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ORIGINAL RESEARCH

Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken

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Abstract

Aim: A safety pharmacology trial was conducted to evaluate the impact of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in Newcastle disease virus vaccinated broiler chicken after pulsed water medication.

Materials and Methods: Experimental group birds were administered with enrofloxacin at recommended therapeutic dose 10mg/Kg body weight, through drinking water for five consecutive days from 43rd to 47th day of age. Zootechnical performance parameters, behavioural and humoral immune response in terms of haemagglutination inhibition (HI) titre were assessed at different time interval during pre-treatment, treatment, post-treatment period. Bursa of Fabricius and spleen tissues collected at each sampling point viz. 1, 3, 5, 7 and 9 days post treatment were subjected to histopathological examination.

Results: A significant reduction in HI titre was noticed in enrofloxacin administered birds. The decreased HI titre was further substantiated by the histopathological changes observed in bursa of Fabricius and spleen which showed a lymphocytic dispersion and depletion with several areas of lymphoblastic degeneration. Conversely, a down regulatory effect on humoral immunity was observed as evidenced by increased HI titre value noticed from 5th day post treatment onwards and a congruent reversible trend in histopathological changes as indicated by repopulation with lymphocytes on 9th day post treatment. However, there was no significant change in body weight, cumulative feed intake, feed efficiency and behaviour in enrofloxacin administered groups.

Conclusion: The present study suggests that the immuno suppressive activity of enrofloxacin may alter the immune response to vaccines, if it is coadministered during vaccination of broilers. On the other hand, enrofloxacin, though it decreased the humoral immune response, it did not have any appreciable effect on broiler's performance.

Keywords: broiler chicken, enrofloxacin, immunohistopathology, safety pharmacology, zootechnical performance

Introduction

In poultry farms, it is a common practice to add antibiotics in drinking water at the time of vaccination. Most farmers administer antibiotics at the time of vaccination without scientific knowledge of the effects on the immune response. Database on the effects of such practices on the immune system is scarce, and more authenticated studies are needed to investigate the impact of antibiotics on the immune system at the time of vaccination [1].

Enrofloxacin, a fluoroquinolone developed exclusively for veterinary use is advocated in poultry in large-scale for treatment of chronic respiratory disease, colibacillosis, salmonellosis and fowl cholera [2]. The effect of enrofloxacin on the immune response is not well documented [1]. However, Tokarzewski [3] revealed that enrofloxacin and chloramphenicol decreased the level of specific IgY in laying hens immunized with living cells of *Salmonella enterica* subsp. *enterica serovar enteritidis* and lipopolysaccharide.

Studies conducted in other species revealed that the presence of circulating antimicrobial residues, especially fluoroquinolones, in nestlings of the three vulture species breeding in central Spain resulted in impaired cellular and humoral immune systems compared with nestlings from the control areas, which did not ingest antibiotics [4].

After administration, enrofloxacin is metabolised in the liver via de-ethylation into pharmacologically active metabolite ciprofloxacin [5-7]. Porchezhian [8] reported that ciprofloxacin significantly reduced the antibody titre against Lasota and Sheep Red Blood Cells (SRBC) antigens in broiler chicks. Consequently there is every possibility of alteration of immune response after enrofloxacin administration in broiler chicken. Further, our earlier study [9] speculated that the reduction in the lymphocyte count at therapeutic dose of enrofloxacin may have influence on the immune response of the broiler chicken.

Hence the present study has been undertaken to

investigate the effect of enrofloxacin on humoral immune response, the key factor that has been underestimated during vaccination programs in the field. Zootechnical performance, behavioural and immunohistopathological response of broiler chicken were also evaluated after recommended therapeutic dosage of enrofloxacin under experimental conditions.

Materials and Methods

Experimental birds: Thirty six one-day-old broiler chicks (Broiler strain B_1) obtained from Institute of Poultry Production and Management, Madhavaram Milk Colony, Chennai-600 051, were wing banded and maintained under brooder management for 14 days and on day 15, birds were caged under standard management conditions. Necessary approval was obtained from Institutional Animal Ethics Committee (IAEC), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), for conducting the experiment.

