salinomycin (12%), Diclazuril + Salinomycin in shuttle programme, and Maduramicin (1 %) at a dose rate of 100 g, 50 g, 100 + 50 g and 50 g per 100 kg broiler feed as coccidiostats, respectively. Group T5 was kept as infected control and Group T6 as uninfected healthy control, both without coccidiostats. Birds of T1 to T5 groups were given experimental infection of 50,000 oocytes of *E. tenella* at three week of age.

Blood samples were taken randomly from 10 birds in each group from wing vein in citrated vials for haematology just before and again 1 week after experimental infection of *E. tenella*. Haemoglobin (Hb, g %) content was estimated by Sahli's acid haematin method and packed cell volume (PCV %) by microhaematocrit method (Coles, 1986). Total erythrocytes count (TEC, $10^6$/µl), total leukocytes count (TLC, $10^3$/µl) and differential leukocyte count (DLC, $10^3$/µl) were estimated as per Jain (1986). Data generated were analyzed statistically (Snedecor and Cochran, 1980) by using completely randomized design on SAS software version 20.00.

## Results and Discussion

Comparative efficacy of different coccidiostats on haematological values is given in Tables 1 and 2. Haematological values are the indicators of the pathological damage caused by the chemicals or infection.

## Haemoglobin (Hb)

All treatment groups had lower Hb value at 3 weeks of age, i.e. just before experimental infection, except Salinomycin group had nearly normal Hb value (12.22 ± 0.14 g/dl) similar to control group, which indicated better efficacy of Salinomycin. After experimental infection the Hb values were significantly decreased. The trend of decrease was highest in T1 group (9.84 ± 0.17 g/dl) followed by shuttle T3 (9.96 ± 0.16 g/dl), T4 (10.76 ± 0.19 g/dl) and T2 (11.51 ± 0.29 g/dl) group among four treatment groups. Positive control T5 group showed significantly lowest Hb value (8.54 ± 0.07 g/dl) as compared to all treatment groups. Result indicated better efficacy of Salinomycin as compared to other coccidiostats. There was marked reduction in Hb values in the infected groups during acute phase of infection and the values returned to normal during recovery. There was no appreciable reduction in Hb values in the infected medicated group when compared to control group. The present findings are in conformity with the observations made by Turk (1985), Padmavathy and Muralidharam (1986), Ogbe *et al.*, (2010) and Adamu *et al.*, (2013) for *E. tenella* infection. The reduction in the value of haemoglobin observed in the infected group of birds might be attributed to haemorrhages in the caeca followed by development of caecal lesions. There may be injury to tissue and liberation of large quantities of histamine, which increase the local blood flow and also increase the permeability of capillaries and venules allowing large quantities of fluid to come out (Padmavathi and Muralidharan, 1986).

## Packed Cell Volume (PCV)

Before experimental infection, PCV value was lowest in shuttle group T3 (26.47 ± 0.75%) and highest in Salinomycin group T2 (29.11 ± 0.18%). Both control groups had non-significantly higher values compared to treated groups. After experimental infection, the highest reduction was observed in T1 and T3 groups followed by T4 and T2 groups compared to pre-infection values. Result indicated better protection on PCV by Salinomycin among four coccidiostats. There was also significant reduction in PCV in post-infection positive control group compared to all treated post-infection groups indicating supportive efficacy of coccidiostats in birds. Significant reduction in PCV was also recorded by Stephens (1965). Similarly, Turk (1985) recorded fall in haematocrit value from the 5$^{th}$ to 10$^{th}$ day with *E. necatrix* infection. The present results were also comparable to those reported previously by number of authors for *E. tenella* infection (Padmavathi and Muralidharan, 1986; Panda *et al.,* 1997; Kumar and Padmavathi, 2000; Jaipurkar *et al.*, 2004; Ogbe *et al.*, 2010; Adamu *et al.*, 2013).

## Total Erythrocytes Counts (TEC)

Before experimental infection, the highest mean TEC count ($10^6$/µl) was observed in T2 group ((2.36 ± 0.03) followed by T1 (2.33 ± 0.02), T4 (2.30 ± 0.03) and T3 (2.29 ± 0.02) groups. Positive control group T5 (2.31 ± 0.01 $10^6$/µl) and negative control group T6 (2.32 ± 0.01× $10^6$/µl) had more or less similar values.

There was no significant difference in TEC counts between control and treatment groups. After experimental infection, there was significant reduction in TEC in all treatment groups with highest reduction in T1 followed by T3, T2, and T4 groups as compared to T6 group (Table 1). In comparison with positive control group, all coccidiostats treated groups showed significant beneficial effect on TEC value with better efficacy of Maduramicin in term of less RBCs damage. Significant reduction in TEC was also recorded by above workers in birds affected with *E. tenella* infection.

