www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of combination *of Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL 1841) on blood parameters of commercial chicks

Milad Manafi[1] and Hossein Bagheri[2]

[1]Department of Animal Science, Chaloos Branch, Islamic Azad University, Chaloos, Iran
[2]Department of Agriculture, Chaloos Branch, Islamic Azad University, Chaloos, Iran

Abstract

The aim of this study is to investigate the combination effect of two mycotoxins in blood parameters of commercial broiler chicks (0-5 weeks) at 7, 14, 21, 28 and 35 days of post intoxication. The broiler chickens were divided into four groups of 40 birds each. Control (group I), OA (1ppm, group II), CTN (12.5ppm, group III) and combination (1ppm OA plus 12.5ppm CTN, group IV) were given in feed up to 35 days of the trial and the control (group I) was fed standard toxin free feed. The levels of blood urea nitrogen (BUN), serum creatinine, uric acid, ALP, AST, ALT and serum triglyceride increased significantly in all the toxin treated groups. However, biochemical alternations were maximum in the combination group than the individual toxin treated group. The interaction of both the toxins was found to be additive.

Keywords: Blood parameters; mycotoxins; commercial chicks

To cite this article Manafi M and H Bagheri, 2011. Effect of combination *of Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL 1841) on blood parameters of commercial chicks. Res. Opin. Anim. Vet. Sci., 1(S), S23-S29.

Introduction

Mycotoxins comprise a structurally diverse family of naturally occurring fungal toxins elaborated by several species of fungi, which cause mycotoxicosis in single or mycotoxicosis in mixed conditions. These toxins directly or indirectly contaminate the feed of animals resulting in toxicities. So far more than 300 mycotoxins have been characterized and this number is growing rapidly. In 1995, the Food and Agricultural Organization (FAO) of the United Nations estimated that 25 per cent of the world grain supply was contaminated with mycotoxins. The clinical toxicologic syndromes caused by ingestion of moderate to high amounts of mycotoxins have been well characterized and range from acute mortality to slow growth and reduced reproductive efficiency (Pier et al.., 1980). 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 spectrum of mycotoxins encountered, ochratoxin and citrinin (CTN) have occupied position in causing toxicosis in poultry. Ochratoxins are known to be potentially nephrotoxic, hepatotoxic, carcinogenic, immunotoxic and teratogenic in nature and are produced by storage moulds predominantly by species of *Aspergillus* and *Pencillium*.

Exposure to low concentration of Ochratoxin A (OA) through diet is known to cause structural and functional changes in different organ systems, especially the kidneys and liver of several domestic and experimental animals. Citrinin is produced by several species of fungi belonging to genus *penicillium* and *aspergillus* and are known to be nephrotoxic besides affecting the growth and productivity of birds. Ocharatoxin and citrinin could interact synergistically and affect greatly the performance and productivity of birds. The study of combined toxicity may throw light on the degree of lesions and involvement of various

Corresponding author: Milad Manafi, Department of Animal Science, Chaloos Branch, Islamic Azad University, Chaloos, Iran; Email: manafi_milad@yahoo.com

organs. Considering the effects of these mycotoxins on health and performance of birds as well as huge economic losses involved, it is necessary to study in detail the effect of individual and combined toxicosis of OA and CTN in broilers. Although both OA and CTN are nephrotoxic and they have been reported to occur naturally as co-contaminants, the interaction study involving OA and CTN is limited. Therefore, the present investigation was undertaken to asses the serum biochemical alterations in broiler chickens fed a diet containing, commonly occurring levels of OA and CTN, either alone or in combination.

Materials and Methods

Pure cultures of *Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL 1841), procured from Department of Animal Science, Malayer University, Malayer, Iran were employed for the 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, India.

Unsexed, day old Vencobb broiler chicks (200 numbers) were obtained from local market. All the experimental procedures were carried out with the prior permission of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC). The day old broiler chicks obtained were weighed, wing banded and housed in battery brooders with *ad libitum* supply of feed and water. They were provided with optimum conditions of brooding and management. Poultry mash, both starter and finisher without addition of toxin binder was procured from local hatchery and 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 labelled bins for further use. On day one of age, the broiler chicks were randomly divided taking care to distribute chicks of uniform body weight and segregated into four different dietary treatment groups of 30 birds each and treated as follows.

