

Received -02.07.2016 Accepted -04.07.2016

Abstract

A study was conducted in 72 day-old broiler birds to evaluate the variation in serum parameters in subinfective dose of coccidiosis under sublethal ochratoxicosis for a period of 21 days. Treatment group was fed with ochratoxin A (OTA) and oocysts of Eimeria tenella and serum parameters were analysed day one, three and seven post-inoculation. Significant increase was observed in serum aspartate amino transferase, creatinine, uric acid and urea concentration while significant decrease was noticed in total protein, albumin, globulin and A/G ratio.

Keywords: Ochratoxicosis, coccidiosis, Eimeria tenella

Broiler industry is one of the rapidly growing segments of agriculture sector, showing tremendous growth in India over the last few decades. Ochratoxicosis is one of the most common mycotoxicosis and coccidiosis

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Mannuthy, Thrissur, Kerala. is an enteric protozoan disease common in broiler chicken in Kerala. Both diseases together contribute to huge economic loss to the industry. Even though OTA is primarily a nephrotoxic agent, it is hepatotoxic as well as immunosuppressant even at very low dose. So this could render the bird highly susceptible to

Eimeria tenella, the most pathogenic species in

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Materials and Methods

poultry.

Present research work was conducted in the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy. Ochratoxin was produced from *Aspergillus ochraceus* culture NRRL 3174 (Trenk *et al.*, 1971). Pooled wheat culture powder yielded 1 ppm of OTA. Oocysts of *E. tenella* obtained from natural infection was artificially sporulated in the laboratory using two percent potassium dichromate solution and standardised to sublethal dose of 5,000-6,000 oocyst/ml (Long *et al.*, 1975).

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Experiment was conducted on 72 davold broiler chicks divided into four groups each containing 18 birds and maintained in deep litter system for a period of 21 days. Group T1 was given normal broiler feed. In group T2, OTA at the rate of 300 ppb was added to the feed with coccidiostat and without any toxin binder. Birds of aroup T3 were given broiler feed with toxin binder and without coccidiostat. Birds of group T4 were given broiler feed with OTA at the rate of 300 ppb without toxin binder and coccidiostat. Sporulated oocyst of E. tenella at the dose rate of 5000-6000 oocyst were instilled orally to treatment groups T3 and T4 on 14th day of the experiment. Birds were sacrificed on first day post inoculation (1 PI), third day post inoculation (3 PI) and seventh day post inoculation (7 PI) to evaluate the variation in serum biochemical parameters. Data was analysed statistically with SPSS version 21.0.

Results and Discussion

Mean $(\pm SE)$ aspartate amino transferase (AST) values of controls and treatment broiler birds of the experiment are presented in Table 1. Birds of group T4 showed a significant increase in serum levels of AST when compared to T2 and T3. This result was in agreement with the finding of Koynarski *et al.* (2007). Significant increase in AST in chicks exposed to both OTA and coccidia could be due to severe degenerative changes in internal organs.

Mean $(\pm SE)$ total protein, albumin, globulin and albumin globulin ratio (A/G) of controls and treatment broiler birds of the

experiment are presented in Table 2.Significant decrease in total protein was observed in T4 when compared to T2 and T3 on 3PI and 7PI. Birds of group T4 showed significant decrease in albumin when compared to T2 and T3. Mean (\pm SE) of T4 on 7PI was 0.98 \pm 0.02. Compared to T1, birds of group T2 showed significant decrease in serum albumin throughout the experiment. There was significant reduction in serum globulin level in T4 birds when compared to T2 birds on 3PI and 7PI, while significant reduction was observed throughout the experiment when compared to T3. There was significant elevation of A/G in T4 birds when compared to T2 on 7PI.

This observation of reduction of TP, albumin and globulin in OTA treated chicks is in agreement with Elaroussi et al. (2008), while Mondal et al. (2011) reported similar significant reduction in these parameters in chicks infected with E tenella. Low protein, albumin and globulin level could be due to impairment of hepatic protein synthesis as well as due to loss in urine as a result of renal damage (Elaroussi et al., 2008). Similar observation of reduction in A/G ratio in broiler chicken exposed to OTA was reported by Pozzo et al. (2013). Reduction in total protein, albumin and globulin in coccidiosis could be as a result of acute haemorrhage followed by rapid movement of interstitial fluid into the plasma compartment (Mondal et al., 2011). So due to the combined effect of both toxin and coccidia, T4 showed significant reduction in these parameters.

