Plasma biochemical indices at various stages of infection with a field isolate of *Eimeria tenella* in broiler chicken

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Abstract

Coccidiosis has a major impact on poultry industry as it affects broiler and layer birds of all age groups. Caecal coccidiosis, caused by *Eimeria tenella* is a very devastating enteric disease in broiler,which involves huge economic loss In present study, experimental infective dose of *Eimeria tenella* isolated from field was determined in broiler chicken and subsequent alterations in different plasma biochemical constituents were evaluated at interval of 5th, 7th and 9th day of post inoculation (PI) with the selected dose of 20000-25000 sporulated oocyst per bird. The dose was selected based on titration. A significant increase in plasma glucose, total cholesterol level and aspartate aminotransferase (AST) activity were observed where as a significant decrease in the level of total plasma protein, albumin, globulin, triglyceride and alanine aminotransferase (ALT) activity were evident during infection. Highest degree of infection was found on 7th day PI. Onward 9th day of PI onward clinical recovery was confirmed on the basis of pathognomonic caecal lesion score, clinical signs and symptoms.

Keywords: Coccidiosis, *Eimeria tenella*; Post Inoculation, Oocyst, Poultry.

Introduction

Poultry industry is one of the rapidly growing major segments of agriculture sector and has been showing a tremendous growth in India during the last few decades. But various diseases have also come into the way of growth. Coccidiosis, an enteric parasitic disease caused by protozoan parasite of various species under the genus Eimeria, is one of the most common and expensive diseases in the poultry industry (Shirley *et al*., 2007). It has a major economic impact on both growers and broiler poultry industry worldwide (Pinard-van der Laan *et al*., 2009). It is responsible for 6–10% mortality in broiler chicken and huge global economic loss due to impaired feed conversion, retarded growth (Tipu *et al*., 2002), additional cost of medication and deterioration of the meat quality (Aliyeva, 1999; Ahmedov *et al*., 2006). Severity of this disease is dependent on type of isolate, site of predilection of the parasite as well as dose of oocyst inoculum. *Eimeria tenella*, one of the most pathogenic species that parasitizes growing chickens and causes considerable financial loss to the poultry industry (Williams *et al*., 1999) through clinical and sub-clinical form of infection. A little information is available on infection pattern in broiler chicken and its subsequent effect on plasma biochemical constituents. Therefore, Present study was conducted with a field isolate of *Eimeria tenella* to determine experimental infective dose of the particular isolate in broiler chicken and plasma biochemical alterations at various stages of infection with the selected dose of innoculum.

Materials and Methods

Animal housing and management: Day old broiler chickens (Vencob-100) were procured from Hi-breed International, Kolkata and kept in deep litter system and coccidian free environment. Birds were provided with *ad libitum* drinking water and feed free from anti-coccidial drugs. On 5th day birds were vaccinated against Ranikhet Disease.

Experimental design: Two trials were conducted separately. Trial-1 for determining the infective dose of *Eimeria tenella* causing 80-90% morbidity with 10-20% mortality and Trial-2 for studying the changes in different plasma biochemical constituents at various stages of infection.

Collection and sporulation of oocysts: Oocysts,

Table-1. Showing determination of experimental infective dose of Eimeria tenella.

No. of birds in each group (n=12)	Number of oocyst inoculated per bird	No. of Sick chicken	No. of Dead chicken	Caecal lesion score * (Mean ± SE)
Infected	5000-6000	4 (33.33%)	0 (0.00%)	1.17 ± 0.166
	10000-12000	6 (50.00%)	0 (0.00%)	2.17 ± 0.207
	20000-25000	10 (83.33%)	2 (16.66%)	3.08 ± 0.193
	50000-55000	10 (83.33%)	6 (50.00%)	3.75 ± 0.130
	95000-100000	10 (83.33%)	8 (66.66%)	3.92 ± 0.083
Healthy	NIL	0 (0.00%)	0 (0.00%)	NIL

* Caecal lesion scored was made based on macroscopic lesion of caecum. A scale of 1-4 was used
1. Very mild degree of infection, 2. Mild degree of infection, 3. Moderate degree of infection, 4. Severe degree of infection.

collected from a field outbreak of caecal coccidiosis, were sporulated in 2.5% potassium dichromate solution. Identification and confirmation was done through morphometry and sporulation time (Conway and McKenzie, 1991).

