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Biochemical Changes in Induced Caecal Coccidiosis in Broiler Birds Given Different Feed Anticoccidials at Anand, Gujarat, India

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A B S T R A C T

Studies on biochemical profile revealed significantly ($P < 0.05$) lower serum glucose and serum total protein, while significant increase in serum total cholesterol, Serum Glutamic Oxalo-acetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (AKP) activities was observed due to coccidial infection as compared to pre infection levels in birds. Biochemical studies indicated comparative less pathological damage by coccidiostats treatment as compared to infected non treated group, but there was no consistent trend for drug choice. Serum glucose values indicated better efficacy of D+S group as compare to Salinomycin and Maduramicin, results of serum total protein indicate better efficacy of Salinomycin, while serum total cholesterol results indicated better efficacy of Maduramicin and Diclazuril in all four treatment group. Serum Glutamic Oxalo-acetic Transaminase (SGOT) showed better efficacy of Salinomycin followed by Maduramicin as compared to Diclazuril and Diclazuril + Salinomycin group, Serum Glutamic Pyruvic Transaminase (SGPT) showed better efficacy of Diclazuril and Salinomycin and Serum Alkaline Phosphatase (AKP) indicated better efficacy of Salinomycin and Maduramicin as compared with Diclazuril and Diclazuril + Salinomycin group.

Introduction

Poultry meat constitutes around 20% of total meat production in the country (Singh, 2012). The per capita availability of poultry meat is 2.15 kg/annum which is very less as against the recommendation of 11 kg meat/annum given by National Institute of Nutrition (Prabhakaran, 2012). Hence, there is tremendous scope for further development in poultry meat sector. Challenges from bacteria, viruses and parasites placed a limit on production potential and high mortality was often noticed at the farms. Poultry sector is still confronted with many enteric diseases like coccidiosis which are hindering its progress (Saima *et al.*, 2010). It is a disease which accounts for 5-10 per cent mortality rate of chickens and an unknown loss due to reduced weight gain and feed efficiency, damage to the digestive tract, decreased egg production and lowered resistance of birds to

other poultry diseases. This disease is one of the most prevalent significant problems in poultry throughout the tropical countries (Chakrabarti, 1989) and the annual worldwide loss due to coccidiosis was estimated about $ 800 million (Williams, 1998). The lesions caused by the parasite disturb nutrient absorption, triggering several changes in carbohydrates, lipid, protein and mineral metabolism (Patra *et al.,* 2010). It produces deviation in the biochemical components of the body (McDougald and Reid, 1991; Deger *et al.,* 2002.; Patra *et al.,* 2010). In the organism, enzyme action is influenced by metabolic activities and pathologic condition. To combat clinical and subclinical forms of poultry diseases, accurate and differential diagnosis of the diseases at early stages of infections is necessary (Talebi *et al.,* 2005). In broiler production, numerous anticoccidial drugs are used for prevention and control of coccidiosis. However, development of tolerance to these drugs have lead to search for newer molecules and different classes of anticoccidials have been discovered and used from time to time for prevention and control of coccidiosis. The knowledge of biochemical parameters will also help in diagnosis and control of coccidiosis in this area. So the comparative study of coccidiostat in broiler by giving experimental infection of *E. tenella* is planned to study the alteration in biochemical parameters.

Materials and Methods

The efficacy of three commonly used feed coccidiostats named Diclazuril (T1) Salinomycin (T2), Diclazuril + Salinomycin (T3) in shuttle programme and Maduramicin (T4) on experimentally induced *Eimeria tenella* coccidial infection and their effects on biochemical changes were undertaken in three hundred Cobb400 strain of broiler at University Poultry Complex, Anand Agricultural University, Anand during year 2012. Birds were given feed containing Diclazuril (T1), Salinomycin (T2), and Maduramicin (T4) coccidiostats at dose rate of 1 ppm, 60 ppm and 5 ppm upto 42 days. Experimental infection of 50,000 oocysts of *E.tenella* was given on 22nd day of age. Blood was collected before experimental infection at 3 weeks and after experimental infection at 4 weeks of age for biochemical study.

