GLOBAL JOURNAL OF BIO-SCIENCE & BIOTECHNOLOGY

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# EFFECT OF FEEDING OCHRATOXIN A AND CITRININ TOXINS ON CERTAIN BIOCHEMICAL PARAMETER IN BROILER CHICKEN

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**ABSTRACT**
The present investigation deals with effect of ochratoxin A (OA), citrinin (CTN) and their combination on certain biochemical parameters in broiler chicken at weekly intervals. The broiler chickens were divided into four groups of 50 chicks each and fed with OA 1ppm, CTN 12.5 ppm and combination of OA+CTN (1 ppm OA + 12.5 ppm CTN) in feed up to 35 days of the experiment. The blood samples were collected from all the groups at weekly interval to study the effect of this toxin on various biochemical parameters. Biochemically, hypoproteinemia, hypoalbuminemia, hypoglobulinemia and hypoglycaemia. The levels of blood urea nitrogen (BUN), serum creatinine, uric acid, AST, ALP, ALT and Serum triglyceride were increased significantly in all the toxin treated groups. However, biochemical alterations were maximum in the combination group than the individual toxin treated group. The interaction of both the toxins was found to be additive.

**KEYWORDS:** biochemical alterations, ochratoxin A, citrinin, hypoproteinemia, broiler chicken.

## INTRODUCTION

Mycotoxins comprise a structurally diverse family of naturally occurring fungal toxins which directly or indirectly contaminate the feed of livestock and poultry resulting in toxicities. In poultry, mycotoxicosis causes reduced growth rate, lowered feed conversion, impaired resistance to infectious disease and reduced vaccination efficacy with lesions in many organs (Coulombe, 1993). Among the mycotoxins encountered, ochratoxin and citrinin have occupied important position in causing toxicosis in poultry. Ochratoxin and citrinin may occur as co-contaminants of feed and feed ingredients. Considering the effects of these mycotoxins on health and performance of birds as well as huge economic losses involved the present work was taken up to study in detail the effect of individual and combined toxicosis of ochratoxin and citrinin in broilers. The present investigation was undertaken to asses the serum biochemical alterations of protein, albumin, globulin, glucose, blood urea nitrogen, serum creatinine, uric acid, ALP, AST, ALT and Serum triglyceride levels in broiler chicken fed a diet containing, commonly occurring levels of OA and CTN, either alone or in combination.

## MATERIAL AND METHODS

Pure cultures of *Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL 1841), maintained at Department of Poultry Science and Animal Nutrition, Veterinary College, KVAFSU, Hebbal, Bangalore were used for OA and CTN production. The concentrations and purity of OA and CTN were estimated using thin layer chromatography at the Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Nammakal – 637 001.

Unsexed, day old Vencobb broiler chicks (200 numbers) were obtained from M/S Akash Hatcheries, Bangalore. They were provided with optimum conditions of brooding and management. Poultry mash, both starter and finisher without addition of toxin binder. They were tested for the presence of mycotoxins such as Aflatoxin, ochratoxin and citrinin. After ascertaining the mycotoxin free status of the feed, they were kept in individual labeled bins for further use. On day one of age, the broiler chicks were randomly divided into four different dietary treatment groups of 50 birds each *viz.,* Group I, fed standard mytoxin free basal diet (control), Group II, diet containing 1 ppm OA, Group III, diet containing 12.5 ppm CTN, Group IV, diet containing 1 ppm OA + 12.5 ppm CTN. Six birds from each group were sacrificed on day 7th, 14th, 21st, 28th and 35th day of the experiment. During each sacrifice, 5ml blood samples were also collected in non- heparinised vials. The serum was separated after eight hours and stored at -20$^{o}$C until further analysis. The sera were harvested and analysed for biochemical parameters such as serum total protein, albumin, globulin, albumin/globulin ratio, glucose, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Blood urea nitrogen (BUN), serum creatinine and uric acid.

Total serum protein and albumin were estimated by modified Biuret and Dumas method, glucose by glucose oxidase method, Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) by IFCC (International Federation of Clinical Chemistry) method, Blood urea nitrogen (BUN) by glutamate dehydrogenase method, uric acid by enzymatic photometric test by IFCC method, creatinine by Jaffe's kinetic method and triglycerides (TG) by colorimetric enzymatic method. The data generated from different parameters of experimental study were subjected to statistical analysis one-way analysis of variance (ANOVA) test using Graph Pad Prism software as per Snedecor and Cochran (1989).