Vaccination: Vaccination against Newcastle Disease Virus (NDV) was carried out in both control and experimental birds via intra ocular route administration of RDVF live (Lentogenic) (L.No.11, Batch No.1, December 2010; Institute of Veterinary Preventive Medicine (IVPM)) on 5th day of age and RDV-Lasota (L.No.11, Batch No.11, December 2010, IVPM) was administered via drinking water on 25th day of age.

Ration: The broilers were fed with standard broiler starter mash (Moisture-7.76%, Crude protein-22.55%, Crude fibre-2.15%, Ether extract-3.63%, Total ash-5.56% and Gross energy-4037 kcal/Kg) and finisher mash (Moisture-9.07%, Crude protein-17.07%, Crude fibre-4.17%, Ether extract-2.59%, Total ash-4.40% and Gross energy-3884 kcal/Kg) *ad libitum* from 0-2 weeks and 3-8 weeks respectively. Non medicated broiler ration (free from feed additives, antibiotics and anticoccodials) was used throughout the study. Broiler feed was screened for mycotoxins, pesticides, heavy metals and coliform count.

Enrofloxacin dosing protocol: Enrofloxacin 10% was obtained *as gratis* from M/s. Neospark Drugs and Chemicals Private Limited, Hyderabad, India. Chicks were randomly divided on the first day of age into six groups. Group I (6 birds) was kept as untreated control, received non medicated water and Groups II, III, IV, V and VI each 6 birds were administered with enrofloxacin at recommended therapeutic dose 10mg/Kg body weight, through drinking water for five consecutive days from 43rd to 47th day of age [10]. Birds received their freshly prepared daily medication during a 4 hours period in the morning, and their water was antibiotic free for the remaining 20 hours of each day. The concentration of enrofloxacin in the water to give the required dose per Kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication [11]. Drinking water was free from pesticides, heavy metals and coliform count. Pulsed water medication was used because oral administration of drugs is the most practical way for treating birds being followed in Indian field condition.

Zootechnical performance parameters: Zootechnical performance parameters such as body weight and cumulative feed intake were recorded at different time point intervals as follows: During the pre-treatment period (Day old to 42nd day) at weekly intervals, during treatment period (43rd to 47th day) daily and during post-treatment period (48th to 56th day) on alternative days i.e. 1,3,5,7 and 9 days post treatment.

Behavioural observation: All the birds were observed thrice daily during pretreatment, treatment and post treatment periods for behavioural alterations and clinical signs based on the subjective evaluation as described by Khan *et al*. [12] and Patel *et al*. [13]. The parameters observed were general alertness, feed intake, water intake, locomotion, palpation of joints, feather appearance, eyes (mucous membrane), comb colour, droppings and mortality.

Haemagglutination inhibition titre: Blood samples were collected from wing vein into sterile test tubes from control and treatment groups at different time points (6 birds each) as follows; at 24 hours interval during the dosing period after administration of the first dose (44th to 47th day of age) and during the post treatment period at 48 hours interval on day 1, 3, 5, 7 and 9 post treatment (48th to 56th day of age). During the treatment period blood samples were collected from 6 birds randomly selected from treatment groups and during the post treatment period blood samples were collected from respective treatment groups. Serum was subjected to haemagglutination inhibition (HI) titre against NDV vaccine following standard procedures [14]. Data were subjected to one way analysis of variance for their significance as per Snedecor and Cochran [15].

Histopathology: After cessation of the last dose of enrofloxacin, six birds from corresponding treatment group were sacrificed ethically at each sampling point viz. 1, 3, 5, 7 and 9 days post treatment. Control birds were sacrificed on day 9 post treatment. Bursa of Fabricius and spleen tissues were collected in 10 % formalin. Paraffin embedded tissues were sectioned to 5 µm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination [16].

Results

Effect on Zootechnical performance parameters: There was no significant change in body weight (Table-1), cumulative feed intake (Table-2) and feed efficiency (Table-3) in enrofloxacin administered groups when compared to that of control.

