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A study on toxico biochemical effects of dietary ochratoxin and citrinin combination in broiler chicken

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Abstract
The present combination deals with toxico biochemical alteration in broiler chicken produced by experimental administration of ochratoxin A (OA) and citrinin (CTN) combination @ 2 ppm and 25 ppm respectively for a period of six weeks and efficacy of ameliorating agents in reducing the toxic effects. Experimental design consisted of four dietary Group including control (Group-I), toxin (Group-II) and ameliorative Group (Group III and IV). Birds were sacrificed at an interval of 14th, 28th and 42nd days of experiment and whole blood samples were collected for the estimation of biochemical profiles. The serum biochemical profile of the toxic Group of birds showed a significant (P<0.05) increase in certain parameters like uric acid, creatinine, blood urea nitrogen (BUN), glucose, albumin: globulin (A/G) ratio, gamma glutamyl transferase (GGT) activity, alkaline phosphatase (ALP) activity and alanine transaminase (ALT), whereas some other parameters like cholesterol, total proteins, albumins, globulins, calcium and phosphorus were significantly (P<0.05) decreased. However toxins did not showed any significant effect on enzymatic activity of aspartate transaminase (AST). These parameters were moderately ameliorated by activated charcoal and yeast when compared to activated charcoal alone.

Keywords: chicken, citrinin, ochratoxin, activated charcoal, serum toxico biochemistry

1. Introduction
Mycotoxins represents one of the serious problems in poultry and other animals. Natural Mycotoxicosis is often caused by exposure to a combination of mycotoxins especially under field conditions as a result of multiple grain sources in poultry feed manufacture [1]. The tropical and sub-tropical countries like India with moderate to high ambient temperature and high relative humidity levels experience the ill-effects of mycotoxins the most. Ochratoxin and citrinin are nephrotoxic mycotoxins produced by common moulds *Aspergillus ochraceus* and *Penicillium viridicatum,* which can grow frequently in common, feed ingredients of farm animals and poultry [2]. Besides nephrotoxic effects of OA, it is also hepatotoxic carcinogenic and immunosuppressive in nature, whereas Citrinin is a "Slow-Acting" mycotoxin. OA toxicity is a major problem of worldwide importance in commercial poultry production resulting in heavy economic losses as it is 2-3 times highly toxic than aflatoxins to the domestic fowl [3]. Ochratoxins rather than occurring alone, it is usually associated with various other mycotoxins especially citrinin [4]. Reports on serum toxicological profile on combined administration of dietary OA+CTN in relation to broiler chicken were scanty. Hence the present study was undertaken with an aim of studying serum biochemical alterations in broiler chicken in detail under experimental feeding OA+CTN @ 2ppm + 25ppm respectively in combination.

2. Materials and methods
An experiment was carried at Poultry Research Station, C.V.Sc, Hyderabad to study the combined effects of dietary OA & CTN and their amelioration with activated charcoal and yeast.

2.1 Experimental birds
Experiment was conducted for a period of six weeks with a total of 80 day-old commercial male broiler chicks (Vencobb Strain) which were obtained from Venkateshwara Hatcheries, Hyderabad. These chicks were divided into 4 experimental Group at random (Table 1).

2.2 Experimental feeds
All the chicks were fed with experimental diets along with their basal diets from 0-3 weeks and finisher diets for 4-6 weeks. All the four Group were maintained by housing under electrically heated battery brooders with feed and water supplementation *ad libitum*.

2.3 Source of toxins
Ochratoxin was produced by inoculating *Aspergillus ochraceus* culture (MTCC-2577), which was obtained from MTCC (Institute of Microbial Technology), Chandigarh, on flake wheat. Citrinin was produced by growing *Penicillium citrinum* (MTCC-2547) on maize flakes using the method of Nelson *et al.,* (1980) [5].

2.4 Source of Ameliorative agents
The physical adsorbant, activated charcoal (Decolorizing Powder) was obtained from "Qualigens fine chemicals, division of GlaxoSmithKline Pharmaceuticals Limited, Mumbai. Likewise the detoxifying agent, lyophilized yeast (*Saccharomyces cervisiae*) was obtained from APNL Biotechnology Project, Department of Veterinary Biochemistry, C.V.Sc., Rajendranagar.

2.5 Experimental design
Completely Randomized Design.

2.6 Serum biochemical studies
From the end of 2nd week onwards 6 birds from each Group were picked and scarified at every fortnight interval (14th, 28th and 42nd days of experiment). Whole blood was collected from each bird by jugular vein puncture in non-heparinized tubes and serum was collected after 8-10 hours and stored at -20 ^{o}C until further analysis.