**Table.1** Haematological values in different coccidiostats treated groups before and 1 week after experimental infection of *E. tenella* (n=10)

| Parameters | BI/ AI | Treatment groups | | | | | | Period Mean | P | | T×P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | | S Em | CD | S Em | CD |
| TEC ($10^6$/µl) | BI | 2.33 ±0.03 | 2.36 ±0.03 | 2.29 ±0.02 | 2.30 ±0.03 | 2.31 ±0.01 | 2.32 ±0.01 | 2.32[a] | 0.03 | 0.08 | 0.07 | 0.20 |
| | AI | 2.02 ±0.12 | 2.13 ±0.12 | 2.05 ±0.12 | 2.11 ±0.12 | 1.69 ±0.01 | 2.42 ±0.01 | 2.07[b] | | | | |
| PCV (%) | BI | 28.28 ±0.55 | 29.11 ±0.18 | 26.47 ±0.75 | 27.90 ±0.72 | 28.80 ±0.15 | 29.51 ±0.14 | 28.35[a] | 0.23 | 0.65 | 0.57 | 1.59 |
| | AI | 24.56 ±0.26 | 28.05 ±1.10 | 24.56 ±0.26 | 26.72 ±1.00 | 22.77 ±0.15 | 30.24 ±0.12 | 26.15[b] | | | | |
| Hb (g%) | BI | 11.16 ±0.23 | 12.22 ±0.14 | 11.43 ±0.27 | 11.63 ±0.32 | 12.35 ±0.06 | 12.42 ±0.11 | 11.87[a] | 0.08 | 0.22 | 0.19 | 0.54 |
| | AI | 9.84 ±0.17 | 11.51 ±0.29 | 9.96 ±0.16 | 10.76 ±0.19 | 8.54 ±0.07 | 12.75 ±0.05 | 10.56[b] | | | | |
| TLC ($10^3$/µl) | BI | 23.14 ±0.21 | 22.91 ±0.13 | 23.04 ±0.12 | 22.70 ±0.20 | 21.51 ±0.06 | 21.51 ±0.06 | 22.47[a] | 0.15 | 0.42 | 0.37 | 1.04 |
| | AI | 41.36 ±0.46 | 33.36 ±0.39 | 37.56 ±0.99 | 34.66 ±0.13 | 52.71 ±0.38 | 21.47 ±0.05 | 36.86[b] | | | | |
| Heterophills ($10^3$ / µl) | BI | 9.84 ±0.09 | 9.74 ±0.06 | 9.90 ±0.08 | 9.56 ±0.16 | 8.40 ±0.09 | 8.29 ±0.03 | 9.29[a] | 0.09 | 0.24 | 0.21 | 0.59 |
| | AI | 18.07±0.07 | 14.92±0.27 | 16.52 ±0.56 | 15.34±0.26 | 17.49±0.14 | 7.44 ±0.02 | 14.96[b] | | | | |
| lymphocytes ($10^3$ / µl) | BI | 9.53 ±0.16 | 9.34 ±0.08 | 9.42 ±0.09 | 9.38 ±0.08 | 9.28 ±0.12 | 9.32 ±0.07 | 9.38[a] | 0.13 | 0.36 | 0.31 | 0.87 |
| | AI | 19.14±0.47 | 13.90±0.57 | 16.66 ±0.62 | 15.06±0.29 | 30.11±0.29 | 9.92 ±0.01 | 17.47[b] | | | | |
| Monocytes ($10^3$ / µl) | BI | 0.72 ±0.02 | 0.68 ±0.02 | 0.73 ±0.02 | 0.69 ±0.02 | 0.75 ±0.01 | 0.75 ±0.01 | 0.72[a] | 0.02 | 0.06 | 0.05 | 0.15 |
| | AI | 0.49 ±0.06 | 0.61 ±0.08 | 0.65 ±0.10 | 0.61 ±0.03 | 0.67 ±0.11 | 0.90 ±0.02 | 0.65[b] | | | | |
| Eosinophills ($10^3$ / µl) | BI | 0.77 ±0.02 | 0.82 ±0.01 | 0.75 ±0.03 | 0.80 ±0.01 | 0.84 ±0.01 | 0.81 ±0.01 | 0.80[a] | 0.01 | 0.05 | 0.05 | 0.13 |
| | AI | 1.84 ±0.07 | 1.68 ±0.08 | 1.80 ±0.08 | 1.46 ±0.07 | 2.37 ±0.04 | 0.93 ±0.01 | 1.68[b] | | | | |
| Basophills ($10^3$ / µl) | BI | 2.28 ±0.02 | 2.34 ±0.03 | 2.25 ±0.02 | 2.28 ±0.03 | 2.26 ±0.02 | 2.33 ±0.03 | 2.29[a] | 0.02 | 0.05 | 0.04 | 0.12 |
| | AI | 1.82 ±0.05 | 2.25 ±0.04 | 1.94 ±0.08 | 2.18 ±0.03 | 2.06 ±0.07 | 2.28±0.05 | 2.09[b] | | | | |