Effect on behaviour: All the birds were active and interested in feed, water and the surroundings throughout the experiment period. Birds did not show

Table-1. Effect of enrofloxacin administration (@ 10mg/Kg body weight, in drinking water for 5 consecutive days) on body weight in broiler chicken (expressed as grams, Mean±SE, n=6)

Group	Pretreatment period						Treatment period						Post treatment period				
	Day 1	1st Week	2nd Week	3rd Week	4th Week	5th Week	42nd Day	43rd Day	44th Day	45th Day	46th Day	47th Day	48th Day	50th Day	52nd Day	54th Day	56th Day
I	36.27 ±0.62	70.33 ±1.33	161.67 ±1.31	298.67 ±2.36	467.83 ±1.99	657.17 ±2.87	862.33 ±3.38	893.17 ±2.50	924.17 ±2.50	956.17 ±2.50	989.17 ±2.50	1023.17 ±2.50	1058.17 ±2.50	1130.17 ±2.50	1204.17 ±2.50	1278.17 ±2.50	1352.17 ±2.50
II	36.93 ±0.89	69.50 ±2.39	161.50 ±2.32	299.50 ±3.43	465.00 ±5.25	656.17 ±4.20	854.17 ±5.31	883.83 ±5.84	913.00 ±5.17	944.83 ±3.56	977.00 ±3.76	1010.67 ±4.24	1044.83 ±3.50	----	----	----	----
III	36.77 ±0.81	68.33 ±3.68	159.33 ±5.28	300.00 ±4.93	473.00 ±2.83	665.00 ±4.68	866.17 ±5.69	895.50 ±5.81	925.50 ±4.40	955.33 ±3.40	985.50 ±3.86	1018.50 ±3.89	1052.50 ±3.67	1123.67 ±3.98	----	----	----
IV	36.27 ±0.89	71.00 ±2.67	162.67 ±4.58	300.17 ±5.66	465.17 ±4.48	660.33 ±4.88	867.00 ±5.26	896.17 ±5.17	926.00 ±4.85	955.00 ±3.28	986.33 ±3.21	1019.00 ±4.1	1053.00 ±3.49	1122.00 ±4.27	1195.17 ±4.22	----	----
V	36.90 0.96	70.00 2.41	160.33 3.59	299.33 4.97	472.17 3.84	655.33 4.79	858.67 5.56	888.67 4.54	918.67 ±4.54	948.67 ±4.54	980.67 ±4.54	1013.67 ±4.54	1046.67 ±4.54	1116.67 ±4.54	1189.67 ±4.54	1263.67 ±4.54	----
VI	36.43 ±0.87	68.17 ±2.52	160.67 ±1.5	298.00 ±2.78	462.50 ±2.43	653.67 ±2.81	858.83 ±3.00	888.00 ±2.03	918.33 ±3.17	949.33 ±3.33	981.83 ±3.69	1015.50 ±4.62	1048.67 ±3.09	1118.83 ±4.28	1192.67 ±4.38	1264.83 ±5.86	1338.17 ±6.64

Table-2. Effect of enrofloxacin administration (@ 10mg/Kg body weight, in drinking water for 5 consecutive days) on cumulative feed intake in broiler chicken (expressed as grams, Mean±SE, n=6)

