

Original Research Article

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Serum Biochemistry of Broiler Chicken Supplemented with Detoxifying Microbial Enzymes to Ameliorate Feed Toxins

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ABSTRACT

A feeding trial was conducted to assess the efficacy of serum profile of broiler chicken supplemented with detoxifying microbial enzymes (DME) on feed toxins using 420 number of day -old male Vencobb broiler chicks were divided into fourteen groups, 30 birds in each for 42 days. Treatment groups: T₁ and T₂ were healthy control and positive control, respectively, T₃ received naturally mycotoxin contaminated feed at field level, T₄ received naturally mycotoxin contaminated feed at field level + DME at 250g/ton of feed, T₅ received naturally contaminated feed with *Clostridium spp.*, T₆ received naturally contaminated feed with *Clostridium spp.*+ DME at 250g/ ton of feed, T₇ received naturally contaminated feed with *E.coli*, T₈ received naturally contaminated feed with *E.coli* + DME at 250g/ton of feed, T₉ infected with *Clostridium spp.*, on day 8th, T₁₀ infected with *Clostridium spp.*, on day 8th + DME at 250g/ton of feed, T₁₁ infected with *E.coli* on day 8th, T₁₂ infected with *E.coli* on day 8th + DME at 250g/ton of feed, T₁₃ received mycotoxin contaminated feed inoculated with *Clostridium spp.*, and *E.coli* from day 8th and T₁₄ received mycotoxin contaminated feed inoculated *Clostridium spp.*, and *E.coli* from day 8th + DME at 250g/ton of feed. At the end of 28th and 42nd day two birds from each replicate were slaughtered for biochemical analysis. The level of serum sodium, potassium, calcium, chloride, glucose, total protein, uric acid, SGOT, SGPT and ALP were measured. All the parameter levels were comparable with the treatment groups and it could be inferred that the supplementation of DME did not cause any adverse health effect on broilers.

Keywords

Broiler, Detoxifying microbial enzymes, Serum biochemistry

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Introduction

Mycotoxins are the toxic metabolites produced by certain fungi, mainly by the *Aspergillus*, *Penicillium* and *Fusarium* genera. They are always a hazard to man and domestic animals and had come to public interest since the past 30 years. Aflatoxins constitute a great

threat to the health of animals and humans due to their teratogenic, carcinogenic, mutagenic and immunosuppressive effects (Guan *et al.*, 2008; Yunus *et al.*, 2011). Additionally, in terms of the livestock industry aflatoxins cause huge economic loss by retarding animal growth, increasing feed consumption and reducing meat production (Fan *et al.*, 2013;

Do and Choi, 2007). Due to its ubiquitous presence and harmful effect on consumption of contaminated feed, animal nutritionists focused their research to minimize the incidence of fungi in feed and toxicity from the toxin variety. In addition to fungal toxins, the poultry is also being exposed to bacterial toxins like *Clostridium spp.*, and *E.coli*. Both these fungal and bacterial toxins known to affect the vital organs like liver, kidney and gut health in poultry. Since the vital organs and gut health are affected, the absorption and utilization of nutrients will be impaired which results in sub-optimum productive performance in broilers. The Food and Agriculture Organization (FAO) estimates that at least 25% of world cereal production is contaminated with mycotoxins (Dowling, 1997). Hence the research was carried out with detoxifying microbial enzymes (DME) which are capable of detoxifying the fungal and bacterial toxins and also contain toxin absorbents like MOS (Mannan Oligo saccharides), antifungal agents and biological antioxidants. Among mycotoxin binder, the use of biological methods, using microorganisms and their metabolites to eliminate aflatoxins can be a highly promising approach owing to its specific, efficient and environmentally sound detoxification (FAO, 2001). The present study was designed to ascertain the efficacy of detoxifying effect of the DME in broiler feed and its effects on serum biochemical parameters in broilers.

Materials and Methods

A biological trail was carried out with detoxifying microbial enzymes in broiler ration at Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal with 420 numbers of day-old Vencobb straight run male chicks. The design of experiment followed was completely randomised design. The chicks were randomly divided in to fourteen groups. The experiment

was conducted in a shed having Mangalore tile roofing and concrete flooring. The birds were housed in deep litter pens using coconut coir pith as litter material and reared from day-old to 6 weeks following standard management practices. Feed and water were provided *ad-libitum*. All the birds were vaccinated against *Ranikhet* disease on 7th day and IBD (Infectious bursal disease) on 14th day of age. Mortality was recorded on occurrence. Post-mortem was done and the cause of death was recorded.