Trial-1: For infective dose determination eighty-four unsexed day old broiler birds were randomly divided into 6 groups of 14 each. Two birds from each group were sacrificed and examined to confirm the absence of any parasitic stage of *Eimeria tenella* at 7th day of age. On the same day, five different doses (5000-6000, 10000-12000, 20000-25000, 50000-55000 and 95000-100000) of oocysts were inoculated to each group by crop inoculation keeping one group as healthy control. Infective dose was determined on 6-7 day post inoculation by clinical symptoms and caecal lesion scoring**.** (Johnson and Reid, 1970).

Trial-2: Twenty-eight unsexed day old broilers chicks were randomly divided into two groups of 14 each. At 7th day of their age two birds from each group were sacrificed to confirm the absence of any parasitic stage of *Eimeria tenella* and one group was inoculated with 20000-25000 of sporulated oocyst by crop inoculation keeping other group as healthy control. Heart blood was collected from both healthy control and infected group on 5th, 7th and 9th day post inoculation.

Sample collection and storage: Blood samples were collected in heparinized vials and immediately centrifuged to separate plasma. Separated plasma was stored at -20^{0}C for biochemical analysis.

Plasma biochemical indices: Plasma glucose was estimated by Glucose Oxidase Method (Trinder, 1969) using 4- amino phenazone as oxygen receptor.

Total plasma protein and plasma albumin of the samples were determined following Biuret Method (Reinfold,1953) using Biuret Reagent [Potassium hydroxide and hydrated copper (II) sulphate, together with potassium sodium tartarate]. Plasma globulin was calculated based on total plasma protein and plasma albumin value.

Automated enzyme method (Fletcher, 1968) was used to estimate plasma triglyceride and direct enzymatic method (Wybenga *et al., 1970*) for estimation of total plasma cholesterol. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activity was determined by method of Bergmeyer and Bernt (1974) using 2, 4- Di-Nitro-Phenyl-Hydrazine through formation of coloured hydrazone complex in presence of sodium hydroxide.

Statistical analysis: Data were partitioned and subjected to statistical analysis by Student t-test and significance at 5% (P<0.05) and 1% (P<0.01) level were tested by Duncan Method (Snedecor and Cochran, 1967).

Results

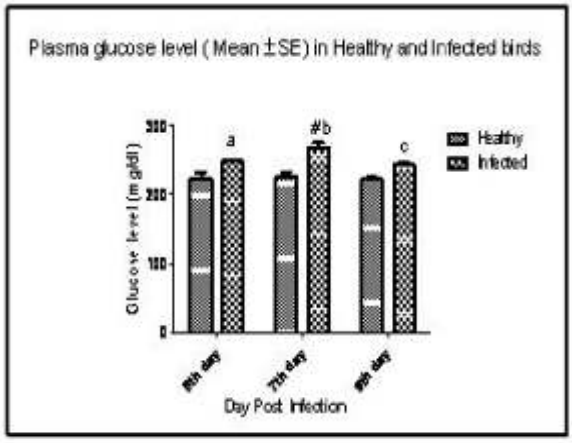
Infective dose for the experiment**:** From Trial-1 the infective dose for caecal coccidiosis with sporulated oocyst was determined based on significant symptoms of droopiness, huddling, anorexia, emaciation, bloody dysentery in live birds and caecal score lesions in dead birds as well birds sacrificed at the end of trial-1. The infective dose of *Eimeria tenella* oocyst for the trial was considered as 20000-25000 of sporulated oocysts per bird (Table-1). Control group showed neither any death nor any symptom.