Group	Pretreatment period						Treatment period						Post treatment period			
	1st Week	2nd Week	3rd Week	4th Week	5th Week	42nd Week	43rd Day	44th Day	45th Day	46th Day	47th Day	48th Day	50th Day	52nd Day	54th Day	56th Day
I	37.17 ±0.6	177.50 ±2.63	425.83 ±3.52	765.33 ±4.66	1180.67 ±4.45	1610.00 ±6.45	1680.33 ±3.48	1765.33 ±4.10	1835.00 ±5.23	1900.83 ±5.83	1972.33 ±4.18	2105.00 ±2.78	2275.00 ±4.08	2435.00 ±5.77	2610.00 ±5.32	2761.00 ±4.76
II	38.17 ±0.54	180.33 ±2.67	423.83 ±3.52	770.50 ±2.68	1190.67 ±4.45	1630.50 ±6.7	1695.67 ±4.94	1778.50 ±4.32	1817.33 ±3.61	1909.50 ±3.36	1966.33 ±5.10	2100.00 ±6.45	----	----	----	----
III	37.5 ±0.67	182.00 ±2.74	434.17 ±3.25	775.33 ±2.89	1185.33 ±3.02	1620.67 ±5.33	1690.83 ±5.83	1772.67 ±5.81	1824.50 ±4.30	1915.83 ±4.73	1973.67 ±6.03	2095.17 ±2.89	2265.50 ±5.80	----	----	----
IV	37.33 ±0.67	179.00 ±1.51	429.33 ±3.09	760.67 ±2.68	1187.50 ±2.93	1624.50 ±4.11	1684.33 ±3.34	1770.83 ±4.21	1830.50 ±3.39	1912.50 ±3.39	1986.00 ±3.36	2112.50 ±3.39	2280.33 ±4.10	2439.50 ±2.81	----	----
V	36.67 ±0.61	181.33 ±3.02	430.83 ±3.52	769.50 ±2.68	1189.17 ±2.7	1617.50 ±2.93	1694.17 ±3.25	1779.17 ±5.53	1827.50 ±4.3	1906.00 ±4.62	1980.83 ±5.78	2110.83 ±5.83	2270.00 ±5.77	2440.00 ±2.63	2600.83 ±5.39	----
VI	37.5 ±0.67	180.33 ±2.67	426.33 ±3.6	765.33 ±4.66	1182.83 ±4.67	1622.17 ±2.46	1687.67 ±3.19	1766.33 ±2.67	1832.50 ±5.34	1909.00 ±4.20	1971.33 ±5.90	2099.17 ±4.09	2275.83 ±4.72	2435.83 ±5.39	2605.00 ±6.45	2750.00 ±5.32

Table-3. Effect of enrofloxacin administration (@ 10mg/Kg body weight, in drinking water for 5 consecutive days) on feed efficiency in broiler chicken (Mean±SE, n=6)

Group	Pretreatment period						Treatment period						Post treatment period			
	1st Week	2nd Week	3rd Week	4th Week	5th Week	42nd Week	43rd Day	44th Day	45th Day	46th Day	47th Day	48th Day	50th Day	52nd Day	54th Day	56th Day
I	1.10 ±0.05	1.42 ±0.03	1.62 ±0.02	1.77 ±0.01	1.90 ±0.01	1.95 ±0.01	1.960 ±0.01	1.99 ±0.01	1.99 ±0.01	1.99 ±0.01	2.00 ±0.01	2.06 ±0.01	2.08 ±0.01	2.09 ±0.01	2.10 ±0.01	2.10 ±0.00
II	1.20 ±0.09	1.45 ±0.04	1.62 ±0.03	1.80 ±0.02	1.92 ±0.02	2.00 ±0.02	2.00 ±0.01	2.03 ±0.02	2.00 ±0.01	2.03 ±0.01	2.02 ±0.01	2.08 ±0.01	----	----	----	----
III	1.25 ±0.13	1.50 ±0.06	1.65 ±0.04	1.78 ±0.01	1.89 ±0.01	1.95 ±0.02	1.97 ±0.01	1.99 ±0.01	1.99 ±0.01	2.02 ±0.01	2.01 ±0.01	2.06 ±0.01	2.08 ±0.01	----	----	----
IV	1.11 ±0.08	1.42 ±0.04	1.63 ±0.04	1.77 ±0.01	1.90 ±0.02	1.96 ±0.01	1.96 ±0.01	1.99 ±0.01	1.99 ±0.01	2.01 ±0.01	2.02 ±0.01	2.08 ±0.01	2.10 ±0.01	2.11 ±0.01	----	----
V	1.15 ±0.1	1.48 ±0.05	1.64 ±0.03	1.77 ±0.02	1.92 ±0.01	1.97 ±0.01	1.99 ±0.01	2.02 ±0.01	2.00 ±0.01	2.02 ±0.01	2.03 ±0.01	2.09 ±0.01	2.10 ±0.01	2.12 ±0.01	2.12 ±0.01	----
VI	1.22 ±0.1	1.45 ±0.03	1.63 ±0.01	1.80 ±0.02	1.92 ±0.01	1.97 ±0.01	1.98 ±0.01	2.00 ±0.01	2.01 ±0.01	2.02 ±0.01	2.01 ±0.01	2.07 ±0.01	2.10 ±0.01	2.11 ±0.01	2.12 ±0.01	2.11 ±0.01