Group I was 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 plus 12.5 ppm CTN. Six birds from each group were sacrificed on day 7th, 14th, 21st, 28th and 35th day post intoxication.

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 total protein, albumin, globulin, albumin/globulin ratio, glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), serum creatinine, uric acid, calcium, Phosphorus, Calcium/phosphorus ratio, sodium, potassium and Serum triglyceride.

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) was determined by glutamate dehydrogenase method. Uric acid was determined by enzymatic photometric test by IFCC method. Creatinine was found by Jaffe's kinetic method. O-Cresophthalein complexone method was used to estimate calcium. Phosphorus was determined by modified Metol method while sodium and potassium were estimated by colorimetric 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. 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 (Kumar et al., 2003). 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. 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 hypoglo- bulinaemia 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

Table 1: Effect of OA, CTN and their combination on biochemical parameters

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 ± 0.87 [a]	2.60 ± 0.89 [b]	2.28 ± 0.90 [bc]	1.98 ± 0.91 [c]
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 ± 0.93 [a]	1.16 ± 0.93 [b]	1.10 ± 0.93 [b]	0.95 ± 0.94 [c]
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 ± 0.92 [a]	1.30 ± 0.93 [b]	1.22 ± 0.93 [bc]	1.04 ± 0.94 [c]
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 ± 0.94 [a]	0.97 ± 0.94 [ab]	1.03 ± 0.94 [b]	1.44 ± 0.92 [c]
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 ± 5.93 [a]	166.97 ± 4.99 [b]	159.16 ± 4.75 [b]	135.83 ± 5.40 [c]

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

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 (Dwivedi and Burns, 1984).

In the present investigation, the ALT levels in mycotoxin treated birds increased significantly than the control. 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. The increase in AST level in the present study could be due attributed to leakage of enzyme due to liver damage (Prakash, 2001). The present study revealed that mycotoxin treated birds showed an increase in serum ALP level as compared to control birds. 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

Table 2: Effect of OA, CTN and their combination on biochemical parameters

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 ± 0.76 [a]	7.18 ± 0.75 [b]	7.51 ± 0.73 [b]	8.31 ± 0.70 [c]
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 ± 4.68 [a]	149.05 ± 4.84 [b]	158.40 ± 4.81 [bc]	168.68 ± 5.22 [c]
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 ± 19.96 [a]	666.93 ± 27.03 [ab]	690.90 ± 28.12 [b]	713.12 ± 30.79 [b]
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 ± 3.22 [a]	115.26 ± 3.03 [b]	135.32 ± 4.02 [cd]	137.52 ± 3.57 [d]

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

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 ± 0.79 [a]	7.25 ± 0.75 [b]	7.79 ± 0.73 [c]	8.91 ± 0.70 [d]
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 ± 0.95 [a]	0.51 ± 0.95 [b]	0.45 ± 0.95 [a]	0.57 ± 0.95 [c]
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 ± 0.75 [a]	7.36 ± 0.74 [a]	7.73 ± 0.74 [ab]	8.05 ± 0.73 [b]

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

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 (Mohiuddin et al., 1993). The BUN values were higher in Group III when compared to