The means bearing different superscript within the column for BI (before infection) & AI (after infection) differ significantly (P<0.05).

**Table.2** Haematological pooled values (Mean ± SE) in different treatment groups (n=10)

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | T | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | S Em | CD |
| TEC ($10^6$/µl) | $2.18^{b}$ | $2.25^{ab}$ | $2.17^{b}$ | $2.21^{b}$ | $2.00^{c}$ | $2.37^{a}$ | 0.05 | 0.14 |
| PCV (%) | $26.42^{cd}$ | $28.58^{b}$ | $25.52^{d}$ | $27.31^{c}$ | $25.79^{d}$ | $29.88^{a}$ | 0.40 | 1.12 |
| Hb (g%) | $10.50^{d}$ | $11.87^{b}$ | $10.70^{d}$ | $11.20^{c}$ | $10.45^{d}$ | $12.59^{a}$ | 0.14 | 0.38 |
| TLC ($10^3$/µl) | $32.25^{b}$ | $28.13^{d}$ | $30.30^{c}$ | $28.68^{d}$ | $37.11^{a}$ | $21.49^{e}$ | 0.26 | 0.73 |
| Heterophills ($10^3$ / µl) | $13.95^{a}$ | $12.33^{c}$ | $13.21^{b}$ | $12.45^{c}$ | $12.95^{b}$ | $7.87^{d}$ | 0.15 | 0.42 |
| lymphocytes ($10^3$ / µl) | $14.34^{b}$ | $11.62^{d}$ | $13.04^{c}$ | $12.22^{d}$ | $19.70^{a}$ | $9.62^{e}$ | 0.22 | 0.62 |
| Monocytes ($10^3$ / µl) | $0.60^{b}$ | $0.64^{b}$ | $0.69^{b}$ | $0.65^{b}$ | $0.71^{b}$ | $0.83^{a}$ | 0.04 | 0.10 |
| Eosinophills ($10^3$ / µl) | $1.31^{b}$ | $1.25^{b}$ | $1.28^{b}$ | $1.13^{c}$ | $1.59^{a}$ | $0.87^{d}$ | 0.03 | 0.09 |
| Basophills ($10^3$ / µl) | $2.05^{d}$ | $2.30^{a}$ | $2.09^{cd}$ | $2.23^{ab}$ | $2.16^{bc}$ | $2.31^{a}$ | 0.03 | 0.08 |

The means bearing different superscripts within the row differ significantly (P<0.05).

However, Stephens *et al.*, (1967) found significant increase in erythrocyte count with *E. acervulina* infection at 6th, 10th and 14th day of inoculation. The reduction observed in TEC during the acute phase of infection is due to haemorrhage.

## Total Leucocytes Counts (TLC)

Just before experimental infection, TLC counts were in the range of 22.70 to 23.14 $\times 10^3$/µl among all four treatment groups, while the TLC counts were significantly lower (21.51±0.06 $\times 10^3$/µl) in both the control groups. After experimental infection TLC counts were significantly increased in four groups than the pre-infection values. Highest count ($10^3$/ µl) was observed in T1 group (41.36 ± 0.46) followed by T3 (37.56 ± 0.99), T4 (34.66 ± 0.13) and T2 (33.36 ± 0.39) groups. TLC count of post-infection positive control group was (52.71 ± 0.38 $\times 10^3$/µl) significantly higher than the pre-infection value of same group (21.51 ± 0.06 $\times 10^3$/µl). The result indicated better efficacy of Salinomycin followed by Maduramicin in *E. tenella* infection. Significant increase in TLC has also been reported by Padmavathi and Muralidharan (1986), Panda *et al.*, (1997), Kumar and Padmavathi (2000), Jaipurkar *et al.*, (2004) and Ogbe *et al.*, (2010). Adamu *et al.*, (2013) also reported similar findings of higher TLC with increased numbers of lymphocytes, eosinophils and heterophils in *E. tenella* and *E. brunetti* infected broilers. The increased total leucocytes count in coccidia-affected birds might be due to suppressed body immune system from the infection (Stephen, 1965). This increase was suggestive of increase leucopoiesis as a first step of defence mechanism to infection (Padmavathi and Muralidharan, 1986).

