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# Combined effect of dietary aflatoxin and ochratoxin on serum biochemical profile in broilers and their amelioration using adsorbents

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## Abstract
The present study was conducted to evaluate the effect of aflatoxin and ochratoxin on serum biochemical changes and their amelioration using adsorbents in birds. Around 128 one day age broiler chicks were taken and divided into 8 birds in each group total amounting to 4 groups with 4 replicates in a complete randomized design. Each group was fed for 6 weeks with four different diets *viz* Group 1: Toxin free basal diet (BD), (Control), group 2: A mixture of BD, aflatoxin (AF) and ochratoxin A (OA). Group 3: Mixture of BD, AF, OA and activated charcoal (AC) and Group 4: Mixture of basal diet, AF, OA, AC and lyophilized yeast culture (Yc). The whole blood was collected on 14$^{th}$, 28$^{th}$ and 42$^{nd}$ day and serum were separated to evaluate the biochemical parameters. The result indicated a significant decrease in the glucose, total proteins, albumin, globulin, cholesterol, triglycerides, calcium, phosphorus and significant increase in the bilirubin in group 2 when compared to control group. Group 3 birds showed significant improvement in the biochemical profile when compared with group 2 and varying significantly from groups 4 and 1, which indicates that AC (@ 0.4%) has only partial amelioration effect on combined toxicity. Group 4 showed significant improvement when compared to group 2 and 3. The results obtained from the present study indicated that only AC has partial amelioration where as a combination of AC (@ 0.4%) and yeast culture (@ 0.2%) has complimentary effect in amelioration of the combined toxicity in broilers. However, the combination of the adsorbents cannot completely ameliorate the combined toxicity when compared to control group.

**Keywords:** Aflatoxin, ochratoxin, adsorbents, biochemical parameters

## Introduction
Mycotoxins are the fungal secondary metabolites which depending on their chemical structure cause deleterious effect on human and animal health (Peng *et al.*, 2018) [29]. These mycotoxins are formed when the feed is contaminated by toxigenic fungi (Tessari *et al.*, 2010) [41]. Aflatoxins and Ochratoxins are considered as the most hazardous mycotoxins causing mortality, decreased egg production, reduced body weight, and feed efficacy in various poultry species (Hoerr, 2020) [11]. Aflatoxins are produced by *Aspergillus spp*., particularly *A. flavus*, *A. parasiticus* and *A. nomius* (Guerre, 2016) [9]. Aflatoxin is most commonly found in the poultry feeds which can be highly toxic to poultry making major problem in poultry production (Loi *et al.*, 2020) [25]. In poultry the aflatoxin toxicity includes fatty liver, kidney disorder, leg and bone deformity, reduced weight gain and productivity, immunosuppression, small and poor-quality eggs, pigmentation problems, etc. (Sana *et al.*, 2019) [36]. Aflatoxin is found to exert detrimental effects on immune system by regression of bursa of Fabricius and lowering of serum immunoglobulin in ducks (Chen *et al.*, 2014) [5]. Prolonged exposure to aflatoxin increases susceptibility to infections and disease outbreaks. Ochratoxins are produced by genera *Aspergillus spp*. and *Penicillium spp*. Ochratoxins are exceedingly known to occur in cereal grains such as maize, wheat, oats, barley, rye, hay, and mixed feed (Bakr *et al.*, 2019) [3]. Ochratoxins are of three types A, B and C with most prevalent being Ochratoxin A (OTA), causing nephrotoxic, hepatotoxic, cytotoxic, immune suppressant effects besides decreased productivity in poultry (Peng *et al.*, 2018) [29]. It can accumulate in meat and eggs of poultry thereby causing carcinogenic and mutagenic effects in human consumers (Tsiouris *et al.*, 2021) [42].