Mean (±SE) creatinine, uric acid and urea values of controls and treatment birds of

Group	AST values (IU/L)					
Group	1PI	3PI	7PI			
T1	202.45 ^{Ac} ±0.54	195.40 ^{Bd} ±0.40	177.98 ^{cd} ±0.45			
T2	214.33 ^{Ab} ±0.38	210.5 ^{Bb} ±0.33	206.20 ^{cc} ±0.15			
Т3	201.25 ^{Cd} ±0.22	204.81 ^{Bc} ±0.16	212.73 ^{Ab} ±0.16			
T4	216.13 ^{ca} ±0.15	219.40 ^{Ba} ±0.14	235.65 ^{Aa} ±0.12			

Means bearing the same superscript within the same column (a-d) and same rows (A-C) do not differ significantly (P<0.05).

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Total protein(g/dl)		Albumin(g/dl)		Globulin(g/dl)			Albumin globulin ratio				
1PI	3PI	7PI	1PI	3PI	7PI	1PI	3PI	7PI	1PI	3PI	7PI
3.91 ^{Ab}	3.94 ^{Ad}	4.15 ^{Bd}	1.29 ^{Ab}	1.41 ^в	1.61 ^{cd}	2.62 ^b	2.53 ^d	2.54 ^d	0.49 ^{cb}	0.56 ^в	0.63 ^{Ab}
±0.03	±0.03	±0.04	±0.01	±0.02	±0.03	±0.04	±0.04	±0.03	±0.01	±0.01	±0.02
3.50 ^{Ba}	3.43 ^{Bb}	3.14 ^{Ab}	1.24ª	1.24ª	1.26 ^b	2.27 ^{Ba}	2.19 ^{вь}	1.88 ^{Ab}	0.54 ^{Ba}	0.57 ^в	0.67 ^{Aa}
±0.04	±0.04	±0.05	±0.02	±0.01	±0.02	±0.03	±0.03	±0.04	±0.01	±0.01	±0.02
3.90 ^{cb}	3.71 ^{Bc}	3.47 ^{A c}	1.31 [♭]	1.31⁵	1.32°	2.59 ^{cb}	2.40 ^{Bc}	2.15 ^{Ac}	0.50 ^{Cb}	0.55 ^в	0.61 ^{Ab}
±0.04	±0.03	±0.02	±0.01	±0.01	±0.01	±0.03	±0.03	±0.03	±0.01	±0.01	±0.01
3.45 ^{ca}	3.22 ^{Ba}	2.58 ^{Aa}	1.22 ^{Ba}	1.20 ^{Ba}	0.98 ^{Aa}	1.23 ^{Aa}	2.02 ^{Ca}	1.60 ^{Ba}	0.55 ^{Ba}	0.60 ^A	0.62 ^{Ab}
±0.02	±0.03	±0.02	±0.01	±0.02	±0.02	±0.02	±0.03	±0.04	±0.01	±0.01	±0.03
	Tota 1PI 3.91 ^{Ab} ±0.03 3.50 ^{Ba} ±0.04 3.90 ^{Cb} ±0.04 3.45 ^{Ca}	Total protein(1Pl 3Pl 3.91 ^{Ab} 3.94 ^{Ad} ±0.03 ±0.03 3.50 ^{Ba} 3.43 ^{Bb} ±0.04 ±0.04 3.90 ^{Cb} 3.71 ^{Bc} ±0.03 3.22 ^{Ba}	Total protein(g/dl) 1Pl 3Pl 7Pl 3.91^{Ab} 3.94^{Ad} 4.15^{Bd} ± 0.03 ± 0.03 ± 0.04 3.50^{Ba} 3.43^{Bb} 3.14^{Ab} ± 0.04 ± 0.04 ± 0.05 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} ± 0.04 ± 0.03 ± 0.02 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} ± 0.04 ± 0.03 ± 0.02 3.45^{Ca} 3.22^{Ba} 2.58^{Aa}	Total protein(g/dl) All TPI 3PI 7PI 1PI 3.91^{Ab} 3.94^{Ad} 4.15^{Bd} 1.29^{Ab} ± 0.03 ± 0.03 ± 0.04 1.29^{Ab} ± 0.03 ± 0.03 ± 0.04 ± 0.01 3.50^{Ba} 3.43^{Bb} 3.14^{Ab} 1.24^{a} ± 0.04 ± 0.04 ± 0.02 ± 0.02 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} ± 0.04 ± 0.03 ± 0.02 ± 0.01 3.45^{Ca} 3.22^{Ba} 2.58^{Aa} 1.22^{Ba}	Total protein(g/dl) Albumin(g/ TPI 3PI 7PI 1PI 3PI 3.91^{Ab} 3.94^{Ad} 4.15^{Bd} 1.29^{Ab} 1.41^{Bc} ± 0.03 ± 0.03 ± 0.04 ± 0.01 ± 0.02 3.50^{Ba} 3.43^{Bb} 3.14^{Ab} 1.24^{a} 1.24^{a} ± 0.04 ± 0.05 ± 0.02 ± 0.01 ± 0.02 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.31^{b} ± 0.04 ± 0.03 ± 0.02 ± 0.01 ± 0.01 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} ± 0.01 3.45^{Ca} 3.22^{Ba} 2.58^{Aa} 1.22^{Ba} 1.20^{Ba}	Total protein(g/dl) Albumin(g/dl) TPI 3PI 7PI 1PI 3PI 7PI 3.91 ^{Ab} 3.94 ^{Ad} 4.15 ^{Bd} 1.29 ^{Ab} 1.41 ^{Bc} 1.61 ^{Cd} ± 