Experimental design and feeding program

The chicks were weighed individually, wing banded and distributed randomly to fourteen treatment groups with three replicates with ten birds in each replicate. The broiler pre-starter, starter and finisher rations were fed from 0-14, 15-28 and 29-42 days of age, respectively. The treatment of the biological experiment was presented in Table 1.

The broiler pre-starter, starter and finisher rations were fed from 0-14, 15-28 and 29-42 days of age, respectively. The experimental rations were formulated to contain the same level of protein; energy, lysine, methionine, calcium and phosphorus by using MS excel^(R). The ingredient and proximate composition of the experimental prestarter, starter and finisher rations are presented in Table 2.

Serum Preparation

At the end of experiment, two birds from each replicate were slaughtered at the end of 28th and 42nd day for biochemical studies. About 10-15 ml blood was collected from each bird in a sterile test tube without any anticoagulant kept in a slanting position at room temperature for preparation of serum. These serum samples were refrigerated overnight at 4°C. The separation of serum from the clotted blood was done by centrifugation at 1000 rpm

for 10 min. These cell free serum samples were preserved at -20°C for further biochemical analysis.

Biochemical Parameters

The biochemical parameters were analyzed with the preserved serum samples. The levels of sodium, potassium and calcium in serum were quantified using flame photometer as per AOAC (2002). The chloride content in feed was determined by thiocyanate method using diagnostic kit supplied by Span Diagnostics Ltd, India. Serum glucose (GOD/POD method), uric acid (kit method), total protein (Biuret method), serum glutamate oxaloacetate transaminase (SGOT) (IFCC method), SGPT (kit method) and alkaline phosphatase (ALP) (using alkaline phosphatase diagnostic kit method) levels were also determined.

Statistical Analysis

The data were collected and subjected to one way ANOVA using SPSS software as per the standard statistical methods given by Snedecor and Cochran (1994).

Results and Discussion

The data on serum profile of broilers fed with DME in different treatment groups during starter and finisher phase are presented in Table 3, 4 and 5, respectively. Sodium (mEq/L) level was significantly differed ($P \leq 0.05$) between the treatment groups in starter and finisher phase. In starter phase, it was lower in T_7 (50.40 ± 1.68) and higher in T_2 group (159.64 ± 5.91). In finisher phase, lower levels were observed in T_7 (121.87 ± 4.09) and higher in T_3 (164.71 ± 6.99) when compared to other treatment groups. However, higher levels in both starter and finisher phase were comparable with control. Potassium (mEq/L) level in starter and finisher phase did not vary

significantly but numerically vary among the treatment groups. In starter phase, lower level was found in T_{11} (4.90 ± 1.55) and higher in T_{14} (20.19 ± 9.80). In finisher phase, potassium level was lower in T_9 (8.26 ± 1.34) and higher in T_4 group (13.07 ± 4.23).

Calcium (mEq/L) level in starter phase significantly differ among the groups lower level was found in T_3 (8.43 ± 0.93) and higher level was observed in T_6 (9.50 ± 0.25). In finisher phase, the calcium level did not vary among the treatment groups and comparable with the control. Chloride (mEq/L) (starter and finisher) levels were comparable and not affected by the supplementation of DME. Uric acid levels (mcg/dl) were comparable and significantly vary among the treatment groups in starter and finisher phase. DME supplementation did not alter the serum glucose (mg/dl), protein (g%) and liver enzymes such as SGOT (U/L), SGPT(U/L) and ALP (U/L) levels in starter and finisher phase.

El-Katcha *et al.*, (2017) reported that the amount of calcium increased in the group treated with biological toxin binders when compared to other groups. Mycotoxin binder supplement significantly decreased the levels of serum urea, uric acid, but increased the liver related enzymes including the GOT and GPT levels when compared to control. However, some reports reported that mycotoxin binder increased serum ALP and ALT in animals (Bagherzadeh Kasmani *et al.*, 2012). Aflatoxin toxicity in broiler may manifested by increased hepatic enzyme activities such as AST and ALT; and if AST and ALP are found together in elevated amount of blood and liver damage (Nyirenda and Makhambra, 1986). Aflatoxin increase lipid peroxidation in liver and kidney tissues and induce cellular damage causing impaired morphology of the organs (Verma and Chakraborty, 2008; Darwish *et al.*, 2011).