Different approaches such as physical and chemical methods have been studied for mycotoxin elimination but have led to losses in nutritional value and biosafety risks and have limited efficacy and high potential costs (Ji *et al.*, 2016) [14]. Li *et al.*, 2018 [24] have described use of agents such as adsorbents that can help in the elimination of mycotoxins by binding or adsorption. In addition, the use of adsorbents is the safest method for reducing deleterious effects of mycotoxins. AC is one of the commonest adsorbents used in primary and secondary detoxification (Hamad *et al.*, 2022) [10]. Due to its porosity and large surface area, the AC is considered as an excellent adsorbent which can remove harmful gases, heavy metals, mycotoxins, pesticides, and other chemicals from aqueous solutions (Dizbay-Onat *et al.*, 2017) [7]. Another mechanism for elimination of mycotoxins is by adsorption to microorganisms. Microbial yeast such as *Saccharomyces cerevisiae* have been known to specifically bind to the cell wall components of the mycotoxins (Jouany, 2007) [16]. The esterified form of β-D-glucan from yeast cell walls can help to protect broiler chickens exposed to aflatoxin B1, ochratoxin A, and T-2 toxin, individually and in combination (Piotrowska, 2021) [30]. These toxin binders are nondigestible and are known to be effective in binding with mycotoxins in small intestines when they are mixed with broiler feed. In addition, these adsorbents alleviate the alterations in biochemical profile of birds such as total proteins, albumins and cholesterol caused by mycotoxins. Therefore, the present study was conducted to investigate the combined effect of two mycotoxins *viz.*, aflatoxin and ochratoxin A on the alterations in serum biochemical profile in broilers and their amelioration by AC and lyophilized yeast.

## Materials and Methods

### Production of aflatoxin and ochratoxin

*Aspergillus parasiticus* NRRL 2999 culture was used on broken rice to produce aflatoxin (Shotwel *et al.*, 1966) [38]. Rice brokens were inoculated with spore suspension obtained from six-day old culture of *A. parasiticus* grown on potato dextrose agar. *Aspergillus ochraceus* culture obtained from IMTC, Chandigarh was used to produce ochratoxin A. Flaked wheat was inoculated with a loopful of *A.ochraceus* culture maintained on oatmeal agar slants. The flasks containing *A. parasiticus* and *A.ochraceus* cultures were incubated in dark at room temperature (28-32 °C) for 6 days and 14 days, respectively, by shaking twice a day. Later at the end of respective periods fungus was killed by autoclaving rice broken and flaked wheat (15PSI, 121°C, 15min) and they were dried over night at 50 °C in hot air oven, powdered and stored in cool dark place. Aflatoxin was extracted and quantified by thin layer chromatography (TLC) method using modified Romer's method (Romer, 1975) [34]. Ochratoxin A was extracted and estimated using TLC as per AOAC 1995 method (AOAC, 1995) [2].

### Experimental design

About 128 one day age old broiler chicks of Vencob strains and distributed as 8 birds in each group with a total of 4 groups with 4 replicates. The four groups of chicks were fed with following experimental diets; Group 1

The experimental design was completely randomized design with four groups of chicks which were fed with the following experimental diets; Group 1 – Basal diet (BD) (control Group), Group 2 – BD + 1 ppm AF + 2 ppm OA, Group 3 – BD+ 1 ppm AF + 2 ppm OA+ 0.4% AC and Group 4 – BD+ 1 ppm AF+ 2 ppm OA + 0.4% AC+ 0.2% lyophilized yeast culture (Yc).

Blood was collected from the birds in each treatment by puncturing the wing vein on 14$^{th}$, 28$^{th}$ and 42$^{nd}$ days. Serum was separated by centrifugation after collection and was stored at -20$^{o}$C for subsequent analysis. The individual serum samples were analysed for glucose (O Toluidine method), Total Protein (Biuret method), Albumin (BCG dye binding method), Globulin, Cholesterol (Wybenga and Pileggi's method), Triglycerides (GPO method), Total bilirubin (Diazo method), Calcium (O-Cresolthalein method) and Phosphorus (Ammonium Molybdate method).

### Statistical analysis

The obtained experimental data for different treatments from the experimental animals has been analyzed for finding significance among the experimental groups as per the procedures of Snedecor and Cochran (1994) [40] by using statistical package for social sciences (SPSS – 20 software.

## Results and Discussion

The mean serum glucose, total protein, albumin, globulin, bilirubin, cholesterol, triglycerides, calcium and phosphorous levels were significantly ($p$<0.01) affected by different diets as well as periods of collection of blood.

**Serum glucose:** The mean values of serum glucose as affected by different diets and periods of collection of blood are shown in Table 1. Serum glucose levels of groups 1, 2, 3 and 4 were 129.40, 104.54, 115.64 and 120.22 (mg %) respectively. The mean serum glucose levels were 105.44, 118.48 and 128.44 (mg %) on 14$^{th}$, 28$^{th}$ and 42$^{nd}$ days respectively. The glucose concentration was lowest in group 2 containing both aflatoxin and ochratoxin A. Hypoglycemia in group 2 may be attributed to reduced levels of glucose-6-phosphatase enzyme due to the effect of aflatoxin. These results were in accordance with Ledoux *et al.*, (1999) [23], Mishra *et al.*, (1996) [27] and Barati *et al.*, 2018 [4]. Though ochratoxin A has a tendency of increasing glucose levels (Kumar and Gopal, 2015) [21, 22], the glucose levels were decreased on diet 2 indicating that aflatoxin had more depressant effect compared to increasing tendency of ochratoxin A. According to Rosa *et al.*, (2001) [35], the breakdown of glycogen in the liver cells and subsequent release into the blood stream may be the cause of the elevated serum glucose level experienced during aflatoxicosis.