Table 4: Effect of OA, CTN and their combination on biochemical parameters

Parameter/Intervals	Group I (control)	Group II (OA)	Group III (CTN)	Group IV (OA+CTN)
Serum calcium (mg/dL)				
7 day	9.57 ± 0.24	7.89 ± 0.06	7.24 ± 0.08	6.86 ± 0.17
14 day	9.96 ± 0.27	8.02 ± 0.09	7.84 ± 0.06	6.99 ± 0.14
21 day	10.26 ± 0.22	8.10 ± 0.05	8.08 ± 0.05	7.71 ± 0.18
28 day	10.73 ± 0.19	9.75 ± 0.19	8.18 ± 0.08	8.00 ± 0.08
35 day	11.02 ± 0.18	10.76 ± 0.17	8.36 ± 0.17	8.16 ± 0.09
Mean value	10.31 ± 0.65 [a]	8.90 ± 0.71 [b]	7.94 ± 0.72 [cd]	7.54 ± 0.73 [d]
Phosphorus (mg/dL)				
7 day	7.18 ± 0.07	7.10 ± 0.06	6.18 ± 0.17	6.16 ± 0.16
14 day	7.15 ± 0.06	7.08 ± 0.04	6.29 ± 0.20	6.16 ± 0.16
21 day	7.09 ± 0.04	7.02 ± 0.03	6.32 ± 0.19	6.07 ± 0.12
28 day	6.55 ± 0.15	6.34 ± 0.18	6.27 ± 0.19	5.78 ± 0.18
35 day	6.36 ± 0.18	6.00 ± 0.14	5.49 ± 0.18	5.38 ± 0.14
Mean value	6.87 ± 0.75 [a]	6.71 ± 0.76 [a]	6.11 ± 0.78 [b]	5.91 ± 0.78 [b]
Calcium/phosphorus ratio				
7 day	1.80 ± 0.04	1.64 ± 0.02	1.39 ± 0.03	1.14 ± 0.02
14 day	1.76 ± 0.03	1.67 ± 0.02	1.41 ± 0.01	1.16 ± 0.01
21 day	1.73 ± 0.02	1.69 ± 0.02	1.46 ± 0.02	1.18 ± 0.02
28 day	1.74 ± 0.01	1.72 ± 0.01	1.52 ± 0.03	1.32 ± 0.04
35 day	1.76 ± 0.03	1.71 ± 0.01	1.59 ± 0.09	1.49 ± 0.02
Mean value	1.76 ± 0.91 [a]	1.69 ± 0.91 [b]	1.47 ± 0.92 [c]	1.26 ± 0.93 [d]
Sodium (mEq/L)				
7 day	148.00 ± 2.58	132.78 ± 2.72	127.06 ± 4.72	121.85 ± 3.77
14 day	147.42 ± 2.39	133.61 ± 2.66	125.67 ± 3.89	122.96 ± 3.42
21 day	146.16 ± 1.95	134.30 ± 2.55	124.70 ± 3.32	123.25 ± 2.51
28 day	143.85 ± 1.90	138.16 ± 2.67	123.92 ± 3.72	123.76 ± 3.61
35 day	142.25 ± 2.45	140.18 ± 3.36	124.98 ± 3.18	123.52 ± 3.46
Mean value	145.54 ± 3.86 [a]	135.81 ± 3.63 [b]	125.27 ± 3.44 [cd]	123.07 ± 3.30 [d]
Potassium (mEq/L)				
7 day	3.72 ± 0.05	3.43 ± 0.11	3.42 ± 0.11	3.15 ± 0.06
14 day	3.71 ± 0.05	3.46 ± 0.10	3.42 ± 0.11	3.14 ± 0.05
21 day	3.60 ± 0.08	3.47 ± 0.08	3.43 ± 0.04	3.16 ± 0.07
28 day	3.98 ± 0.05	3.74 ± 0.08	3.53 ± 0.12	3.25 ± 0.17
35 day	4.41 ± 0.26	3.59 ± 0.05	3.38 ± 0.06	3.10 ± 0.04
Mean value	3.88 ± 0.85 [a]	3.54 ± 0.85 [b]	3.44 ± 0.86 [b]	3.16 ± 0.87 [c]

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

Group I. 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 levels of serum creatinine were 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. 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 (Gupta et al., 2005).

Significant difference was noticed in the uric acid value between the Groups I and IV. 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 overall mean values for calcium were significantly decreased in all the mycotoxin fed groups when compared to the control. Among the mycotoxin fed groups no significant difference was observed between Group III and IV. The decreased calcium level could be attributed to reduced feed intake and poor intestinal absorption in the mycotoxin treated birds. Significant differences were noticed between Group III, IV and control birds. However, a numerical decrease in phosphorus level was noticed in Group II when compared to the control. The decreased phosphorus level could be attributed to reduced feed intake, excess renal excretion and poor intestinal absorption in the mycotoxin treated groups. There was a significant decrease in the sodium levels of birds fed with mycotoxin when compared to the Group I. The Group III and IV did not show significant difference between them. The overall mean value of sodium in Group II was significantly lower than the Group I. Low dose of

CTN employed in this study coupled with decrease sodium absorption due to reduced feed intake might be the reason for decrease in sodium level (Raina and Singh, 1991). There was a significant decrease in the potassium level in the mycotoxin fed groups when compared to the control. Among the mycotoxin fed groups, there was no difference in the mean potassium value between group II and III. Whereas there was a significant difference between Group III and IV. The hypokalaemia could be attributed to decreased feed intake, malabsorption and excess loss of potassium in urine due to nephritis (Rajeev, 2001). 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. 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. The commonly occurring dietary mycotoxin exposure to different animal species and human populations suggests need to assess the impact of these environmental food born contaminants.