Plasma Biochemical Constituents:

Plasma glucose and total plasma protein: Trial -2 revealed a significant (P<0.05) variation in blood glucose level between healthy control and infected group on 5th day, 9th day and a highly significant (P<0.01) variation on 7th day of PI. In infected group, a significant (P<0.05) variation was observed on 7th day when compared to 5th day and 9th day of PI (Figure-1).

Hypopreoteinemia in infected group with highly significant (P<0.01) variation from healthy group was evident on 5th, 7th and 9th day PI. Although a highly significant (P<0.01) variation was observed between 5th and 7th day as well as 7th and 9th day but no significant (P>0.05) variation was noted between 5th and 9th day PI in the infected group (Figure-2).

Plasma albumin and globulin: Plasma albumin



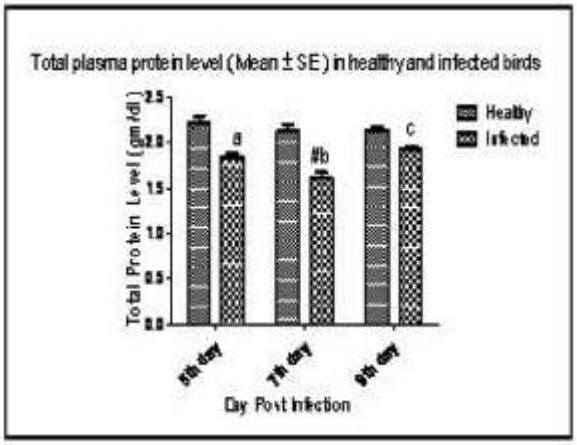
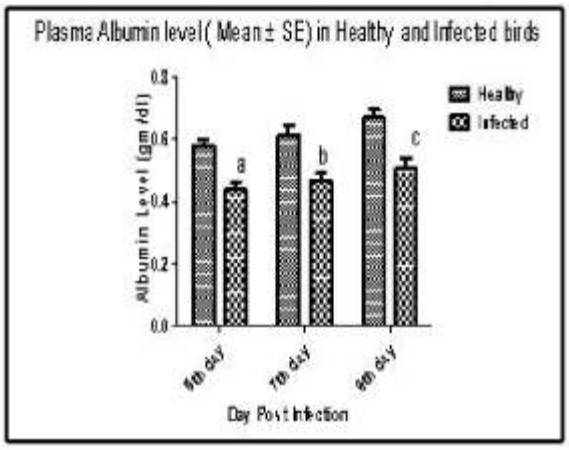


Figure-1. Plasma glucose and Figure-2. Plasma total protein level in healthy and infected birds on different days of Post infection. Different superscripts (a,b,c) denote that mean values differ significantly as compared to healthy control group at the period of observation and # denotes significant difference within infected group between different periods of observation.



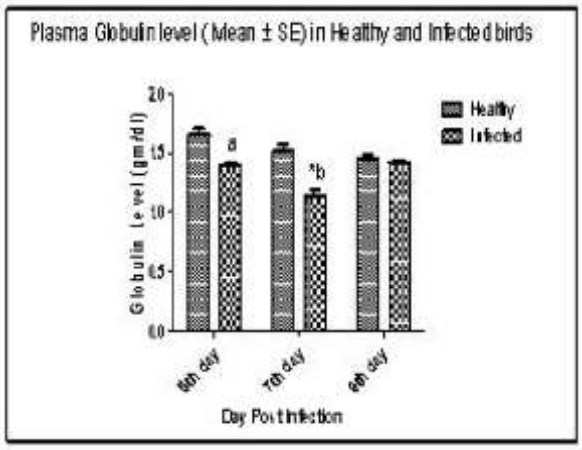


Figure-3. Plasma Albumin and Figure-4. Plasma Globulin level in healthy and infected birds on different days of Post infection. Different superscripts (a,b,c) denote that mean values differ significantly as compared to healthy control group at the period of observation and * denotes significant difference within infected group between different periods of observation.

level showed a highly significant (P<0.01) variation between healthy and infected group on 5th, 7th and 9th day PI but no variation was observed in infected group on various days PI(Figure-3).