Table-4. Effect of enrofloxacin administration (10mg/Kg body weight, in drinking water for 5 consecutive days) on haemagglutination inhibition titre (log 2) in broiler chicken.

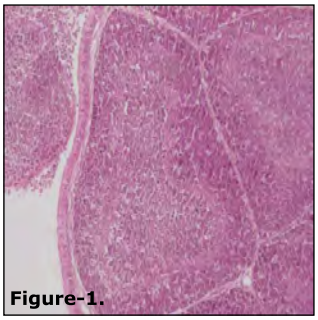
Group	Treatment period				Days post treatment				
	After 1st dose	2nd dose	3rd Dose	4th Dose	1	3	5	7	9
Control	2.67±0.21	2.83±0.40	2.67±0.33	2.83±0.40	2.83±0.31	2.67±0.21	2.67±0.33	2.50±0.22	2.50±0.22
Treatment	2.83±0.31	2.50±0.34	2.33±0.49	2.00±0.58	1.33[a]±0.42	1.33[a]±0.42	1.50[a]±0.22	1.67[a]±0.21	1.83[a]±0.17

Means bearing different superscript (a) within the column differ significantly ($p<0.05$), Mean±SE, n=6

pain on palpation of joints, no clinical signs and mortality were observed during the study period.

Effect on humoral immune response: There was significant reduction in HI titre of enrofloxacin administered birds when compared to that of control (Table-4). The reduction in HI titre noticed during the treatment period i.e. after 4th dose of enrofloxacin was not statistically significant, whereas, it was significant ($p<0.05$) after 5th dose and during the post treatment period. However, a tendency in increased HI titre was noticed from 5th day post treatment onwards.