References

Abdelhamid, A.M. and Dorra T.M. 1990. Study on the effects of feeding laying hens on separate myctotoxins (aflatoxins, patulin or citrinin) contaminated diets on the egg quality and tissue constituents. *Archives Tierenahr*, 40(4): 305-316.

Ahamad, D.B. and Vairamuthu, S. 2000a. Occurrence of citrinin mycotoxin in compounded feeds and various feed ingredients of poultry ration in Namakkal area (Tamilnadu). *Indian Journal of Environmental Toxicology*, 10 (2): 84-86.

Ahamad, D.B. and Vairamuthu, S. 2000b. Production of citrinin mycotoxin under laboratory condition. *Indian Journal of Environmental Toxicology*, 10 (2): 99-101.

Ahamad, D.B. and Vairamuthu, S. 2001. Individual and combined effects of citrinin and aflatoxin B1 in broiler chicks: A pathomorphological study. *Indian Journal of Veterinary Pathology,* 25 (1&2): 32-34.

Ahamad, D.B. 1999. Pathology of citrinin mycotoxicosis in broiler chicken. M.V.Sc. thesis, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.

Ames, D.D., Wyatt, R.D. Marks, H.L. and Washburn, K.W. 1976. Effect of citrinin, a mycotoxin produced by *Penicillium citrinum* on laying hens and young broiler chicks. *Poultry Science,* 55: 1294-1301.

Amita, P. 2001. Pathology of experimental ochratoxicosis in broilers and assessment of efficacy of a toxin binder. M.V.Sc. thesis, Univ. Agric. Sci., Bangalore, India.

Anandkumar. 2006. Pathological study on the combined effect of citrinin and aflatoxin in broiler chicken. Ph.D. thesis, Univ. TANUVAS, Chennai, India.

Anil Kumar, P. 2002. Pathology of aflatoxicosis, ochratoxicosis and ameliorating effect of vitamin E and selenium in broiler chicken. M.V.Sc. thesis. Univ. Agric. Sci., Bangalore, India.

Anil Kumar, P., Satyanarayana, M.L., Vijayasarathi, S.K., Sreenivas Gowda, R.N. and Suguna Rao. 2003. Pathology of lymphoid organs in aflatoxicosis and ochratoxicosis and immuno-modulatory effect of vitamin E and selenium in broiler chicken. *Indian Journal of Veterinary Pathology,* 27: 102-106.

Anitha, 2007. Pathology of ochratoxicosis and its amelioration with herbal oil and toxin binder in broiler chicken. M.V.Sc. thesis. Univ. KVAFSU, Bangalore, India.

Bailey, C.A., Gibson, R.M., Kubena, L.F., Huff, W.E. and Harvey, R.B. 1989. Ochratoxin A and dietary protein: 2. effects on haematology and various clinical chemistry measurements. *Poultry Science,* 68: 1664 – 1671.

Bhanuprakash, Sathyanarayana, M.L., Vijayasarathi, S.K. and Suguna Rao. 2004. Pathomorphological changes in broiler chickens exposed to individual and combined toxicity of aflatoxin and ochratoxin, and assessment of efficacy of a toxin binder. *Indian Journal of Animal Science,* 74: 747-749.

Braunberg, R.C., Gantt, O., Batan, C. and Friedman, L. 1992. In vitro-effect of the nephrotoxins ochratoxin A and citrinin upon biochemical function of porcine kidney. *Archives of Environmental Contamination and Toxicology,* 22 (4): 70-464.

Carlton, W.W., Sansing, G., Szczech G.M. and Tuite, J. 1974. Citrinin mycotoxicosis in beagle dogs. *Food and Cosmetics Toxicology,* 12: 479-490.

Ciegler, A., Vesonder, R.F. and Jackson, L.K. 1977. Production and biological activity of patulin and citrinin from *Penicillium expansum*. *Applied Environmental Microbiology,* 33: 1004-1006.

Creepy, E.E., Schlegel, M., Roschenthaler, R. and Dirheimer, G. 1980. Phenylalanine prevents acute poisoning by ochratoxin A in mice. *Toxicological Letters,* 60: 77-80.