A highly significant (P<0.01) variation in plasma globulin level was evident on 5th and 7th day PI between healthy and infected group but without any observable variation on 9th day PI. A significant (P<0.05) variation in infected group was noted on 7th day PI compared to 5th and 9th day PI (Figure-4).

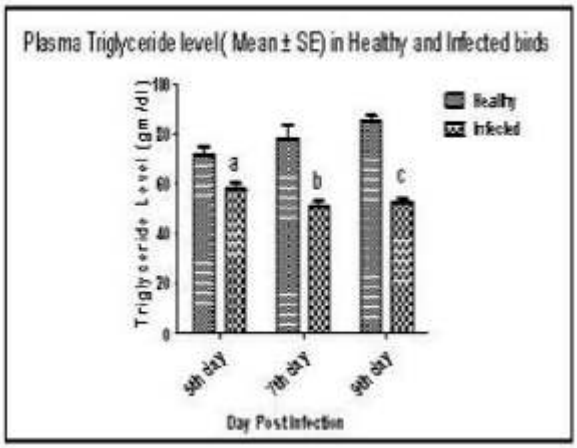
Plasma triglyceride and cholesterol: A highly significant (P<0.01) fall in plasma tri-glyceride (Figure-5) and subsequent rise plasma cholesterol (Figure-6) level was evident in infected group compared to healthy control group on different days (5th, 7th and 9th) of PI but no significant (P>0.05) variation (fall/rise) in the same was observed in infected group on different days of PI.

Plasma AST and ALT activity: A highly significant (P<0.01) rise in plasma AST activity in infected group was observed compared to healthy control group on different days PI but no significant (P>0.05) variation was observed in infected group on different days (5th, 7th and 9th) PI(Figure-7).

Highly significant (P<0.01) fall in plasma ALT activity in infected group on different day PI was noted compared to healthy control group but with no significant (P>0.05) variation in infected group on different days (5th, 7th and 9th) PI (Figure-8).

Discussion

Coccidiosis is caused by the development and



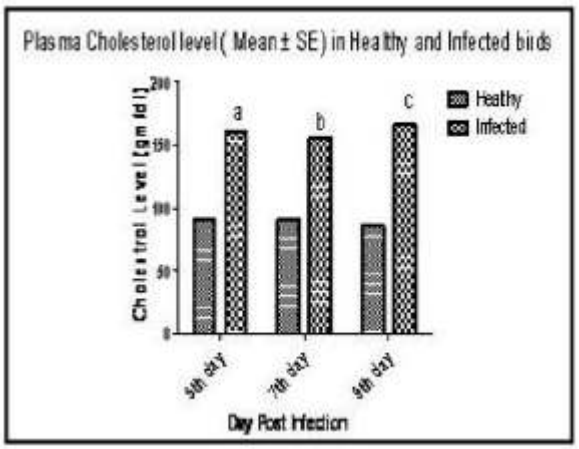
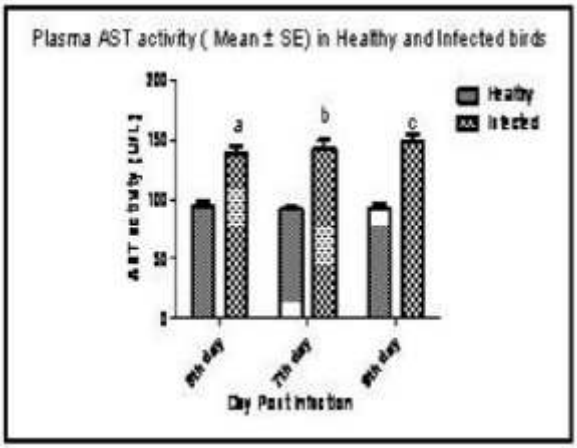


Figure-5. Plasma Triglyceride and Figure-6. Plasma Cholesterol level in healthy and infected birds on different days of Post infection. Different superscripts (a,b,c) denote that mean values differ significantly as compared to healthy control group at the period of observation.