Approximately 4 ml of blood was collected from wing vein for separation of serum. Blood sample will be taken randomly from 10 birds in each group before experimental infection of *E. tenella* at 3 weeks and at 4 weeks of age after experimental infection of *E. tenella* with same group birds. Serum samples were stored at -20^{o}C in deep-freeze until analyzed for the biochemical and enzymatic attributes. In all, 60 blood samples before experimental infection and 60 blood samples after experimental infection of six groups of broilers were used for biochemical alterations. Estimation of serum glucose, Serum total protein and serum total cholesterol were carried out as per the Glucose Oxidase and Peroxidase (GOD/ POD), Biuret and Cholesterol Oxidase (CHOD) Methods, respectively by using kits of Crest Biosystems India Ltd., Goa.

Serum Glutamic Oxalo-acetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were estimated by modified International Federation of Clinical Chemistry (IFCC) method (1986), while Serum Alkaline Phosphatase (AKP) activity was estimated by P- Nitro Phenyl phosphate (PNPN) method (1954) using kits of Crest Biosystems India Ltd., Goa, and the values were expressed as U/L. Data so generated were statistically analyzed as per the method of Snedecor and Cochran (1980) by using completely randomized design on SAS software version 2000.

Results and Discussion

Coccidia can produce subtle change in metabolism. In disease condition, enzyme action is also influenced by metabolic activities (Allen, 1988). The result of serum biochemical and enzymatic profile in different coccidiostat treatment before and after experimental infection are presented in Table 1 and 2.

Effects on serum glucose

Before experimental infection at 3 weeks of age, serum glucose values were more or less similar in treatment as well as in control group. After experimental infection there was significant reduction in glucose value. The highest reduction (mg %) was observed in T2 group (210.40 ± 1.63) followed by T4 group (224.20 ± 5.05), T1 group (237.40 ± 1.58) and T3 group (238.40 ± 1.36). T5 positive control group had shown significant lower post infection glucose value (203.60 ± 2.73 mg %) as compare to pre infection control value (267.90 ± 2.58 mg %). Results indicates better efficacy of Diclazuril + Salinomycin group as compare to Salinomycin and Maduramicin in relation to effect on glucose.

Pangasa *et al.,* (2007) reported significant low glucose in infected and non medicated broilers as compared to control and Salinomycin coccidiostat fed broilers given 50,000 oocyst of *E. tenella* during acute phase of disease. Salinomycin had given better result with less glucose reduction similar to our findings. Reduction in glucose might be due to defect in absorption of glucose, leakage of glucose in plasma and increase demand of glucose by developing stage of parasite as reported by Mondal *et al.,* (2011). Padmavathi and Muralidharam (1986) who recorded hypoglycaemia with 50,000 *E.tenella* oocysts infection at 7 day PI and Constantinescu (1976) found hypoglycaemia in mixed infection, and Freeman (1970) and Basith *et al.,* (2000) failed to see any change in plasma glucose.

Effect on serum total protein

Serum Total Protein Value are more or less similar in treatment group at 3 week of age, but it was significantly reduced after experimental infection. Highest decrease in serum protein value (g %) observe in T4 group (3.46 ± 0.08) followed by T1 group (3.58 ± 0.08), T3 group (3.62 ± 0.07) and T2 group (3.75 ± 0.03). Post infection value of positive control was significant lower (3.28 ± 0.06 g %) as compared to pre infection (4.62 ± 0.06 g %). Result indicated better efficacy of Salinomycin among four treatments. The study revealed that there was significant reduction in levels of serum protein among the infected birds. Infected birds had hypoproteinaemia from Day 7 Day PI.

Mondal *et al.,* (2011) reported decrease in Protein in broilers given 20000-25000 doses of *E.tenella* oocysts infection. They also stated that protein decrease is due to rapid movement of interstitial fluid without protein into the plasma and also due to acute stress responsible for cortisol secretion and disturbances in protein catabolism.

Padmavathi and Muralidharan (1986) and Conway *et al.,* (1993) who all recorded lowered total serum protein values in *E. tenella* infected birds. The significant reduction in serum total protein observed in affected birds might be due to reduced feed intake and/or haemorrahges through the gut leads reduced absorption of amino acids and formation of inflammatory exudates rich in blood proteins (Basith *et al.,* 2000). It might also be due to decreased retention and change in the protein metabolism (Conway *et al.,* 1993).