Da Losso, E.J., Oliveira, M.B.M. and Carnieri, E.G.S. 1998. Citrinin induced mitochondria permeability transition. *Journal of Biochemical Toxicology,* 12: 291-297.

Devegowda, G., Raju, M.V.L.N., Afzal, N. and Swamy, H.V.L.N. 1998. Mycotoxin picture worldwide. Novel solution for their counteraction. In: Biotechnology in the feed Industry.Eds. T.P. Lyons and K.A Jacques, Proc. 14th Alltechs Annual Symp., KY, USA pp. 241-255.

Dwivedi, P. and Burns, R.B. 1984. Pathology of ochratoxicosis A in young broiler chicks. *Research in Veterinary Science,* 36: 92-103.

El-Bagoury, A.M. and Abdel Khalek, M.M. 1997. Incidence of ochratoxicosis on a poultry farm in Egypt. *Veterinary Medicine Journal,* 45: 11-16.

Gupta, S., Jinda, N., Khokhar, R.S., Gupta, A.K., Ledoux, D.R. and Rottinghaus, G.E. 2005. Effect of ochratoxin A on broiler chicks challenged with *Salmonella gallinarum*. *British Poultry Science,* 46: 443-450.

Hanika, C., Carlton, W.W., Boon, G.D. and Tuite, J. 1984. Citrinin mycotoxicosis in the rabbit: clinicopathological alternations. *Food Chemistry Toxicology,* 22 (12): 999-1008.

Hnatow, L.L. and Robert, F.W. 1985. Kidney function of single comb White Leghorn pullets following acute renal portal infusion of the mycotoxin citrinin. *Poultry Science,* 64: 1553-1561.

Huff, W.E. and Doerr, J.A. 1981. Synergism between aflatoxin and ochratoxin in broiler chickens. *Poultry Science, 60*: 550-555.

Huff, W.E., Kubena, L.F. and Harvey, R.B. 1988. Progression of ochratoxicosis in broiler chickens. *Poultry Science,* 67: 1139-1146.

Huff, W.E., Wyatt, R.D., Tucker, T.L. and Hamilton, P.B. 1975. Nephrotoxicity of dietary ochratoxin A in broiler chicken. *Poultry Science,* 53: 48-51.

Kubena, L.F., Huff, W.E., Harvey, R.B. and Corrier, D.E. 1988. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. *Poultry Science, 67*: 253-260.

Kumagai, S. 1985. Ochratoxin A: Plasma concentration and excretion into bile and urine in albumin-deficient rats. *Food and Chemistry Toxicology,* 23: 941-943.

Kumar, A., Jinda, N., Shukla, C.L., Yash Pal, Ledoux, D.R. and Rottinghaus, G.E. 2003. Effect of ochratoxin A on *E. coli* - challenged broiler chickens. *Avian Disease,* 47: 415-424.

Mohiuddin, S.M., Warasi, S.M.A. and Vikram Reddy, M. 1993. Haematological and biochemical changes in experimental ochratoxicosis in broiler chicken. *Indian Veterinary Journal,* 70: 613-617.

Prakash, G.C. 2001. Pathology of combined effects of aflatoxin and ochratoxin in broiler chicken. M.V.Sc. thesis, Univ. Agric. Sci., Bangalore, Idnia.

Raina, J.S. and Singh, B. 1991. Prevalence and pathology of mycotoxicosis in poultry in Punjab. *Indian Journal of Animal Science,* 61: 671-676.

Rajeev, K.. 2001. Pathology of ochratoxin and T-2 toxin and their combined toxicity in broiler chickens. M.V.Sc. thesis, Univ. Agric. Sci., Bangalore, India.

Ravikumar, 2007. Studies on interactive effects of ochratoxicosis and coccidiosis and evaluation of herbal anticoccidial efficacy in broiler chickens. M.V.Sc. thesis. Univ. KVAFSU, Bangalore, India.

Snedecor, G.W. and Cochran, W.G., 1989. Statistical methods, 8th Ed., Iowa state university press. Ames.

Trenk, H.L., Butz, M.E. and Chu, F.S. 1971. Production of ochratoxins in different cereal products by Aspergillus ochraceus. *Applied Microbiology,* 21: 1032-1035.