## RESULTS AND DISCUSSION

The values of various biochemical parameters are presented in Tables 1 and 2. Serum total protein, albumin and globulin levels of the mycotoxin treated groups were significantly decreased when compared to the control. These observations were akin to the findings of earlier workers[5,8,15,17] fed with ochratoxin. Decrease in the serum total protein albumin and globulin values in OA fed birds could be due to inhibition of hepatic protein synthesis which occurred at the post transcription level by competitive inhibition of phenylalanine-tRNA phenylsynthetase, so that amino-acylation and peptide elongation were stopped. One of the primary effects of albumin binding on OA was to retard its elimination by limiting the transfer of OA from the blood stream to the hepatic and renal cells contributing to its long half-life. In the present study, the Group III (CTN fed) birds showed significantly low protein, albumin and globulin values when compared to the Group I. Similar observation were also made in broiler chicks[2,3,4,13] fed with citrinin. CTN affects the synthesis of DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) and subsequently the protein synthesis (Braunberg *et al*., 1992). The authors observed that the effect of CTN was more profound than ochratoxin A on the inhibition of respiratory cycle of mitochondria and organic ion tetra ethyl ammonium acetate transport and on protein leakage by membrane perturbation when effect of 0.01 mM CTN over renal cortical explants was studied. The hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia observed in mycotoxin treated groups could be ascribed to the reduction in feed consumption as observed in this study and inactivation of biosynthetic enzymes and impairment of protein synthesis as evinced by hepatic damage observed histologically in this study. Besides, affection of lymphoid organs could have contributed for hypoglobulinaemia. The Group IV birds showed significantly low protein, albumin and globulin values when compared to the individual toxin fed groups. This indicated that OA and CTN combination caused a severe hypoproteinaemia than individual OA and CTN toxicities. This could be due to additive effect of OA and CTN. A significant reduction in serum glucose level was observed in the mycotoxin treated group when compared to the control (Group I).

Serum glucose levels were similar in groups II and III although a significant reduction in serum glucose value was observed in Group IV when compared to Group I and individual toxin treated groups. This indicated that OA and CTN combination caused a severe hypoglycaemia than individual OA and CTN toxicities. The hypoglycaemia observed might be attributed not only to the impaired digestion and absorption but also to hepatic and pancreatic damage observed in this study. Similar observation were also made in broiler chicks[4,13] fed with citrinin.

In the present investigation, the ALT levels in mycotoxin treated birds increased significantly than the control. Similar observation were also made in broiler chicks fed with ochratoxin[5,7,14] and citrinin[2,4]. Increase in ALT values in birds fed with ochratoxin could be attributed to the hepatic damage caused by ochratoxin. The ALT level in Group III and IV birds increased significantly than the Group I. The increased level of ALT in Group IV might be attributed to the liver damage observed in the present study. In the present investigation, the AST levels in mycotoxin treated birds increased significantly than the control birds. Similar observations were also made in broiler chicks fed with ochratoxin[12]. The increase in AST level in the present study could be due attributed to leakage of enzyme due to liver damage. The present study revealed that mycotoxin treated birds showed an increase in serum ALP level as compared to control birds. Similar observations were also made in broiler chicks fed with ochratoxin[16] and citrinin[1]. The increased level of this enzyme could be correlated to the degenerative changes noticed in the liver leading to seepage of enzyme into serum. The ALP level in Group III was significantly higher than the Group I. All mycotoxin treated groups showed a significant increase in the BUN values when compared to the control. Higher BUN values reported in OA fed birds could be attributed to kidney damage observed in the present study. Similar observation were also made in broiler chicks fed with ochratoxin[10]. The BUN values were higher in Group III when compared to Group I. Similar observation were also made in broiler chicks fed with citrinin[1,4]. The increase in the BUN value could be attributed to the renal damage. The BUN value was higher in Group IV when compared to the individual toxin fed groups and this could be due to combined effect of OA and CTN.

The level of serum creatinine was increased in birds fed with ochratoxin (Group II) as compared to the control (Group I). Further, significant increase in relative weights of kidney observed in the present study is indicative of renal damage. Extensive renal damage was noticed histologically in the OA fed birds. Similar observation were also made in broiler chicks fed with ochratoxin[5,6,9,11] and citrinin[3]. No significant difference was noticed between the Group I and III. In the present study, significant increase in Creatinine value was recorded in combined toxicity group as compared to control birds and this could be ascribed to synergistic effect of combined toxicity of ochratoxin and citrinin. Significant difference was noticed in the uric acid value between the Groups I and IV. Similar observation were also made in broiler chicks fed with citrinin[2,3,4,13]. However, there was no significant difference in the uric acid value in Groups II and III. Also, no significant difference was noticed between the Group III and I. The mycotoxin fed broiler chicken (G-II, III and IV) showed significant increase in the overall mean values of triglycerides when compared to the control. Similar observation were also made in broiler chicks fed with citrinin[4]. However, among the mycotoxin fed birds there was no difference in the triglycerides level between Group III and IV. Increase in the triglyceride level observed in the present study could be attributed to hepatic damage and altered fat metabolism during individual and combined toxicosis. The comparative evaluation of the above biochemical observations of OA and CTN in the present study indicated that the simultaneous exposure of OA and CTN was found to be additive in broiler chicken.