Effect on serum total cholesterol

Before experimental infection at 3 week of age, serum total cholesterol values were more or less similar except Maduramicin group. This T4 group showing significantly higher serum total cholesterol values (119.40 ± 1.19 mg %). After experimental infection of *E.tenella* serum total cholesterol value were significant increases in control as were as treatment group. Higher Serum cholesterol values (mg %) were observed in T3 shuttle group (131.90 ± 3.12) followed by T2 group (130.50 ± 3.58), T1 group (121.60 ± 0.65) and T4 group (122.00 ± 0.60) in post infection treatment group. Positive control T5 group shown significant high post infection value (142.50 ± 1.44 mg %), as compare to pre infection value (112.80 ± 2.37 mg %). Result indicate better efficacy of Maduramicin in all four treatment group at four week of age. Mondal *et al.,* (2011) reported increase in cholesterol value in broilers given 20000-25000 doses of *E.tenella* oocysts infection. Increase in cholesterol might be due to decrease billiary excretion of cholesterol in anorexia. Our results were in agreement with the observations of Singh *et al.,* (1976), Padmavathi and Muralidharan (1986) and Basith *et al.,* (2000). The hypercholesteremia observed in present study among the infected birds might be due to disturbed fat metabolism and loss of fluid resulting in apparent increase (Padmavathi and Muralitharan, 1986) or due to impaired liver function leads to disturbed fat metabolism consequent to injury to intestinal epithelium in coccidiosis (Basith *et al.,* 2000).

Effect on serum glutamic oxalo-acetic transaminase

At 3 week of age SGOT value were about 30-35 I.U higher in treatment group as compare to control birds. The post infection values (U/L) were significantly higher in T1 group (320.20 ± 3.87) and T3 group (314.60 ± 5.24) among four treatment groups. Post infection value in control group was significantly higher (314.80 ± 4.52 U/L) as compare with pre infection control (240.60 ± 2.94 U/L). The result showed better efficacy of Salinomycin followed by Maduramicin as compared to Diclazuril and Diclazuril + Salinomycin group.

Rizvi *et al.,* (2008) reported higher AST and ALT value in salinomysin 60-120 ppm given coccidiostat feed at 12 week of age in layer type birds similar to our findings. Significant increase in SGOT activity observed in coccidia affected birds under study might be due to damage to liver and intestine. Coles, (1986) and Montogomery *et al.,* (1990) also reported SGOT increase due to cellular membrane and tissue degeneration.

Effect on serum glutamic pyruvic transaminase

At 3 week of age, SGPT values were in range of 36 U/L to 39 U/L between control and treatment groups which were not differing significantly. After experimental infection significant higher value were observed in T1 and T2 group as compare to pre infection. After infection, positive control bird showing significant higher value (46.60 ± 0.85 U/L) as compared to pre infection value (36.00 ± 1.13 U/L). Result indicated the effect of infection on SGPT value.

Among coccidiostat treatment used in experiments, Diclazuril and Salinomycin causing less pathogenic damage as compare to Maduramicin. Biu *et al.,* (2006) also reported similar findings of increased ALT level in mixed coccidia infected chickens. Deger *et al.,* (2002) also found comparable higher SGPT values in coccidia-affected birds before treatment of coccidiostats.

Table.1 Biochemical value (Mean + S.E.) in different treatment group before and after experimental infection of *E. tenella*

