SEROLOGICAL ALTERATIONS IN BROILER BIRDS INFECTED WITH SUB-INFECTIVE COCCIDIOSIS UNDER SUB-LETHAL AFLATOXICOSIS

Nithya Chacko[1], Ajith Jacob George[2*], N. Vijayan[3], Mammen J. Abraham[2], Bindu Lakshmanan[4] and V.L. Gleeja[5]
Department of Veterinary Pathology
College of Veterinary and Animal Sciences
Mannuthy - 680 651, Thrissur- Kerala

Received -02-07-2016
Accepted -05-07-2016

Abstract

Poultry farming is the most organized and economically viable sector in animal agriculture. As sub-lethal AF is a constant threat to broiler industry and produces immunosuppression, birds would be more prone to coccidiosis even under reduced infective dose. So it is important to study the response of sub-infectious dose of coccidia in an immune-compromised state. The susceptibility of broiler chicks to sub-infective dose of sporulated oocyst of Eimeria tenella in sub-lethal aflatoxicosis was revealed by significant alterations in serological parameters.

Keywords: *Aflatoxicosis, coccidiosis, broiler birds*

Poultry farming is the most organized and economically viable sector in animal agriculture. In the last few decades, the broiler industry had made tremendous changes with an annual growth rate of 10-12 per cent. This spurt in poultry production imparts severe pressure on proper feeding in order to meet the gap between requirement and availability. Arulmozhi (1999) opined that 24 per cent of poultry feed are contaminated with aflatoxin (AF) with a range of 20 to 200 ppb. Even at 20 ppb level cellular and subcellular damage to the tissues with marginal economic loss was observed. Regular sub-lethal exposure of AF leads to chronic aflatoxicosis which results poor production performance and immune suppression. As sub-lethal AF is a constant threat to broiler industry and produces immunosuppression, birds would be more prone to coccidiosis even under reduced infection. Hence, it is important to study the alterations in serological parameters in sub-infective dose of Eimeria under low level of aflatoxicosis. Hence, this study was undertaken.

1. MVSc scholar
2. Associate Professor (Corresponding Author)*
3. Professor and Head
4. Assistant Professor, Dept. of Veterinary Parasitology, CVAS, Mannuthy
5. Assistant Professor, Dept. of Statistics, CVAS, Mannuthy
Email: ajithjacob@kvasu.ac.in

Materials and Methods

AF production was carried out using *Aspergillus parasiticus* NRRL 2999 culture as per the procedure followed by Shotwell *et al*. (1966). Pooled rice culture yielded 2.5 ppm AF. Oocyst of *Eimeria tenella* collected from natural infection were sporulated artificially in laboratory and standardized to a dose rate of 5,000-6,000 sporulated oocysts/ml (Long *et al*., 1976). One-day-old broiler birds were divided into four groups T1 (control), T2 (toxin control with 150 ppb AF), T3 (coccidia control) and T4 (treatment group received both 150 ppb AF and coccidia). T3 and T4 birds inoculated with sub-infective dose of 5000-6000 sporulated oocysts on day 14. The data were recorded at three stages of sacrifice viz; one day post inoculation (1 PI), three day post inoculation (3 PI) and seven day post inoculation (7 PI). Serum samples were collected for estimating Aspartate Amino Transferase (AST), creatinine, globulin, total protein, albumin, AG ratio, triglyceride, cholesterol on all days of observation. Data were analysed statistically using SPSS version 21.0.

Results and Discussion

Mean (±SE) values of serum parameters of controls and treatment birds are presented in Table 1 to 3. There was no significant difference in AST values between T2 and T4 at 7 PI, though significant increase existed between these groups in 1 PI and 3 PI stages. This observation is in accordance with Toulah (2007) who reported significant elevation in AST on birds exposed to 1ppm AF and coccidia. The elevated AST values during aflatoxicosis might be due to degenerative changes in hepatic parenchyma and leakage of enzymes into the circulation (Indu, 2009). Damage caused by coccidia to intestine, liver and kidneys and seepage of enzyme to circulation might be the reason for elevated AST level during coccidiosis. The extensive tissue damage in birds exposed to both AF and coccidia might be the reason for significant elevation in AST in that group. Creatinine values showed significant elevation in T4 birds when compared to T2 and T3 during all days of sacrifice. Creatinine values of T2 and T3 were elevated compared to control. This observation is in accordance with Sakhare *et al*. (2007) and Harfoush *et al*. (2010). In addition to toxic damage in kidney by AF, severe dehydration and blood loss during coccidiosis might be the reason for increased creatinine level in treatment group received both AF and coccidia.