Table.1 Dietary treatment for the biological experiment

Treatment Group	Purpose	Particulars and Dosage
T ₁	Healthy Control	Non - contaminated and non – medicated
T ₂	Positive Control	Non - contaminated and non – medicated + DME @ 250 g/ ton feed.
T ₃	Natural Contaminant*	Naturally mycotoxin contaminated feed at field level.
T ₄	Prophylaxis*	Naturally mycotoxin contaminated feed at field level + DME @ 250 g/ ton feed.
T ₅	Infected Control	Naturally contaminated feed with <i>Clostridium spp.</i> ,
T ₆	Prophylaxis	Naturally contaminated feed with <i>Clostridium spp.</i> , + Supplementation of DME @ 250 g/ ton feed.
T ₇	Infected Control	Naturally contaminated feed with <i>E.coli</i> .
T ₈	Prophylaxis	Naturally contaminated feed with <i>E.coli</i> . + Supplementation of DME @ 250 g/ ton feed.
T ₉	Challenge study	Infected with <i>Clostridium spp.</i> , on day 8 th .
T ₁₀	Prophylaxis	Infected with <i>Clostridium spp.</i> , on day 8 th + Supplementation of DME @ 250 g/ ton feed.
T ₁₁	Challenge study	Infected with <i>E.coli</i> on day 8 th .
T ₁₂	Prophylaxis	Infected with <i>E.coli</i> on day 8 th + Supplementation of DME @ 250 g/ ton feed.
T ₁₃	Challenge study*	Mycotoxin contaminated feed inoculated with <i>Clostridium spp.</i> , and <i>E.coli</i> from day 8 th .
T ₁₄	Prophylaxis*	Mycotoxin contaminated feed inoculated with <i>Clostridium spp.</i> , and <i>E.coli</i> from day 8 th + Supplementation of DME @ 250 g/ ton feed.

* Naturally Aflatoxin contaminated feed in the field level @ 50 ppb

Each bird in treatment group 9, 10, 13 and 14 were infected orally with approx. 0.4 ml inoculum (bacterial count 1×10^6 CFU/ml) containing *Clostridium spp.*, on 8th day of age. Each bird in group 11, 12, 13 and 14 were infected orally with approx. 0.4 ml inoculum (bacterial count 1×10^6 CFU/ml) containing *E.coli* on 8th day of age.

Table.2 Ingredient (%) composition and proximate composition of the broiler pre-starter, starter and finisher rations

Feed Ingredients (%)	Pre-starter	Starter	Finisher
Maize	55.10	56.30	61.80
Soyabean meal	39.50	37.20	30.70
Salt	0.30	0.30	0.30
Calcite	1.70	1.70	1.60
Di-Calcium Phosphate (DCP)	1.00	0.90	0.90
Rice bran oil	1.80	3.10	4.20
Additives (%)			
NSP degrading enzyme	0.050	0.050	0.050
Phytase-2500 IU	0.02	0.02	0.02
DL-Methionine	0.27	0.27	0.25
Lysine	0.16	0.16	0.18
Threonine	0.02	0.02	0.03
Sodium bicarbonate	0.14	0.07	0.05
Broiler mineral premix (Trouw)	0.20	0.20	0.20
Broiler vitamin premix	0.10	0.10	0.10
Salinomycin	0.05	0.05	0.05
Anti-oxidant (Endoxdry)	0.01	0.01	0.01
Vitamin E (50 %)	0.010	0.008	0.005
Lysoforte	0.05	0.05	0.05
Choline chloride (60%)	0.10	0.10	0.10
Hepatocare (Liver protectants)	0.10	0.10	0.10
Grand Total	100	100	100
Proximate composition and ME			
Dry matter (%)	91.30	90.93	91.74
Crude protein (%)	22.47	21.55	19.20
Crude fibre (%)	3.62	3.34	3.09
Ether extract (%)	4.18	5.67	6.89
Total ash (%)	7.96	7.85	7.49
Nitrogen free extract (%)	53.07	52.52	55.07
Metabolisable energy (kcal/kg) calculated	3,000	3,100	3,200

Table.3 Serum mineral profile of broilers fed with DME during starter (28th day) and finisher (42nd day) phase