Parameter	(BI or AI)	T_1	T_2	T_3	T_4	T_5	T_6	Period Mean	P		T×P	
									S Em	C.D.	S Em	C.D.
Serum Glucose (mg %)	BI	262.50 ±3.44	273.90 ±2.73	263.30 ±60.26	268.70 ±3.11	267.90 ±2.58	270.00 ±2.70	267.72	1.18	3.32	2.90	8.13
	AI	237.40 ±1.58	210.40 ±1.63	238.40 ±1.36	224.20 ±5.05	203.60 ±2.73	270.00 ±2.70	230.67				
Serum Total Protein (gm %)	BI	4.50 ±0.11	4.26 ±0.09	4.28 ±0.15	4.52 ±0.10	4.62 ±0.06	4.43 ±0.10	4.44	0.04	0.10	0.09	0.25
	AI	3.58 ±0.08	3.75 ±0.03	3.62 ±0.07	3.46 ±0.08	3.28 ±0.06	4.49 ±0.07	3.70				
Serum Total Cholesterol (mg %)	BI	115.60 ±2.93	114.60 ±2.78	109.40 ±2.50	119.40 ±1.19	112.80 ±2.37	116.60 ±2.48	114.73	0.94	2.64	2.31	6.46
	AI	121.60 ±0.65	130.50 ±3.58	131.90 ±3.12	122.00 ±0.60	142.50 ±1.44	115.20 ±1.61	127.28				
SGOT (U/L)	BI	276.60 ±2.44	271.80 ±2.14	276.20 ±2.88	274.80 ±2.27	240.60 ±2.94	239.80 ±2.33	263.30	1.64	4.59	4.01	11.25
	AI	320.20 ±3.87	278.40 ±5.92	314.60 ±5.24	286.00 ±7.13	314.80 ±4.52	242.10 ±2.52	292.68				
SGPT (U/L)	BI	37.50 ±0.83	36.60 ±0.95	38.80 ±0.68	36.30 ±1.01	36.00 ±1.13	35.60 ±0.76	36.80	0.51	1.42	1.24	3.48
	AI	42.00 ±1.33	41.20 ±1.55	40.80 ±1.84	38.80 ±2.15	46.60 ±085	36.60 ±0.78	41.00				
AKP (U/L)	BI	752.60 ±4.47	728.80 ±5.71	734.80 ±6.70	729.80 ±5.01	676.60 ±4.42	680.24 ±4.37	717.14	2.91	8.17	7.14	20.01
	AI	759.80 ±2.48	751.00 ±1.87	760.80 ±2.69	750.00 ±1.98	896.35 ±20.29	679.20 ±4.19	766.19				

The means bearing different superscript within same row differ significantly from each other (P<0.05)
BI = Before Infection AI = After Infection

Table.2 Biochemical value (Mean + S.E.) showing treatment mean in different treatment group

Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T		T×P	
							S Em	C.D.	S Em	C.D.
Serum Glucose (mg %)	249.95^{b}	242.15^{c}	250.85^{b}	246.45^{bc}	235.75^{d}	270.00^{a}	2.05	5.75	2.90	8.13
Serum Total Protein (gm %)	4.04^{b}	4.01^{b}	3.95^{b}	3.99^{b}	3.95^{b}	4.46^{a}	0.06	0.18	0.09	0.25
Serum Total Cholesterol (mg%)	118.60^{bc}	122.55^{b}	120.65^{b}	120.70^{b}	127.65^{a}	115.90^{c}	1.63	4.57	2.31	6.46
SGOT (U/L)	298.40^{a}	275.10^{b}	295.40^{a}	280.40^{b}	277.70^{b}	240.95^{c}	2.84	7.96	4.01	11.25
SGPT (U/L)	39.75^{ab}	38.90^{ab}	39.80^{ab}	37.55^{bc}	41.30^{a}	36.10^{c}	0.88	2.46	1.24	3.48
AKP (U/L)	756.20^{b}	739.90^{c}	747.80^{bc}	739.90^{c}	786.48^{a}	679.72^{d}	5.05	14.15	7.14	20.01

The means bearing different superscript within same row differ significantly from each other (P<0.05)

Effect on Serum Alkaline Phosphatase: At 3 week of age AKP value were significant higher in all four treatment group as compare to control group. The highest value (U/L) was observed in T1 group (752.60 ± 4.47) followed by T3 group (734.80 ± 6.70), T4 group (729.80 ±5.01) and T2 group (728.80 ± 5.71) indicating better efficacy of Salinomycin and Maduramicin as compared with Diclazuril and Diclazuril + Salinomycin group. Post experimental infection of *E.tenella* causing less damage in birds given Diclazuril and Salinomycin as compare with other group. Significantly higher post infection AKP value (896.35 ± 20.29 U/L) was observed as compared with pre infection T5 value (676.60 ± 4.42 U/L).

Kogut and Powel (1993) stated that AKP activity may be the sensitive marker of the pathogenesis in coccidial infection of the caecum. Kalra *et al.*, (1996) reported significant increase in serum AKP activity among infected birds. The present increase in serum AKP activity might be due to damage to liver, intestine and kidneys, which liberate the enzymes into the circulation (Kalra *et al.*, 1996). The noticeably increased serum activities of AKP found in the present study might be associated with the metabolic alteration and damage of the bone marrow as compensation for the blood losses; the bone marrow might be forced to produce excessive blood cellular components as reported by Adamu *et al.*, (2013). As a result of cellular damage several enzymes like ALT, AST and AP beach out into serum and hence their levels are increased depending upon the type and extent of damage (Hussain and Rahman, 2005).

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