**TABLE 1:** Effect of OA, CTN and their combination on biochemical parameters in broiler chicken (Mean ± SE)

| Parameter/Intervals | Group I (control) | Group II (OA) | Group III (CTN) | Group IV (OA+CTN) |
|---|---|---|---|---|
| **Total protein (g/dl)** | | | | |
| 7 day | 2.48 ± 0.10 | 2.21 ± 0.09 | 1.75 ± 0.01 | 1.71 ± 0.02 |
| 14 day | 2.55 ± 0.06 | 2.20 ± 0.07 | 1.83 ± 0.01 | 1.80 ± 0.00 |
| 21 day | 3.72 ± 0.10 | 2.67 ± 0.04 | 2.10 ± 0.05 | 2.00 ± 0.02 |
| 28 day | 3.96 ± 0.01 | 2.88 ± 0.02 | 2.77 ± 0.04 | 2.06 ± 0.03 |
| 35 day | 4.10 ± 0.06 | 3.07 ± 0.07 | 2.96 ± 0.01 | 2.35 ± 0.09 |
| Mean value | $3.36^{a}$ ± 0.87 | $2.60^{b}$ ± 0.89 | $2.28^{bc}$ ± 0.90 | $1.98^{c}$ ± 0.91 |
| **Albumin (g/dl)** | | | | |
| 7 day | 1.10 ± 0.03 | 0.99 ± 0.01 | 0.93 ± 0.02 | 0.88 ± 0.01 |
| 14 day | 1.15 ± 0.02 | 1.00 ± 0.02 | 0.94 ± 0.01 | 0.90 ± 0.03 |
| 21 day | 1.25 ± 0.03 | 1.03 ± 0.01 | 0.98 ± 0.01 | 0.95 ± 0.01 |
| 28 day | 1.41 ± 0.01 | 1.33 ± 0.04 | 1.20 ± 0.01 | 1.01 ± 0.01 |
| 35 day | 1.51 ± 0.03 | 1.46 ± 0.04 | 1.45 ± 0.04 | 1.03 ± 0.01 |
| Mean value | $1.28^{a}$ ± 0.93 | $1.16^{b}$ ± 0.93 | $1.10^{b}$ ± 0.93 | $0.95^{c}$ ± 0.94 |
| **Globulin (g/dl)** | | | | |
| 7 day | 1.05 ± 0.01 | 0.83 ± 0.02 | 0.82 ± 0.01 | 0.74 ± 0.01 |
| 14 day | 1.45 ± 0.04 | 1.18 ± 0.07 | 1.10 ± 0.03 | 0.83 ± 0.02 |
| 21 day | 1.62 ± 0.02 | 1.22 ± 0.01 | 1.21 ± 0.01 | 1.10 ± 0.03 |
| 28 day | 2.18 ± 0.06 | 1.56 ± 0.02 | 1.44 ± 0.04 | 1.23 ± 0.01 |
| 35 day | 2.56 ± 0.07 | 1.72 ± 0.02 | 1.53 ± 0.03 | 1.30 ± 0.04 |
| Mean value | $1.77^{a}$ ± 0.92 | $1.30^{b}$ ± 0.93 | $1.22^{bc}$ ± 0.93 | $1.04^{c}$ ± 0.94 |
| **Albumin/Globulin ratio** | | | | |
| 7 day | 1.05 ± 0.01 | 0.89 ± 0.01 | 0.92 ± 0.02 | 0.91 ± 0.02 |
| 14 day | 0.90 ± 0.03 | 0.96 ± 0.01 | 0.99 ± 0.01 | 1.30 ± 0.04 |
| 21 day | 0.92 ± 0.01 | 0.99 ± 0.01 | 1.12 ± 0.03 | 1.50 ± 0.03 |
| 28 day | 0.89 ± 0.01 | 1.01 ± 0.01 | 1.07 ± 0.01 | 1.73 ± 0.02 |
| 35 day | 0.72 ± 0.01 | 1.03 ± 0.01 | 1.05 ± 0.01 | 1.77 ± 0.01 |
| Mean value | $0.90^{a}$ ± 0.94 | $0.97^{ab}$ ± 0.94 | $1.03^{b}$ ± 0.94 | $1.44^{c}$ ± 0.92 |
| **Glucose (mg/dl)** | | | | |
| 7 day | 199.06 ± 2.11 | 160.15 ± 5.69 | 158.48 ± 3.64 | 156.00 ± 5.64 |
| 14 day | 198.70 ± 2.16 | 170.13 ± 2.23 | 165.14 ± 2.77 | 163.12 ± 3.80 |
| 21 day | 194.10 ± 2.60 | 179.40 ± 4.51 | 169.86 ± 3.58 | 110.06 ± 5.64 |
| 28 day | 176.03 ± 2.62 | 169.11 ± 3.00 | 156.99 ± 4.79 | 121.81 ± 3.79 |
| 35 day | 156.71 ± 4.64 | 156.06 ± 5.59 | 145.33 ± 5.92 | 128.15 ± 5.91 |
| Mean value | $184.92^{a}$ ± 5.93 | $166.97^{b}$ ± 4.99 | $159.16^{b}$ ± 4.75 | $135.83^{c}$ ± 5.40 |