3. Results
There was a significant (P<0.05) increase in the concentration of serum uric acid, serum creatinine, blood urea nitrogen, serum glucose, ALP, GGT and ALT levels in the present study in the toxin Group birds. Similarly there is a significant (P<0.05) decrease in the parameters like cholesterol, total proteins, albumin and globulin levels and serum calcium and phosphorus levels, while, there was no significant effect in altering the serum AST activity of toxin Group (OA + CTN combined toxicity) birds in comparison with other Group (I, III & IV). Group III and IV Group showed marked amelioration in the above altered parameters, whereas the values in Group I are apparently normal. (Table 2-16)

4. Discussion
The significant increase in the serum uric acid concentration in the present study closely agree with the results of other investigations [6]. This increase could be due to renal damage leadings to accumulation of uric acid in the blood. Group III & IV showed marked amelioration.
The increase in the creatinine was in co-occurrence with the findings of earlier reports [6]. Out of ameliorative Group III & IV, Group IV recorded low creatinine levels indicating beneficial effect of activated charcoal and yeast combination. The increased BUN concentration by the end of 6th week against I, III & IV Group was strongly comparable with experimental studies of previous workers. The probable cause for this increase might be due to hepatotoxic nature of OA. Group IV revealed low BUN levels compared to Group III which could be due to the ameliorating action of activated charcoal and yeast combination to activated charcoal alone.
The similar findings as increase in the glucose levels in the present study was reported by earlier workers [7]. The significant elevation in serum glucose concentration was the sensitive indicator of liver damage which interfered with carbohydrate metabolism.
The decrease in the serum cholesterol concentration was in agreement with the previous report [6] and it was due to hepatotoxic nature of OA and inhibition of cholesterol biosynthesis in the liver and perhaps a shift in the cholesterol concentration from blood to the liver. Among ameliorative Group, Group IV was significantly (P<0.05) more effective compared to Group III.
The results of the present study shows decrease in the overall mean values of hyperproteinemia, hypoalbuminemia and hypoglobulinemia were similar to the reports of several investigators [8]. The mechanism by which OA causes disruption in serum protein levels was thought to include, inhibition of hepatic protein synthesis through competitive inhibition of phenyl alanyl-t-RNA-synthetase with phenylalanine. Another possible contributing factor could be renal leakage of albumin resulting from kidney lesions induced by OA. The improvement in the protein profile of Group III & IV can be attributed to toxin adsorption capacity of activated charcoal and detoxifying capacity of yeast.
The increase in the ALP levels in the present study was in co-occurrence with the experimental conclusions of many workers [9]. This increase might be due to hepatotoxic nature of OA. Group IV showed significant reduction in ALP levels compared to Group III. The mechanism of action for this decrease of serum Ca^{2+} might be due to OA induced lipid peroxidation was an early event in OA toxicity, which results in structural alterations in the cell membrane sufficient to allow influx of cellular Ca^{2+} ions. This results in a change in all metabolisms and ultimately causes cell necrosis.
The increase in the serum GGT activity of the present study was in accordance with the previous authors [8]. The increased GGT activity was due to hepatotoxic nature of OA. However, a precise mechanism involved for this effect was unknown. The result of the AST of the present study was in agreement with reports of earlier workers. The increase in the ALT in the present study could be due to hepatotoxic nature of OA.
The decrease in the serum Ca^{2+} and P levels in the toxin Group is strongly supported by the findings of previous reports [9]. The mechanism of action responsible for this decreased serum Ca^{2+} from calcium loaded microsome and it was also hypothesized that OA induced lipid peroxidation was an early event in OA toxicity, which results in structural alterations in the cell membrane is sufficient to allow influx of cellular Ca^{2+} ions. This results in a change in all metabolisms and ultimately causes cell necrosis [10].

Table 1: Experimental protocol.

Group	Number of Birds	Types of diets
I	20	Basal diet
II	20	Basal diet + Ochratoxin (2ppm) + Citrinin (25ppm)
III	20	Basal diet + Ochratoxin (2ppm) + Citrinin (25ppm) +Activated Charcoal (0.4%)
IV	20	Basal diet + Ochratoxin (2ppm) + Citrinin (25ppm) +Activated Charcoal (0.4%) + Lyophilized yeast (0.2%)

Table 2: Mean values of serum uric acid (mg %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	4.65	4.82	5.28	4.92^{a}±0.19
Group II	5.30	6.24	8.76	6.77^{b}±1.03
Group III	4.92	5.87	7.74	6.18^{a}±0.83
Group IV	4.63	5.02	6.02	5.22^{a}±0.41

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 3: Mean values of serum creatinine (mg %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	0.205	0.277	0.302	0.26^{a}±0.03
Group II	0.285	0.370	0.460	0.37^{c}±0.05
Group III	0.260	0.305	0.377	0.31^{b}±0.03
Group IV	0.225	0.282	0.335	0.28^{ab}±0.03

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 4: Mean values of BUN (mg %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	6.90	7.86	8.42	7.73^{a}±0.44
Group II	7.91	10.82	12.45	10.39^{c}±1.33
Group III	7.43	9.29	11.14	9.29^{bc}±1.07
Group IV	7.16	8.39	9.03	8.19^{ab}±0.55

