SERUM BIOCHEMICAL ALTERATIONS IN EXPERIMENTAL *Escherichia coli* INFECTION IN BROILER CHICKEN

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Abstract

An experiment was conducted to study the serum biochemical changes in Escherichia coli infection in broiler chicken. Sixty numbers of day-old broiler chicks were randomly divided into two groups of thirty chicks each. The group one was determined as control group (T1) and group two as treatment group (T2). Treatment group was inoculated intra-tracheally with 0.25ml of inoculum containing E.coli (3×10^7 cfu/ml) was administered intra-tracheally and pathological studies were done seven days post inoculation. The serum biochemical analysis revealed significant reduction in total serum protein, albumin, globulin and significant increase in albumin: globulin ratio, Alanine amino transferase, Aspartate amino transferase, cholesterol and creatinine concentrations.

Key words: Biochemical alterations, Broiler chicken, *Escherichia coli*.

Broiler production is well flourished in India and it plays a vital role in providing employment and thereby improving economic status of the country. This rapid growth was possible due to the availability of high producing germ plasm, better feeding and managemental practices, better health care and financial aids offered by government to support poultry farmers. The main reasons were less requirement of capital, less space for broiler farms, quick and early returns and higher demands of broiler meat due to cheap price and remunerative value in comparison to other types of meat.

Colibacillosis, caused by *Escherichia coli* is very common in poultry. The high production efficiency and faster growth rate makes the poultry highly vulnerable to diseases. The toxin produced by the organism and the clinical disease cause high mortality among domestic and wild birds. A little information is available on the effect of *E. coli* infection on serum biochemical factors. Therefore, present study was conducted to determine serum biochemical alterations caused by experimental *E. coli* infection in broiler chicken.

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Materials and Methods

Sixty numbers of day-old broiler chicks, belonging to Cobb 400 procured from a local broiler hatchery were used for the study. All the birds were maintained under standard management practices. All the birds were vaccinated on 7th day of age (7 D) against Ranikhet disease. Feed and water were provided *ad lib*.

Escherichia coli was isolated from birds brought for postmortem to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy which had died due to natural cases of colibacillosis. The isolate which was characterized by Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy was used for the study.

The inoculum was prepared from the pure culture of *Escherichia coli.* A loop full of the culture was transferred to brain-heart infusion broth and incubated at 37ºC overnight to ensure the growth of the bacteria. The concentration of bacteria in the broth was assessed by measuring optical density at 600 nm using spectrophotometer. The concentration was adjusted in such a way that one ml of brain-heart infusion broth contains 3×10^7 cfu of *E.coli.* From this inoculum, 0.25 ml was administered intra-tracheally to six chicks each in T2 on 1, 7, 14, 21 and 28 days of age (Goren, 1978). The serum biochemical analysis was done seven days post inoculation (7 D,14 D,21 D,28 D and 35 D).

Blood samples were collected from the birds on the day of sacrifice in vials without anticoagulant for serum separation. The separated serum was aliquoted in sterile tubes and stored at -20ºC for biochemical analysis. Serum analysis was carried out in semi-automated biochemical analyser (Make-Hospitex, Italy; Model- Master T). The individual serum samples were analysed for total protein by Biuret method (Inchiosa, 1964), albumin by bromocresol green dye binding method (Doumas *et al.,* 1971), creatinine by modified Jaffe's method (Fabiny and Ertinghausen, 1971), aspartate amino transferase (AST) and alanine amino transferase (ALT) by IFCC (International Federation of Clinical Chemistry) method and cholesterol (Siedel *et al.,* 1983) by calorimetric enzymatic methods using standard diagnostic kits from Spinreact, Spain. Serum globulin values were determined by deducting serum albumin values from total serum protein values. Albumin: Globulin ratio was also calculated.

Data on different parameters were analysed by repeated measures ANOVA using SPSS version 21.0.