## Differential Leucocyte Counts (DLC)

At 3 weeks of age, all treatment groups had 1 to 1.5 $\times 10^3$/µl heterophils, which were higher as compared to both control groups. After experimental infection heterophills counts ($10^3$/µl) were significantly increased in all treatment groups with highest increase in T1 group (18.07 ± 0.07) followed by T3 (16.52 ± 0.56), T4 (15.34 ± 0.26) and T2 (14.92 ± 0.27) groups suggesting better efficacy of Salinomycin and Maduramicin. The increase in the heterophills was observed because heterophils also contain a variety of granules that contribute to the first line host defence against bacteria, fungi, protozoa and some viruses. Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds (Irizaary-Rovira, 2004) because monocytes,

macrophages and dendritic cells are important hematopoietic cells that play critical role in defence and in maintaining homeostasis.

Slightly higher lymphocytic values were observed in all treatment groups as compared to control group at the age of 3 weeks, but significant increase in lymphocytes count ($10^3$/µl) was observed after experimental infection with highest increase in T1 group (19.14 ± 0.47) followed by T3 (16.66 ± 0.62), T4 (15.06 ± 0.29) and T2 (13.90 ± 0.57) groups. Post-infection positive control group showed significant increase in lymphocytes count (30.11 ± 0.29 $10^3$/µl) as compared to pre-infection value (9.28 ± 0.12 × $10^3$/µl). Better efficacy of Salinomycin followed by Maduramicin was seen in infected birds compared to Diclazuril in terms of pathological damage. The increase in the lymphocyte count may be attributed to the effect of the inflammation of the caeca and intestine. Chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are immunological response, humoral antibody formation and cell mediated immunity (Irizaary-Rovira, 2004).

Coccidiostats used in the experiment decreased monocytes counts after infection with lowest decrease in Salinomycin treatment. There was more decrease in post-infection monocyte value in positive control group (0.08 × $10^3$/µl). Padmavati and Murlidharan (1986) also reported decrease in monocytes count at 7-day post-experimental infection of *E. tenella.*

*E. tenella* infection at 3 weeks of age caused significant increase in eosinophilic count ($10^3$/µl) with highest value in T1 group (1.84 ± 0.07) followed by shuttle group T3 (1.80 ± 0.08), T2 (1.68 ± 0.08) and T4 (1.46 ± 0.07). Significant increase of eosinophilic count observed in T5 positive control group (2.37 ± 0.04 ×$10^3$/µl) as compared to treated groups indicated more damage by infection which can be reduced by coccidiostat treatment. Similar increase of eosinophils was reported by Adamu *et al.*, (2013). Eosinophilia rarely occurs in birds, but may be associated with parasitism and is known to interact with homocytotropic antibodies (IgE and IgG), mast cells and basophils. The antibody and T lymphocytes provide specificity to the reaction and the IgE on mast cells attracts eosinophils to modulate the inflammatory reaction (Irizaary-Rovira, 2004).

Basophils counts ($10^3$/µl) were more or less similar in both control as well as all four treatment group at 3 weeks of age, but more reduction was observed in T1 (1.82 ± 0.05) and T3 (1.94 ± 0.08) groups as compared to T2 (2.25 ± 0.04) and T4 (2.18 ± 0.03) groups after experimental infection indicating better efficacy of Salinomycin and Maduramicin. Significant reduction was observed in post-infection control group (2.06 ± 0.07 ×$10^3$/µl) as compared to pre-infection value (2.26 ± 0.02 ×$10^3$/µl). Padmavathi and Muralidharan (1986) however did not find basophills after 7$^{th}$ day of experimental *E. tenella* infection of 50,000 oocysts.

## Acknowledgement

The authors thank Principal Scientist and Head, Poultry Complex, and Principal and Dean, College of Veterinary Science & AH, Anand for providing the necessary facilities.

## Conflict of Interest

All authors declare no conflict of interest.

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**How to cite this article:**

Hirani, N.D., J.J. Hasnani, S.S. Pandya and Patel, P.V. 2018. Haematological Changes in Broiler Birds with Induced Caecal Coccidiosis following Prophylaxis with Different Coccidiostats. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 1094-1100. doi: https://doi.org/10.20546/ijcmas.2018.704.119