0.03 ± 0.04 ± 0.01 ± 0.02 ± 0.03 ± 0.03 3.50 ^{Ba} 3.43 ^{Bb} 3.14 ^{Ab} 1.24 ^a 1.24 ^a 1.26 ^b ± 0.04 ± 0.05 ± 0.02 ± 0.02 ± 0.02 ± 0.02 3.90 ^{Cb} 3.71 ^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} ± 0.01 ± 0.04 ± 0.03 ± 0.02 ± 0.01 ± 0.02 ± 0.01 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} ± 0.01 3.045^{Ca} 3.22^{Ba} 2.58^{Aa} 1.22^{Ba} 1.20^{Ba} 0.98^{Aa}	Total protein(g/dl) Albumin(g/dl) GI 1PI 3PI 7PI 1PI 3PI 7PI 1PI 3.91 ^{Ab} 3.94 ^{Ad} 4.15 ^{Bd} 1.29 ^{Ab} 1.41 ^{Bc} 1.61 ^{Cd} 2.62 ^b ± 0.03 ± 0.04 ± 0.01 ± 0.02 ± 0.03 ± 0.04 3.50 ^{Ba} 3.43 ^{Bb} 3.14 ^{Ab} 1.24 ^a 1.24 ^a 1.26 ^b 2.27 ^{Ba} ± 0.04 ± 0.05 ± 0.02 ± 0.01 ± 0.02 ± 0.03 ± 0.03 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} 2.59^{Cb} ± 0.04 ± 0.03 ± 0.02 ± 0.01 ± 0.01 ± 0.03 ± 0.03 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} 2.59^{Cb} ± 0.03 3.45^{Ca} 3.22^{Ba} 2.58^{Aa} 1.22^{Ba} 1.20^{Ba} 0.98^{Aa} 1.23^{Aa}	Total protein(g/dl) Albumin(g/dl) Globulin(g/ 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 3.91^{Ab} 3.94^{Ad} 4.15^{Bd} 1.29^{Ab} 1.41^{Bc} 1.61^{Cd} 2.62^{b} 2.53^{d} ± 0.03 ± 0.03 ± 0.04 ± 0.01 ± 0.02 ± 0.03 ± 0.04 ± 0.04 3.50^{Ba} 3.43^{Bb} 3.14^{Ab} 1.24^{a} 1.24^{a} 1.26^{b} 2.27^{Ba} 2.19^{Bb} ± 0.04 ± 0.05 ± 0.02 ± 0.01 ± 0.02 ± 0.03 ± 0.03 ± 0.03 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} 2.59^{Cb} 2.40^{Bc} ± 0.04 ± 0.02 ± 0.01 ± 0.01 ± 0.03 ± 0.03 ± 0.03 3.90^{Cb} 3.71^{Bc} 3.47^{Ac} 1.31^{b} 1.32^{c} 2.59^{Cb} 2.40^{Bc} $\pm 0.04^{Ca}$ ± 0.03 $\pm 0.03^{c}$ $\pm 0.03^{c}$ $\pm 0.03^{c}$ $\pm 0.03^{c}$ $\pm 0.03^{c}$ <td< td=""><td>Total protein(g/dl) Albumin(g/dl) Globulin(g/dl) 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 3.91^{Ab} 3.94^{Ad} 4.15^{Bd} 1.29^{Ab} 1.41^{Bc} 1.61^{Cd} 2.62^b 2.53^d 2.54^d ± 0.03 ± 0.03 ± 0.04 ± 0.01 ± 0.02 ± 0.03 ± 0.04 ± 0.03 ± 0.03 ± 0.04 ± 0.03 3.50^{Ba} 3.43^{Bb} 3.14^{Ab} 1.24^a 1.24^a 1.26^b 2.27^{Ba} 2.19^{Bb} 1.88^{Ab} ± 0.04 ± 0.05 ± 0.02 ± 0.01 ± 0.02 ± 0.03 ± 0.03 ± 0.03 ± 0.04 ± 0.03 ± 0.04 ± 0.04</td><td>$\begin{array}{ c c c c c c c c c c c c c c c c c c c$</td><td>Total protein(g/dl) Albumin(g/dl) Glouin(g/dl) Albumin(g/dl) 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 3P</td></td<>	Total protein(g/dl) Albumin(g/dl) Globulin(g/dl) 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 3.91 ^{Ab} 3.94 ^{Ad} 4.15 ^{Bd} 1.29 ^{Ab} 1.41 ^{Bc} 1.61 ^{Cd} 2.62 ^b 2.53 ^d 2.54 ^d ± 0.03 ± 0.03 ± 0.04 ± 0.01 ± 0.02 ± 0.03 ± 0.04 ± 0.03 ± 0.03 ± 0.04 ± 0.03 3.50 ^{Ba} 3.43 ^{Bb} 3.14 ^{Ab} 1.24 ^a 1.24 ^a 1.26 ^b 2.27 ^{Ba} 2.19 ^{Bb} 1.88 ^{Ab} ± 0.04 ± 0.05 ± 0.02 ± 0.01 ± 0.02 ± 0.03 ± 0.03 ± 0.03 ± 0.04 ± 0.03 ± 0.04 ± 0.04	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Total protein(g/dl) Albumin(g/dl) Glouin(g/dl) Albumin(g/dl) 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 7Pl 1Pl 3Pl 3P