Results and Discussion

Mean (± SE) total protein values of T1 and T2 birds is presented in Table 1. Birds of T2 showed significant reduction in serum total protein value when compared with control birds (T1) during all stages of sacrifice. Zaki *et al.* (2012) and Ahmed *et al.* (2013) reported that the reduction in serum total protein observed may be due to the injury of liver and kidney induced by the bacteria.

Mean (± SE) albumin and globulin values of T2 and control birds is presented in Table 2. The birds of T2 group showed a significant reduction in albumin value on comparison with their respective control birds except on 7D and 28D. Rath *et al*. (2017), Christie and Halliday (1979) and Zaki *et al*. (2012) observed a reduction in albumin levels in broiler chicken experimentally infected with *E.coli.* The infection could have caused injury to liver causing failure of albumin synthesis. The globulin values of T2 birds were significantly reduced than that of control birds (T1) during the entire observation period. Mean (± SE) albumin : globulin ratio of treatment and control birds in the experiment are presented in Table 3. The albumin globulin ratio of the T2 birds were higher than the control group throughout the experiment. A:G ratio of T2 were significantly higher than T1 on 21 D and 35 D. The highest value of A:G was observed on 21 D. In this study, the reduction in total serum protein, albumin and globulin fractions is due to the liver damage caused by the infective agent leading to a decrease in protein synthesis and absorption. The reduction in globulin fractions shows that

Table 1. Mean (± SE) serum total protein of broiler birds

Parameter / Age	Total protein (g/dl)	
	T1	T2
7 D	2.552± 0.049^{Ba}	2.203± 0.049^{Aa}
14 D	2.761± 0.092^{Bb}	2.307± 0.092^{Aa}
21 D	2.856± 0.117^{Bbc}	2.317± 0.117^{Aab}
28 D	3.138± 0.108^{Bbd}	2.225± 0.108^{Aa}
35 D	3.326± 0.096^{Bde}	2.709± 0.096^{Ab}

Means bearing the same superscript within the same column (a-e) and same rows (A-B) do no differ significantly (P≤ 0.05)

Table 2. Mean (± SE) albumin and globulin of broiler birds

Parameter / Age	Albumin		Globulin	
	T1	T2	T1	T2
7 D	1.393±0.027^{Ba}	1.212±0.027^{Aa}	1.158±0.044^{Ba}	0.992±0.044^{Aa}
14 D	1.447±0.76^{Aab}	1.464±0.76^{Ab}	1.314± 0.074^{Ba}	0.846± 0.074^{Aabc}
21 D	1.717±0.086^{Abd}	1.712±0.086^{Abc}	1.138± 0.089^{Ba}	0.605± 0.089^{Ab}
28 D	2.206±0.076^{Bc}	1.481±0.076^{Abd}	1.132±0.068^{Ba}	0.744± 0.068^{Abc}
35 D	1.774±0.085^{Acd}	1.595±0.085^{Abcde}	1.557±0.046^{Bb}	1.114±0.046^{Ad}

Means bearing the same superscript within the same column (a-e) and same rows (A-B) do not differ significantly (P≤ 0.05)

Table 3. Mean (± SE) Albumin : Globulin ratio of broiler chicks

Parameter / Age	Albumin : Globulin ratio	
	T1	T2
7 D	1.209± 0.067^{Aa}	1.241± 0.067^{Aa}
14 D	1.102± 0.280^{Aa}	1.931± 0.280^{Ab}
21 D	1.580± 0.357^{Bab}	3.168± 0.357^{Abc}
28 D	1.829± 0.133 ^{Ab}	1.992± 0.133 ^{Abd}
35 D	1.146± 0.085^{Ba}	1.439± 0.085^{Abc}

Means bearing the same superscript within the same column (a-c) and same rows (A-B) do not differ significantly (P≤ 0.05)

Table 4. Mean (± SE) Alanine amino transferase and Aspartate amino transferase of broiler chicks