Mean values bearing at least one common superscripts indicates no significant difference (P≥0.05) with each other

**TABLE 2 :** Effect of OA, CTN and their combination on biochemical parameters in broiler chicken (Mean ± SE)

| Parameter/Intervals | Group I (control) | Group II (OA) | Group III (CTN) | Group IV (OA+CTN) |
|---|---|---|---|---|
| **ALT (u/L)** | | | | |
| 7 day | 7.20 ± 0.12 | 7.73 ± 0.21 | 7.90 ± 0.08 | 8.46 ± 0.16 |
| 14 day | 7.17 ± 0.11 | 7.69 ± 0.23 | 7.86 ± 0.10 | 8.41 ± 0.14 |
| 21 day | 7.13 ± 0.08 | 7.35 ± 0.21 | 7.63 ± 0.19 | 8.37 ± 0.16 |
| 28 day | 6.40 ± 0.16 | 6.61 ± 0.14 | 7.10 ± 0.06 | 8.27 ± 0.10 |
| 35 day | 5.46 ± 0.13 | 6.52 ± 0.14 | 7.04 ± 0.02 | 8.07 ± 0.05 |
| Mean value | $6.67^{a}$ ± 0.76 | $7.18^{b}$ ± 0.75 | $7.51^{b}$ ± 0.73 | $8.31^{c}$ ± 0.70 |
| **AST (u/L)** | | | | |
| 7 day | 161.43 ± 3.45 | 165.14 ± 2.81 | 171.37 ± 1.75 | 180.00 ± 4.32 |
| 14 day | 142.02 ± 4.74 | 159.24 ± 5.34 | 169.05 ± 2.96 | 179.20 ± 4.35 |
| 21 day | 136.15 ± 3.83 | 152.10 ± 3.16 | 157.15 ± 4.89 | 178.19 ± 3.59 |
| 28 day | 133.32 ± 5.61 | 140.24 ± 3.58 | 152.25 ± 3.23 | 153.25 ± 2.37 |
| 35 day | 116.23 ± 2.98 | 128.51 ± 5.82 | 142.19 ± 4.65 | 152.78 ± 2.14 |
| Mean value | $137.83^{a}$ ± 4.68 | $149.05^{b}$ ± 4.84 | $158.40^{bc}$ ± 4.81 | $168.68^{c}$ ± 5.22 |
| **ALP (u/L)** | | | | |
| 7 day | 566.29 ± 7.07 | 591.85 ± 4.43 | 596.00 ± 4.57 | 605.28 ± 4.75 |
| 14 day | 580.78 ± 7.07 | 589.45 ± 4.43 | 616.10 ± 4.43 | 625.19 ± 4.43 |
| 21 day | 589.72 ± 4.43 | 601.30 ± 4.43 | 636.45 ± 4.43 | 639.80 ± 4.43 |