 Table 2. Mean (±SE) serum total protein, albumin, globulin and albumin globulin ratio of broiler birds

Means bearing the same superscript within the same column (a-d) and same rows (A-C) do not differ significantly (P<0.05).

Group	Creatinine				Uric acid		Urea		
	1PI	3PI	7PI	1PI	3PI	7PI	1PI	3PI	7PI
T1	0.34 ^{вь}	0.35 ^{Ac}	0.36 ^{Ad}	4.68 ^{сь}	4.91 ^{Bd}	5.59 ^{Ad}	12.47 ^{ac}	12.39 ^{₿d}	12.28 ^{cd}
	±0.01	±0.04	±0.03	±0.08	±0.10	±0.10	±0.02	±0.03	±0.02
Т2	0.41 ^{ca}	0.43 ^{вь}	O.44 ^{Ab}	7.48 ^{ca}	7.87 ^{вь}	8.60 ^{Ab}	14.31 ^{сь}	14.54 ^{вь}	14.86 ^{Ab}
	±0.01	±0.01	±0.01	±0.06	±0.09	±0.06	±0.02	±0.02	±0.02
тз	0.34 ^{c₅}	0.36 ^{Bc}	0.38 ^{Ac}	4.85 ^{сь}	5.32 ^{Bc}	7.91 ^{Ac}	12.51 ^{c₀}	12.56 ^{в₀}	12.62 ^{Ac}
	±0.001	±0.001	±0.001	±0.03	±0.02	±0.01	±0.01	±0.01	±0.02
Т4	0.43 ^{ca}	0.46 ^{Ba}	0.51 ^{Aa}	7.56 ^{ca}	8.18 ^{Ba}	14.31 ^{Aa}	14.44 ^{Ca}	15.11 ^{ва}	16.46 ^{Aa}
	±0.01	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01	±0.02	±0.10

Table 3. Mean (±SE) creatinine, uric acid and urea values of broiler birds, mg/dl

Means bearing the same superscript within the same column (a-d) and same rows (A-C) do not differ significantly (P<0.05).

the experiment are presented in Table 3. Means of group T4 revealed significant elevation in creatinine level on 3PI and 7PI when compared to T2 and throughout the experiment when compared to T3 group. Significant increase in creatinine level in ochratoxicosis was in accordance with Agawane *et al.* (2004) while elevation in values in case of coccidiosis is in accordance with Harfoush *et al.* (2010). Elevation in creatinine could be attributed to nephrotoxic action of OTA. So simultaneous effect of toxin and coccidia could have resulted in significant increase in T4, when compared to control groups though low dose was used.

TreatmentgroupT4revealed significant increase in serum uric acid concentration when compared to T2 and T3 on 3PI and 7PI. Both T2 as well as T3 revealed significant increase in serum uric acid concentration when compared to T1. Similar observation was reported by Stoev *et al.* (2002a) at higher doses. But in this experiment similar result was obtained following sublethal exposure to toxin and subinfective dose of *E. tenella*. So low toxin level of 300 ppb and 5000-6000 oocyst of *E. tenella* could cause severe damage to kidney resulting in significant increase in serum uric acid concentration in T4.

Treatment group T4 showed significant elevation of serum urea level when compared to T2 and T3. Toxin control T2 also showed significant increase compared to T1 and this was in agreement with Stoev *et al.* (2002b). Significant increase in T4 could be due to the nephrotoxic effect of OTA besides the role played by coccidia.

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