The birds belonging to T4 group showed significant reduction in total protein and albumin values from all other control groups. T2 and T3 birds had significant reduction in total protein and albumin values compared to control. There was significant reduction in serum globulin values of T4 and T2 birds when compared to the control and T3 birds during the entire observation period. Basith *et al*. (1998) reported that the significant reduction in serum total protein and albumin in birds with coccidiosis might be due to anorexia, malabsorption due to haemorrhages throughout the gut and formation of inflammatory exudates rich in blood proteins. The decrease in total serum proteins and albumin observed in aflatoxicosis might be due to the inhibition of RNA synthesis for protein production (Clifford

Table 1. Mean (±SE) values of Aspartate amino transferase and creatinine of control and treatment groups of broiler birds

	AST (IU/ml)			Creatinine (mg/dl)		
	1 PI	3 PI	7 PI	1 PI	3 PI	7 PI
T1	$137.90^{Ad} \pm 7.80$	$126.85^{Ac} \pm 3.78$	$139.30^{Ac} \pm 4.12$	$0.39^{Ac} \pm 0.31$	$0.42^{Ac} \pm 0.02$	$0.41^{Ac} \pm 0.02$
T2	$219.72^{Bb} \pm 18.53$	$243.35^{Bb} \pm 6.91$	$307.27^{Aa} \pm 7.64$	$0.61^{Cb} \pm 0.23$	$0.67^{Bb} \pm 0.02$	$0.82^{Ab} \pm 0.02$
T3	$166.35^{cB} \pm 11.64$	$223.60^{Ab} \pm 6.83$	$231.17^{Ab} \pm 3.74$	$0.43^{Ac} \pm 0.01$	$0.45^{Ac} \pm 0.01$	$0.44^{Ac} \pm 0.01$
T4	$245.60^{Ba} \pm 6.05$	$267.30^{Ba} \pm 6.35$	$327.42^{Aa} \pm 6.46$	$0.87^{Ca} \pm 0.01$	$0.96^{Ba} \pm 0.02$	$1.06^{Aa} \pm 0.02$

Means bearing the same superscript (a-d within the same column and A-C within row) do not differ significantly. P≤0.05). PI- Post inoculation

and Rees., 1967), inactivation of amino acids in liver and poor intestinal absorption of amino acids (Mani *et al*., 1993). Ellakany *et al*. (2011) observed significant reduction in total protein in combination group even with sub-infective dose of coccidian. This might be due to the combined effect of reduced protein synthesis by liver and malabsorption of nutrients from intestine. In this study, a relative decrease was seen in both albumin and globulin values due to protein loss from intestine and hepatotoxic effect of AF, which resulted in a non-significant AG ratio.

Treatment group birds showed significant reduction in triglyceride and cholesterol values compared to T1, T2 and T3. This finding is in line with that of Mondal *et al*. (2011) who reported significant reduction in triglyceride values in coccidiosis. Anorexia, malabsorption and severe thiamine deficiency affect lipogenesis (West *et al*., 1966) in birds during coccidiosis. Sakhare *et al*. (2007) reported significant reduction in these parameters in birds fed with 200 ppb AF. This might be due to interference with lipogenesis or inhibition of lipid transport by AF (Tung *et al*., 1972). In the present study, combination of AF and coccidia caused severe reduction in triglyceride and cholesterol due to their combined effect on lipogenesis and due to severe mal-absorption.

Severe serological alterations were observed in broiler birds exposed to sub-infective coccidiosis under the influence of sub-lethal aflatoxicosis compared to controls. . This point out the possibility of immune suppression against *E. tenella* under the influence of even low level of AF in poultry feeds. The birds become more susceptible to pathogenic effects of coccidia even in subclinical doses. Hence, it could be concluded that broiler chicks would be more prone to coccidiosis even under reduced infective dose due to suppression of mucosal immunity, under the influence of sub-lethal AF which is a constant threat to broiler industry.

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

	Total protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			Albumin Globulin Ratio		
	1 PI	3 PI	7 PI	1 PI	3 PI	7 PI	1 PI	3 PI	7 PI	1 PI	3 PI	7 PI
T1	2.91^{Ba} ± 0.03	2.99^{Ba} ± 0.03	3.29^{Aa} ± 0.03	1.43^{Ba} ±0.03	1.46^{Ba} ±0.02	1.66^{Aa} ±0.04	1.48^{Ba} ±0.04	1.53^{ABa} ±0.03	1.64^{Aa} ±0.04	0.97 ±0.03	0.96 ±0.03	1.01 ±0.04
T2	2.25^{Bc} ± 0.04	2.42^{Bc} ± 0.03	2.65^{Ac} ± 0.03	1.13^{Cc} ±0.04	1.22^{Bc} ±0.02	1.35^{Ac} ±0.03	1.13^{Bb} ±0.06	1.20^{ABb} ±0.39	1.29^{Ac} ±0.03	1.02 ±0.08	1.02 ±0.04	1.04 ±0.04
T3	2.69^{Bb} ± 0.02	2.79^{Bb} ± 0.03	3.01^{Ab} ± 0.05	1.32^{Bb} ±0.02	1.35^{Bb} ±0.02	1.48^{Ab} ±0.01	1.38^{Ba} ±0.03	1.43^{ABa} ±0.02	1.53^{Ab} ±0.04	0.96 ±0.03	0.94 ±0.01	0.98 ±0.03
T4	2.09^{Bc} ± 0.03	2.20^{Ad} ± 0.15	2.34^{Ad} ± 0.13	0.97^{Bd} ±0.03	1.02^{Ad} ±0.02	1.07^{Ad} ±0.05	1.12^{Bb} ±0.03	1.32^{Ab} ±0.06	1.27^{Ac} ±0.13	0.86 ±0.03	0.97 ±0.01	0.87 ±0.09