| | | | | |
|---|---|---|---|---|
| 28 day | 633.19 ± 4.43 | 714.84 ± 4.43 | 736.30 ± 4.43 | 776.59 ± 4.43 |
| 35 day | 668.17 ± 4.43 | 837.20 ± 4.43 | 869.66 ± 4.43 | 918.76 ± 4.43 |
| Mean value | $607.63^{a}$ ± 19.96 | $666.93^{ab}$ ± 27.03 | $690.90^{b}$ ± 28.12 | $713.12^{b}$ ± 30.79 |
| **Triglycerides (mg/dL)** | | | | |
| 7 day | 111.40 ± 4.50 | 112.25 ± 3.85 | 137.12 ± 2.23 | 114.27 ± 2.75 |
| 14 day | 110.25 ± 5.48 | 118.20 ± 2.15 | 138.25 ± 1.95 | 139.05 ± 2.32 |
| 21 day | 109.65 ± 5.20 | 117.50 ± 2.36 | 138.91 ± 2.28 | 139.05 ± 2.32 |
| 28 day | 108.18 ± 4.76 | 115.25 ± 1.78 | 137.28 ± 2.13 | 141.44 ± 2.24 |
| 35 day | 105.06 ± 4.02 | 113.12 ± 3.95 | 136.05 ± 1.67 | 142.80 ± 2.40 |
| Mean value | $108.91^{a}$ ± 3.22 | $115.26^{b}$ ± 3.03 | $135.32^{cd}$ ± 4.02 | $137.52^{d}$ ± 3.57 |

Mean values bearing at least one common superscripts indicates no significant difference (P≥0.05) with each other

**TABLE 3:** Effect of OA, CTN and their combination on biochemical parameters in broiler chicken (Mean ± SE)

| Parameter/Intervals | Group I (control) | Group II (OA) | Group III (CTN) | Group IV (OA+CTN) |
|---|---|---|---|---|
| **BUN (mg/dL)** | | | | |
| 7 day | 6.01 ± 0.14 | 6.59 ± 0.18 | 7.08 ± 0.06 | 8.29 ± 0.09 |
| 14 day | 5.98 ± 0.15 | 6.89 ± 0.14 | 7.10 ± 0.06 | 8.36 ± 0.10 |
| 21 day | 5.86 ± 0.14 | 7.00 ± 0.05 | 7.17 ± 0.08 | 8.46 ± 0.16 |
| 28 day | 5.65 ± 0.16 | 7.29 ± 0.13 | 8.60 ± 0.15 | 9.25 ± 0.33 |
| 35 day | 5.33 ± 0.17 | 8.49 ± 0.15 | 9.03 ± 0.27 | 10.18 ± 0.40 |
| Mean value | $5.77^{a}$ ± 0.79 | $7.25^{b}$ ± 0.75 | $7.79^{c}$ ± 0.73 | $8.91^{d}$ ± 0.70 |
| **Creatinine (mg/dL)** | | | | |
| 7 day | 0.48 ± 0.01 | 0.56 ± 0.02 | 0.49 ± 0.01 | 0.59 ± 0.02 |
| 14 day | 0.46 ± 0.01 | 0.55 ± 0.02 | 0.48 ± 0.01 | 0.58 ± 0.01 |
| 21 day | 0.44 ± 0.01 | 0.54 ± 0.02 | 0.47 ± 0.01 | 0.57 ± 0.02 |
| 28 day | 0.42 ± 0.01 | 0.48 ± 0.01 | 0.42 ± 0.01 | 0.56 ± 0.02 |
| 35 day | 0.38 ± 0.01 | 0.41 ± 0.00 | 0.41 ± 0.00 | 0.55 ± 0.02 |
| Mean value | $0.44^{a}$ ± 0.95 | $0.51^{b}$ ± 0.95 | $0.45^{a}$ ± 0.95 | $0.57^{c}$ ± 0.95 |
| **Uric acid (mg/dL)** | | | | |
| 7 day | 7.99 ± 0.09 | 8.01 ± 0.10 | 8.53 ± 0.14 | 8.87 ± 0.22 |
| 14 day | 7.88 ± 0.07 | 7.90 ± 0.08 | 8.43 ± 0.08 | 8.79 ± 0.16 |
| 21 day | 7.68 ± 0.17 | 7.81 ± 0.07 | 8.33 ± 0.06 | 8.75 ± 0.13 |
| 28 day | 6.80 ± 0.14 | 6.91 ± 0.11 | 7.12 ± 0.06 | 7.42 ± 0.20 |
| 35 day | 5.96 ± 0.15 | 6.19 ± 0.18 | 6.26 ± 0.22 | 6.41 ± 0.15 |
| Mean value | $7.26^{a}$ ± 0.75 | $7.36^{a}$ ± 0.74 | $7.73^{ab}$ ± 0.74 | $8.05^{b}$ ± 0.73 |

Mean values bearing at least one common superscripts indicates no significant difference (P≥0.05) with each other

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