Parameter / Age	ALT		AST	
	T1	T2	T1	T2
7 D	30.013± 1.086^{Ba}	46.092± 1.086^{Aa}	114.183± 5.528^{Ba}	139.808± 5.528^{Aa}
14 D	38.630± 0.954^{Bb}	97.280± 0.954^{Ab}	143.390± 2.549^{Bb}	174.238± 2.549^{Ab}
21 D	39.972± 2.553^{Bbc}	120.058± 2.553^{Ac}	141.738± 1.536^{Bbc}	194.760± 1.536^{Ac}
28 D	43. 062± 1.748^{Bcd}	138.328± 1.748^{Ad}	141.688± 1.973^{Bbcd}	208.838± 1.973^{Ad}
35 D	47.717± 1.597^{Bde}	145.272± 1.597^{Ae}	145.437± 1.158^{Bbcde}	214.957± 1.158^{Ae}

Means bearing the same superscript within the same column (a-e) and same rows (A-B) do not differ significantly (P≤ 0.05)

Table 5. Mean (± SE) Cholesterol and Creatinine of broiler chicks

Parameter / Age	Cholesterol		Creatinine	
	T1	T2	T1	T2
7 D	122.278±1.524^{Ba}	138.395±1.524^{Aa}	0.346± 0.033^{Ba}	0.768±0.033^{Aa}
14 D	141.672±1.959^{Ab}	146.240±1.959^{Ab}	0.365±0.064^{Bab}	0.905± 0.064^{Aab}
21 D	151.418±3.443^{Bc}	165.842±3.443^{Ac}	0.519±0.042^{Bb}	1.088±0.042^{Ab}
28 D	159.405±8.122^{Abcd}	182.950±8.122 ^{Acd}	0.762±0.035^{Bc}	1.315±0.035^{Ac}
35 D	175.478± 6.055 ^{Ae}	179.033± 6.055 ^{Acde}	0.934± 0.045^{Bd}	1.693±0.045^{Ad}

Means bearing the same superscript within the same column (a-e) and same rows (A-B) do not differ significantly (P≤ 0.05)

the birds are having very low immune status. The A: G ratio was higher in the infected birds compared to control and this shows that the albumin and globulin were diluted to the same extent in serum.

Mean (± SE) ALT and AST values of T2 and control birds in the experiment are presented in Table 4. The ALT values of T2 were significantly higher than T1 during all stages of sacrifice. Ghany *et al.* (2013) reported that *E.coli* infection led to increased activity of liver enzymes like ALT. The AST values of T2 group significantly increased from 7 D to 35 D in comparison with the control group. The elevation of liver enzymes may be observed in avian species due to damage of liver parenchyma or bile duct system damage. The enzymes like ALT and AST are located in cytoplasm and mitochondria of hepatocytes and during injury they leak out into blood plasma. The elevation in serum AST levels may be due to hepatic injury caused by the bacteria. In our study, the elevated levels of ALT and AST are due to the enhanced cell membrane permeability of hepatocytes induced by the bacteria or its toxin.

Mean (± SE) cholesterol and creatinine values of T2 birds and control birds of the experiment are presented in Table 5. The mean cholesterol values of treatment group (T2) were significantly higher than the control group (T1) on 7 D and 21 D. The elevated levels of cholesterol noticed in this study could be due to impaired liver function of production of bile. Bile is mainly responsible for digestion of fat and processing of cholesterol. Due to the injury to liver, the production of bile was reduced leading to elevated cholesterol levels in serum. The creatinine values of T2 were significantly higher than the control groups on all days of sacrifice. The creatinine values were significantly increased from 7 D to 35 D within the T2 group. The elevated levels of creatinine could be due to the kidney damage caused by the bacteria and their toxin leading to increased catabolism of protein or decreased elimination of creatinine.

In this study, *E.coli* induced toxic damage to liver and kidneys was manifested as a decrease in total serum protein, albumin and globulin levels but an elevation in A:G ratio, ALT, AST, creatinine and cholesterol levels.

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