Treatment	Sodium (mEq/L)		Potassium (mEq/L)		Calcium (mEq/L)		Chloride (mEq/L)	
	28 th day	42 nd day	28 th day	42 nd day	28 th day	42 nd day	28 th day	42 nd day
T ₁	158.67 ± 9.64 ^e	158.19 ± 3.50 ^{cd}	13.75 ± 8.55	11.34 ± 1.96 ^a	9.12 ± 0.87 ^{ab}	9.50 ± 0.75	904.54 ± 23.63	789.09 ± 23.63
T ₂	159.64 ± 5.91 ^e	140.59 ± 2.17 ^{abcd}	11.01 ± 3.98	15.38 ± 6.53 ^{ab}	9.31 ± 0.81 ^a	9.68 ± 0.31	900.00 ± 70.90	832.72 ± 19.09
T ₃	108.27 ± 3.13 ^b	164.71 ± 6.99 ^d	8.34 ± 2.42	25.76 ± 2.69 ^b	8.43 ± 0.93 ^a	8.56 ± 0.18	862.72 ± 15.45	834.54 ± 21.81
T ₄	142.28 ± 9.16 ^{bcde}	129.50 ± 4.09 ^{ab}	11.80 ± 6.26	13.07 ± 4.23 ^{ab}	9.25 ± 0.37 ^a	9.50 ± 0.87	857.27 ± 33.63	737.27 ± 77.27
T ₅	62.70 ± 2.89 ^a	154.34 ± 2.41 ^{bcd}	7.84 ± 2.92	20.76 ± 9.23 ^{ab}	9.25 ± 0.37 ^a	9.56 ± 0.68	870.00 ± 20.90	865.45 ± 13.63
T ₆	124.19 ± 2.98 ^{bcde}	124.19 ± 2.17 ^a	8.26 ± 2.11	16.53 ± 3.46 ^{ab}	9.50 ± 0.25 ^{ab}	9.87 ± 0.75	872.72 ± 16.36	792.72 ± 23.63
T ₇	50.40 ± 1.68 ^a	121.78 ± 4.09 ^a	5.75 ± 1.17	16.34 ± 1.57 ^{ab}	9.31 ± 0.56 ^a	9.62 ± 0.37	831.81 ± 59.09	791.81 ± 30.00
T ₈	115.99 ± 8.92 ^{bc}	154.82 ± 3.98 ^{bcd}	11.92 ± 4.23	24.80 ± 2.50 ^b	9.25 ± 0.12 ^a	9.37 ± 0.87	895.45 ± 71.81	793.63 ± 44.54
T ₉	154.09 ± 0.24 ^{de}	151.92 ± 4.82 ^{bcd}	6.94 ± 1.51	8.26 ± 1.34 ^a	9.25 ± 0.37 ^a	9.00 ± 0.75	859.09 ± 50.00	798.18 ± 23.63
T ₁₀	109.48 ± 8.32 ^b	134.56 ± 4.82 ^{abc}	9.42 ± 3.65	14.23 ± 1.15 ^{ab}	9.68 ± 0.43 ^{ab}	9.00 ± 0.37	860.00 ± 32.72	796.36 ± 45.45
T ₁₁	148.07 ± 2.96 ^{cde}	130.94 ± 8.44 ^{ab}	4.90 ± 1.55	11.53 ± 1.38 ^a	9.81 ± 0.93 ^a	9.87 ± 0.62	849.09 ± 78.18	757.27 ± 99.09
T ₁₂	111.65 ± 9.05 ^{bc}	134.32 ± 7.95 ^{abc}	7.76 ± 2.69	10.76 ± 2.69 ^a	9.37 ± 0.62 ^b	9.93 ± 0.93	818.18 ± 10.90	826.36 ± 17.27
T ₁₃	140.35 ± 1.92 ^{bcde}	132.15 ± 6.27 ^{abc}	6.05 ± 1.28	9.23 ± 1.53 ^a	9.13 ± 0.98 ^{ab}	9.06 ± 0.06	794.54 ± 15.45	862.72 ± 13.63
T ₁₄	117.20 ± 3.37 ^{bcd}	145.90 ± 3.36 ^{abcd}	20.19 ± 9.80	10.38 ± 2.69 ^a	9.43 ± 0.31 ^{ab}	9.74 ± 0.37	797.27 ± 48.18	824.54 ± 50.00

Means with different superscript within the same column differ significantly (* - P<0.05)

Table.4 Serum biochemical parameters of broilers fed with DME during starter (28th day) and finisher (42nd day) phase