Histopathological study: Bursa of Fabricius of control group showed normal medullary areas populated with lymphocytes (Fig-1). Histopathological lesions in the bursa of fabricius of 1 day post treatment group revealed the presence of lymphocyte depletion especially in the central portion of the lymphoid follicles in the medullary areas and increased lymphophagocytosis were also noticed (Fig-2). 3rd day post treatment group showed dispersion and depletion of lymphocytes in medullary areas and few areas of lymphoblastic degeneration (Fig-3). Whereas, lymphocyte depletion was mild with lymphocytosis (histiocyte proliferation) in 5th and 7th day post treatment groups (Fig- 4). Few birds from 9th day post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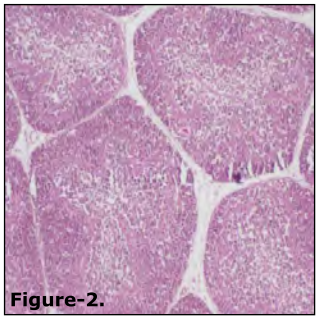
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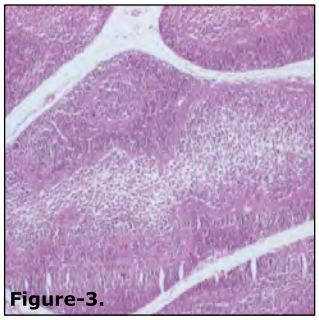
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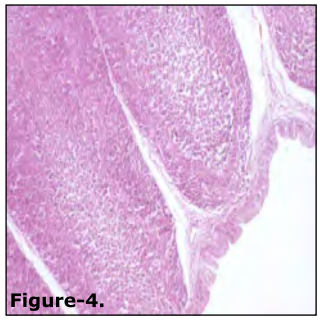
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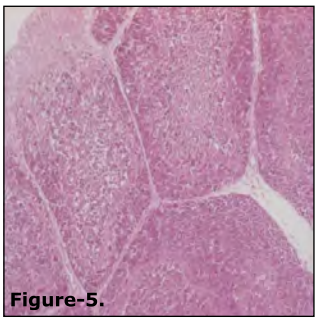
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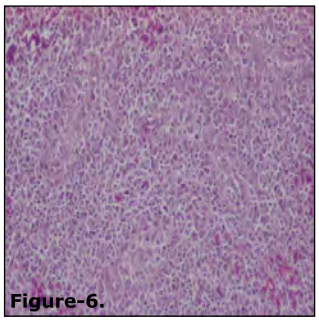
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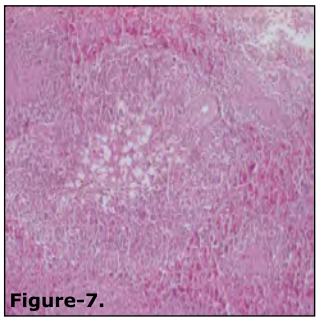
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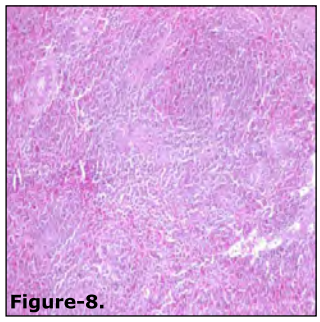
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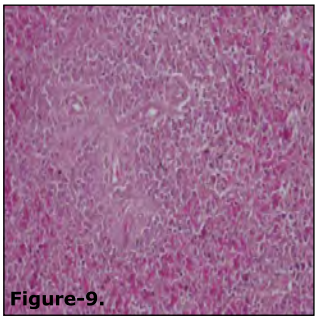
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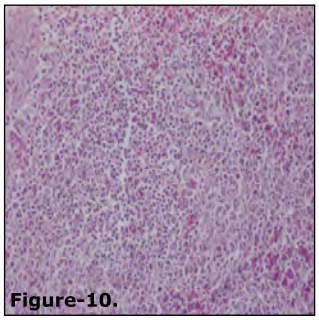
Figure-10.

Fig.1. Bursa of Fabricius of control group showing normal medullary areas populated with lymphocytes. H&E, 100X Fig.2. Bursa of Fabricius of 1st day post treatment group showing lymphocyte depletion from the medullary areas and increased lymphophagocytosis. H&E, 100X Fig.3. Bursa of Fabricius of 3rd day post treatment group showing dispersion and depletion of lymphocytes in medullary areas and areas of lymphoblastic degeneration H&E, 100X Fig.4. Bursa of Fabricius of 7th day post treatment group showing mild depletion of lymphocytes with lymphocytosis (histiocyte proliferation), H&E, 100X Fig.5. Bursa of Fabricius of 9th day post treatment group showing mild depletion of lymphocytes and areas of repopulation with lymphocytes, H&E, 100X Fig.6. Spleen of control group showing normal architecture. H&E, 200X Fig.7. Spleen of 1st day post treatment group showing severe lymphocytolysis and depletion with areas of haemorrhages. H&E, 100X Fig.8. Spleen of 3rd day post treatment group showing depletion of lymphocytes and proliferation of few blast cells. H&E, 100X Fig.9. Spleen of 7th day post treatment group showing mild depletion of lymphocytes and more proliferation of blast cells. H&E, 100X Fig.10. Spleen of 9th day post treatment group repopulated with lymphocytes. H&E, 200X

treatment group showed mild depletion and areas of repopulation with lymphocytes (Fig- 5).