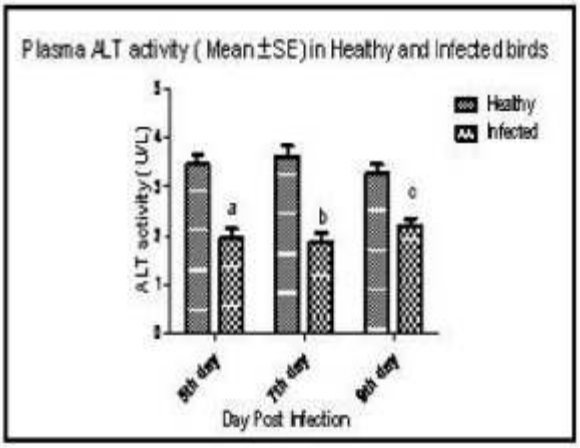


Figure-7. Plasma AST and Figure-8. Plasma ALT level in healthy and infected birds on different days of Post infection. Different superscripts (a,b,c) denote that mean values differ significantly as compared to healthy control group at the period of observation.

multiplication of coccidia oocyst in the epithelial cells of the intestine. Positive correlations exist between number of oocysts inoculated and degree of infection in poultry birds. With the increase in inoculum titre, pathognomonic lesion score increases along with percentage of sick and dead birds. Table-1 showed that low dose of oocyst caused a morbidity of around 50%, where as higher dose led to more than 80% morbidity with a high death percentage. Due to inoculation of higher number of sporulated oocysts, gut system succumbs to infection being unable to fight against the high parasitic load.

The significant rise in plasma glucose level may be due to increased glycogenolysis caused by stress induced release of adreno-corticoid leading to hyperglycemia or disturbed carbohydrate metabolism because of interference with phosphorylative carbohydrate dissimilation by an unidentified factor present in caecum of poultry as suggested by Daugherty and Herrick (1952). These results corroborate with the findings of Kumar and Rawat (1975). Highest level of blood glucose on day 7 PI and gradual fall in following days suggests highest degree of stress due to maximum infection load on day 7 PI, after which birds passes through recovery phase as confirmed by the PM lesion. Low feed intake during acute infective stage may lead to secretion of adrenal cortisones/ corticosteroids promoting glycogenolysis. (Patra *et al.*, 2010).

Fall in total plasma protein (hypo-proteinemia) in the coccidia infected birds might be due to acute stress that leads to cortisol secretion and catabolism of

protein (Kaneko *et al.*, 1997). Acute hemorrhage on day 7 PI causes large loss of plasma protein followed by rapid movement of interstitial fluid without protein into the plasma compartment to induce acute hypo-proteinemia. On day 9 PI protein level reaches normal suggesting a recovery state that was confirmed by post mortem examination.

Allen (1988) and Pascalon (1998) reported fall in plasma triglyceride in chick and duck infected with *Eimeria tenella* and *Eimeria mulardi* respectively. Decreased plasma level of triglyceride in *Eimeria acervulina* infected chicken may be due to anorexia and malabsorption of nutrients in chicken host Turk (1978). Anorexia may be a major reason for declined triglyceride level in the coccidia affected birds. In malnutrition, high rate of fat mobilization from fat depot to their depositions and the disappearance of most of the adipose tissue are evident. Severe thiamine deficiency cause decreased lipogenesis from carbohydrate (West *et al.*, 1966). Disturbance in B-vitamin synthesis in severe coccidiosis may hinder lipogenesis from carbohydrate, but discussion on this matter is beyond scope of the present study. Moreover glucagons inhibit fatty acid synthesis, which may be the reason behind decreased triglyceride level in plasma. Allen and Mc Murtry (1984) reported that non-pancreatic glucagons activity is greatly increased in infected chick plasma on day 7 PI. Increased cholesterol level with subsequent decrease in plasma triglyceride may be attributed to decreased billiary excretion of cholesterol in anorexia causing increased blood cholesterol despite of reduced synthesis.