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 5: Mean values of serum glucose (mg %) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	184.55	185.78	186.70	185.68^{a}±0.62
Group II	217.40	235.38	226.70	226.49^{b}±5.19
Group III	187.25	192.54	194.26	191.35^{a}±2.11
Group IV	187.73	187.92	189.50	188.38^{a}±0.56

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 6: Mean values of serum cholesterol (mg %) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	117.84	126.1	136.88	126.94^{c}±5.51
Group II	107.47	113.85	118.86	113.39^{a}±3.30
Group III	108.43	115.76	121.51	115.23^{a}±4.56
Group IV	110.78	120.19	126.46	119.14^{b}±4.56

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 7: Mean values of serum total protein (g %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	3.41	3.81	4.56	3.93^{c}±0.34
Group II	2.34	2.46	2.19	2.33^{a}±0.08
Group III	3.11	3.23	3.44	3.26^{b}±0.10
Group IV	3.16	3.36	4.07	3.53^{bc}±0.28

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 8: Mean values of serum albumins (g %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	1.58	1.83	2.12	1.84^{b}±0.16
Group II	1.48	1.40	1.27	1.38^{a}±0.06
Group III	1.50	1.56	1.58	1.55^{ab}±0.02
Group IV	1.53	1.70	1.79	1.67^{ab}±0.08

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 9: Mean values of serum globulins (g %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	1.83	1.93	2.44	2.08^{c}±0.18
Group II	0.86	1.06	0.92	0.95^{a}±0.06
Group III	1.61	1.67	1.86	1.71^{b}±0.08
Group IV	1.63	1.66	2.28	1.86^{bc}±0.21

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 10: Mean values of A/G ratio as effected by various experimental diets in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	0.86	0.92	0.86	0.88^{a}±0.02
Group II	1.72	1.32	1.38	1.47^{b}±0.12
Group III	0.93	0.93	0.84	0.90^{a}±0.03
Group IV	0.93	1.02	0.78	0.91^{a}±0.07

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 11: Mean values of ALP (KA Units) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	62.13	68.60	74.63	68.45^{a}±3.61
Group II	67.94	72.54	80.21	73.56^{b}±3.58
Group III	66.34	70.48	80.19	72.34^{b}±4.10
Group IV	64.09	69.21	76.15	69.82^{a}±3.49

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 12: Mean values of serum GGT (IU/L) different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	6.42	7.29	9.85	7.85^{a}±1.03
Group II	7.19	9.52	14.12	10.28^{c}±2.04
Group III	7.12	9.23	12.39	9.58^{bc}±1.53
Group IV	6.83	7.86	11.20	8.63^{ab}±1.32

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 13: Mean values of serum AST (IU/L) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	119.00	127.32	130.16	125.49±3.35
Group II	124.90	116.59	134.83	125.44±5.03
Group III	121.30	127.08	132.04	126.81±3.10
Group IV	120.40	127.10	130.37	125.96±2.93

S.E = Standard Error

Table 14: Mean values of serum ALT (IU/L) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	121.14	128.70	134.56	128.13^{a}±3.88
Group II	126.60	134.20	141.62	134.14^{d}±4.34
Group III	125.82	132.60	139.05	132.49^{c}±3.82
Group IV	122.24	129.31	136.31	129.29^{b}±4.06

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 15: Mean values of serum calcium (mg %) in different Group of birds

Group	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	10.70	11.40	12.35	11.48^{c}±0.48
Group II	9.62	10.13	9.13	9.63^{a}±0.29
Group III	9.82	10.25	10.62	10.23^{ab}±0.23
Group IV	9.91	10.96	11.96	10.94^{bc}±0.59

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

Table 16: Mean values of serum phosphorus (mg %) in different Group of birds

GROUP	Age in days			Mean ±S.E
	14 days	28 days	42 days	
Group I	6.81	7.63	8.81	7.75^{c}±0.58
Group II	6.47	7.13	7.55	7.05^{a}±0.31
Group III	6.63	7.17	7.97	7.26^{ab}±0.39
Group IV	6.72	7.48	8.23	7.48^{bc}±0.44

S.E = Standard Error

Means bearing common superscripts do not differ significantly (P<0.05)

5. Conclusion

In conclusion, the results of the present study revealed that feeding of OA+CTN (2ppm and 25ppm) diets to the broiler chicken for a period of 6 weeks caused significant (P<0.05) alterations in almost all of the serum biochemical parameters indicating altered physiological mechanisms of the birds. It is strongly suggesting the severity of OA+CTN toxicity in inducing nephrotoxicity and hepatotoxicity. The ameliorating agents used to study their efficacy showed mild to moderate protection of the birds as evidenced by the improved results in those parameters. Among these, Group IV has showed moderate beneficial effects when compared to Group III.

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