Means bearing the same superscript (a-d within the same column and A-C within row) do not differ significantly (P≤0.05). PI- Post inoculation

Table 3. Mean (±SE) values of Serum triglyceride and cholesterol (mg/dl) of broiler birds of control and treatment groups of broiler birds

	Triglyceride			**Cholesterol**		
	1 PI	3 PI	7 PI	1 PI	3 PI	7 PI
T1	95.89^{Ba} ± 0.53	95.38^{Ba} ± 0.77	98.70^{Aa} ±1.14	147.38^{Ba} ± 0.90	147.47^{Ba} ± 1.05	154.48^{Aa} ± 1.80
T2	56.40^{Ac} ± 0.49	52.72^{Bc} ± 0.80	51.85^{Bc} ± 0.82	124.83^{Ac} ± 0.41	117.91^{Bc} ± 0.80	110.75^{Cc} ± 0.68
T3	72.31^{Ab} ± 0.98	71.91^{Ab} ± 0.79	67.44^{Bb} ± 0.70	137.49^{Ab} ± 0.69	134.77^{Bb} ± 0.45	128.79^{Cb} ± 0.66
T4	52.39^{Ad} ± 0.74	51.48^{Ac} ± 0.54	44.44^{Bd} ± 0.55	104.20^{Bd} ± 0.48	109.96^{Ad} ± 0.62	103.01^{Bd} ± 0.86

Means bearing the same superscript (a-d within the same column and A-C within row) do not differ significantly (P≤0.05). PI- Post inoculation

References

Arulmozhi, A. 1999. Determination of permissible level of aflatoxin in broiler chicken feed. *M.V.Sc*. *thesis*, Kerala Agricultural University, Thrissur, 70p.

Basith, Abdul, S., Rajavelu, G. and Manohar, B.M. 1998. Biochemical studies in experimental *Eimeria necatrix* infection in chickens. *Indian Vet. J*. **75**: 876-878.

Clifford, J.T. and Rees, K.R. 1967. The action of aflatoxin on the rat liver. *Biochem. J.* **102**: 65-72.

Ellakany, H.F., Abuakkada, S.S., Oda, S.S. and El-Sayed, Y.S. 2011. Influence of low levels of dietary aflatoxins on *Eimeria tenella* infections in broilers. *Trop. Anim. Hlth Prod*. **43**: 249–257.

Harfoush, M.A., Hegazy, A.M., Soliman, A.H. and Amer, S. 2010. Drug resistance evaluation of some commonly used anticoccidial drugs in broiler chickens. *J. Egypt Soc. Parasitol.* **40(2)**:337-48.

Indu, K. 2009. Effect of a composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* on aflatoxicosis in rabbits. *M.V.Sc*. *thesis*, Kerala Agricultural University, Thrissur, 57p.

Long, P.L., Joyner, L.P., Millard, B.P. and Norton, C.C. 1976. A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. *Folia Veterinaria Latina.* **6**: 535-541.

Mani, K., Narhari, D., Kumara, J.R. and Ramamoorthy, N. 1993. Influence of dietary aflatoxin B1 on certain haematological and biochemical characters of broiler chicken. *Indian Vet. J.* **70**: 801–804.

Mondal, D.K., Chattopadhyay, S., Batabyal, S., Bera, A.K. and Battacharya, D. 2011. Plasma biochemical indices at various stages of infection with a field isolate of *Eimeria tenella* in broiler chicken. *Vet. Wld*. **4**: 404-409.

Sakhare, P.S., Harne, S.D., Kalorey, D.R., Warke, S.R., Bhandarkar, A.G. and Kurkure, N.V. 2007. Effect of Toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *Vet. Arhiv.* **77**:129–146.

Shotwell, O.L., Hesseltine, C.W., Stubblefield, R.D. and Sorenson, W.G. 1966. Production of aflatoxin on rice. *Appl. Microbiol.* **14**: 425-428.

Toulah, F.H. 2007. Effect of aflatoxin on the coccidial infection in broilers. *J. Egypt Soc. Parasitol.* **37(3)**: 785-92.

Tung, H.T., Donaldson, W.E. and Hamilton, P.B. 1970. Effects of aflatoxin on some marker enzymes of lysosomes. *Biochem. Biophys*. *Acta Gen. Subj*. **222:** 665–667.

West, E.S., Todd, W. R., Mason, H.S. and Van Bruggen, J. T. 1966. *Challenges in the successful control of the avian coccidian: Textbook of Biochemistry*. (4th Ed.). Oxford and IBH Publishing Company, New Delhi, 1028p. ■