Treatment	Glucose (mg/dl)		Protein (g%)		Uric Acid (mcg/dl)	
	28 th day	42 nd day	28 th day	42 nd day	28 th day	42 nd day
T ₁	277.17±6.30	275.00±4.13	4.94±0.45	4.39±0.13	501 ^a ±2.66	603 ^a ±2.28
T ₂	285.86±8.08	278.47±9.78	4.10±0.68	4.39±0.19	507 ^a ±3.80	617 ^a ±0.05
T ₃	280.43±3.04	288.04±7.60	4.67±0.43	4.43±0.78	553 ^c ±6.90	651 ^b ±13.91
T ₄	283.69±6.13	274.34±2.73	4.28±0.55	4.55±0.08	511 ^a ±14.34	621 ^a ±12.71
T ₅	299.13±7.82	284.13±5.43	4.10±0.24	4.66±0.28	501 ^a ±5.43	606 ^a ±0.10
T ₆	279.78±2.82	288.47±4.21	4.11±0.74	4.71±0.16	515 ^a ±8.47	611 ^a ±0.86
T ₇	291.52±6.30	280.65±3.26	4.11±1.14	4.96±0.69	565 ^c ±2.66	632 ^{ab} ±7.17
T ₈	286.95±8.69	282.39±4.13	4.63±0.80	4.26±0.37	503 ^a ±0.32	619 ^a ±5.00
T ₉	276.08±6.52	271.73±3.13	4.63±0.09	4.41±0.24	508 ^a ±2.01	637 ^{ab} ±12.55
T ₁₀	299.34±7.34	282.39±3.26	4.36±0.16	4.57±0.26	518 ^{ab} ±16.63	622 ^a ±4.56
T ₁₁	285.86±7.82	273.04±5.34	4.88±0.28	4.54±0.45	522 ^{ab} ±3.75	609 ^a ±1.73
T ₁₂	287.39±3.91	293.47±9.00	4.30±0.44	4.94±0.01	501 ^a ±0.16	614 ^a ±11.63
T ₁₃	282.60±7.39	286.52±2.73	4.00±1.07	4.95±0.68	545 ^{bc} ±0.59	654 ^c ±6.90
T ₁₄	289.52±5.26	273.91±4.34	4.97±0.55	5.02±0.09	520 ^{ab} ±1.08	629 ^a ±8.31

Means with different superscript within the same column differ significantly (* - P<0.05)

Table.5 Serum enzyme levels of broilers fed with DME during starter (28th day) and Finisher (42nd day) phase

Treatment	SGOT (U/L)		SGPT (U/L)		ALP (U/L)	
	28 th day	42 nd day	28 th day	42 nd day	28 th day	42 nd day
T ₁	513±14	649±16	424±08	561±19	848±17	930±22
T ₂	567±15	680±18	459±06	566±17	843±14	980±24
T ₃	570±13	676±19	462±07	557±18	868±16	1003±27
T ₄	500±11	686±21	483±09	550±16	833±21	981±21
T ₅	567±11	647±17	471±03	533±13	825±25	992±19
T ₆	561±09	622±14	473±04	518±19	803±18	980±20
T ₇	508±07	614±12	478±09	589±12	876±16	944±18
T ₈	554±12	685±16	404±08	558±17	851±14	921±16
T ₉	517±08	616±17	405±06	598±16	863±17	972±21
T ₁₀	515±11	699±14	449±07	502±18	855±12	960±23
T ₁₁	534±15	622±11	458±09	558±13	830±16	994±28
T ₁₂	528±11	681±15	441±08	589±15	812±15	989±19
T ₁₃	578±09	644±10	486±10	515±18	801±17	999±16
T ₁₄	530±06	610±16	466±09	516±21	831±21	991±17

Means with different superscript within the same column differ significantly (* - P<0.05)

Both liver and kidney are critical in detoxification and excretion of mycotoxin contamination in animals hence, their morphological changes leading to high concentrations of the hepatic and nephritic enzymes in the blood (Ademola *et al.*, 2015). However, in present study there was no increase in GOT, AST and ALP during the starter and finisher phase, it could be concluded that supplementation of DME did not cause any liver damage or other disorders where from these enzymes secreted. Detoxifying enzyme is an alternative to the use of live microbes to neutralize mycotoxins in animal feed. It is the application of enzymes responsible for the degradation of mycotoxins (Devreese *et al.*, 2012). So it could be inferred that the supplementation of DME did not cause any adverse health effect on broilers.

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