Significant damage of cell lining of the caecal wall along with their inflammation and severe blood loss causing tissue loss from the body may attribute to increased AST activity as supported by Coles (1986) and Montgomery *et al.*, (1990) with their findings of significant alteration of serum SGOT profile in cellular membrane degeneration, inflammation and diffused tissue degeneration and loss.

Fukuta *et al.*, (1997) reported slight change in ALT activity in chicken infected with *E. tenella* and *E. acervulina*. Little but not significant, alteration in plasma ALT activity in the present study may be due to less feed intake as suggested by Gessler (1965).

Therefore it may be concluded that, present study may be regarded as a tool to estimate severity of infection on different days of *Eimeria tenella* infection on the basis of biochemical profile along with signs, symptoms and lesion score.

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biochemical changes in broiler chicken naturally infected with Eimeria tenella. A Polymerase Chain Reaction. (PCR) based assay was also done for the detection, identification and differentiation of pathogenic species. of Eimeria in poultry. Post mortem examination revealed petechial haemorrhages, oedema, necrosis and.Â In regard to biochemical parameter, in this experiment (AST, ALT activities) showed a significant elevation in serum activities of these enzymes in infected group with Eimeria acervulina when compared with non-infected group. Similar out comes have been observed by (Patra et al., 2010;Dar et al., 2014) and can explained by them as increase in the level of serum enzymes might be due to cellular damage particularly of hepatocytes. Antioxidant status of broiler chickens, infected with Eimeria acervulina. V. KOINARSKI*1, N. GEORGIEVA2, V. GADJEVA3 and P. PETKOV4. 1 Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Studentâ€™s Campus, 6000, Stara Zagora, Bulgaria.Â The high levels of infection with this Eimeria species result in significant lower live body weights of chickens [15] and a more severe aflatoxicosis in the infec-ted birds [31]. The invasion is reported to induce serious his-. topathological alterations in avian intestines and parenchy-mal organs [20]. There are several reports evidencing the presence of antioxidant desequilibrium in birds with parasitic diseases. Plasma biochemical indices at various stages of infection with a field isolate of Eimeria tenella in broiler chicken. Vet World.; 4 (9): 404â€“409. [27]. Basith AS, Rajavelu G and Manohar MB (1998). Biochemical studies in experimental Eimeria necatrix infection in chickens. Indian Vet J.; 75 (10): 876â€“878. [28]. Patra G. Rajkhowa, Ayub M, Tiwary JG and Sailo L (2009). Studies on clinical, gross, histopathological and biochemical parameters in broiler birds suffered from Eimeria necatrix infection in Aizawl District of Mizoram, India. Int J Poult Sci.; 8 (11): 1104â€“6.Â The economic impact of infection with Eimeria spp. in broiler farms from Romania. Rev Bras Zootec.; 45 (5): 273â€“280. sub-clinical form of infection. A little information is available on infection pattern in broiler chicken and its subsequent effect on plasma biochemical constituents. Therefore, Present study was conducted with a field isolate of Eimeria tenella to determine experimental infective dose of the particular isolate in broiler chicken and plasma biochemical alterations at various stages of infection with the selected dose of innoculum. Materials and Methods. Animal housing and management: Day old broiler chickens (Vencob-100) were procured from Hi-breed International, Kolkata and kept in deep litt