Spleen of control group revealed normal architecture (Fig-6). Whereas, severe lymphocytolysis, depletion of lymphocytes with several areas of haemorrhages were noticed in spleen of 1 day post treatment group (Fig-7). 3rd day post treatment group showed depletion of lymphocytes around arterioles with mild haemorrhagic areas. In few birds blast cell proliferation were also noticed (Fig-8). Mild depletion of lymphocytes with lot of blast cell proliferation is the only appreciable lesion observed in 5th day and 7th day post treatment groups (Fig-9). Repopulation of lymphocytes was observed in 9th day post treatment group (Fig-10).

Discussion

In the present study, the enrofloxacin treated group showed no significant change in body weight, cumulative feed intake and feed efficiency when compared to that of control. These observations are in accordance with Roura *et al.* [17] who indicated that antibiotics are known to produce no significant improvement on growth performance under cleaner environment despite continuous administration in low doses. Further Porchezhian [8] also documented that ciprofloxacin did not have any influence on body weight in healthy broilers and suggested that fluoroquinolones has influence in increasing body weight only in infection, not in healthy condition. Indeed, Ahmad *et al.* [18] also described that enrofloxacin (25%) administration through drinking water did not have any effect on mean body weights and feed conversion ratio.

In the present study, enrofloxacin administration did not have any influence on the behaviour of the broilers and all the birds showed normal activity. Droppings of all the birds were semisolid, dark green and with a white uric acid deposition on the top. These findings were in conformity with Patel *et al.* [13], who studied the safety evaluation of levofloxacin following repeated oral dose administration in White Leg Horn layer birds and reported similar observations.

There was a significant reduction in humoral immune response as measured by haemagglutination inhibition (HI) titre against Newcastle Disease virus

(NDV) antigen of enrofloxacin dosed birds when compared to that of control. These findings which are indicative of immunosuppressive action of enrofloxacin are in accordance with the observations of Khalifeh *et al*. [1], who reported that enrofloxacin reduced the production of Newcastle Disease (ND) antibody in the first 3 week after the last ND vaccination as measured by HI test. The authors suggested that enrofloxacin exert its effect mainly on the IgM isotype and reduced the humoral immune response. Ellakany *et al*. [19] found that both the therapeutic (10mg/Kg body weight) as well as overdose (100mg/Kg body weight) of enrofloxacin had decreased the rate of protection against Newcastle Disease Virus vaccine (vNDV) challenge and opined that this could be attributed to the adverse effect of enrofloxacin on phagocytosis, low HI antibody titre and histopathological deformities in the lymphoid organs.

In conformity to Ellakany *et al*. [19], the decreased HI titre in the present study was in harmonious with histopathological changes observed in bursa of Fabricius and spleen which showed lymphocytic dispersion and depletion with several areas of lymphoblastic degeneration during the post treatment period.

However, a tendency in increased HI titre value was noticed in the present study from 5th day post treatment onwards. A similar downregulatory effect on humoral immunity with the use of other antibiotics such as ciprofloxacin, moxifloxacin, and clarithromycin in humans has been reported [20]. Further, the histopathological changes observed in bursa of Fabricius and spleen of enrofloxacin administered birds showed reversible trend as evidenced by repopulation with lymphocytes on 9th day post treatment. These findings are in compliance with Lemus and Blanco, [4] who suggested that immunodepression may occur shortly after antibiotic administration, but also that the immune system may recover quickly after antibiotic therapy. In contrast with other toxicants that cause an irreversible effect on the immune system, some effects of antibiotics may be quickly reversed [21, 22].

Conclusion

The immunosuppressive effect of enrofloxacin was found to be dose dependent as evidenced by reversal in HI titre and associated histopathological changes in lymphoid organs observed during post treatment period. The present study suggests that the immuno-modulatory activity of enrofloxacin may alter the immune response to vaccines if it is coadministered during vaccination of broiler chicken. However, enrofloxacin though it decreased the humoral immune response, it did not have any appreciable effect on performance of the broilers.

Authors' contributions

Part of the Ph.D. research work of the first author VS. GS was the adviser and designed the experiment. VS carried out the research work. JR involved in conduct of the biological trial and collection of blood and tissue samples during the experiment. GS and VS analysed the data, prepared and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interest.

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