**Total proteins:** The mean values of serum total proteins as affected by different diets and periods of collection of blood are shown in Table 1. The protein levels for groups 1, 2, 3 and 4 were 4.09, 2.24, 3.29 and 3.54 (g %) respectively. The mean serum total protein levels were 3.20, 3.50 and 3.18 (g %) on 14$^{th}$, 28$^{th}$ and 42$^{nd}$ days respectively. The total serum protein values were lowest in group 2. The reason for the depression of serum total proteins might be due to antagonistic interaction between the two toxins (Raju and Devegowda, 2000) [32]. Aflatoxin inhibits DNA-dependent RNA polymerase and causes impairment of DNA template function resulting in general inhibition of protein synthesis. As a result, hypoproteinemia is a common effect of aflatoxicosis (Huff *et al.*, 1986) [12]. Creppy *et al.*, (1983) [6] reported that ochratoxin A causes depression in serum protein levels through competitive inhibition of phenyl alanine-tRNA synthesis with A. Hypoproteinemia recorded in group 2 might be due to Phenyl alanine of ochratoxin synergistic effect of aflatoxin

and ochratoxin A present in the diet. These results were in accordance to the results obtained by Al-Masad and Rao *et al.,* (2018) [33] where decrease of total proteins was observed in broilers when fed with mycotoxins.

**Serum albumin:** The total serum albumin levels for the groups 1, 2, 3 and 4 were 2.65, 0.89, 1.26 and 1.42 (g %) respectively. The mean Serum albumin levels were 1.72, 1.52 and 1.43 (g %) on 14th, 28th and 42nd days, respectively. The serum albumin levels were lowest ($p$<0.01) in group 2. The reason for the decrease may be attributed to liver damage. Similar toxicity were also reported by Kubena *et al.* (1991) [18] and Kalorey *et al.* (2005) [17].

**Serum globulins:** The mean globulin levels were 1.44, 1.35, 2.03 and 2.12 (g %) for groups 1, 2, 3 and 4, respectively. The mean levels were 1.48, 1.97 and 1.75 (g %) on 14th, 28th and 42nd days respectively. Group 2 fed with aflatoxin and Ochratoxin A showed lowest serum globulin content significantly ($p$<0.01) compared to other groups. This indicates that aflatoxin and ochratoxin A synergistically inhibited the globulin synthesis. This was in accordance with the report of Kumar *et al.,* (2005) [20].

**Serum bilirubin:** The mean serum bilirubin values were 3.38, 3.84, 3.56 and 3.47 (mg %) for groups 1, 2, 3 and 4, respectively. The mean serum bilirubin values were 3.27, 3.54 and. 3.88 (mg %) respectively on 14th, 28th and 42nd days. Bilirubin values were highest for the group receiving aflatoxin and ochratoxin A in the diet. The highest serum bilirubin levels in group 2 containing aflatoxin and ochratoxin A indicates the impaired liver function caused by aflatoxin majorly. A similar increase in bilirubin was observed by Raj *et al.,* 2021 [42] where broilers were fed with aflatoxin and ochratoxin A.

**Serum cholesterol:** The mean cholesterol levels in groups 1, 2, 3 and 4 were 118.15, 79.41, 98.79 and 106.97 (mg %). The mean serum cholesterol levels were 85.04, 99.21 and 118.24 (mg %) on 14th, 28th and 42nd days, respectively. Serum cholesterol concentrations (Table 1) were significantly ($p$<0.01) lower in group 2. Marked depression in serum cholesterol due to synergistic effect of aflatoxin and ochratoxin A was reported by Kubena *et al.* (1991) [18] and Kalorey *et al.* (2005) [17]. Raj *et al.* (2021) [42] observed hypocholesterolaemic in broiler chicks fed with a diet containing aflatoxin and Ochratoxin A.

**Serum triglycerides:** The mean serum triglycerides values for groups 1, 2, 3 and 4 were 119.77, 66.83, 78.89 and 86.38 (mg %), respectively. The mean serum triglycerides levels were 91.28, 87.33 and 85.29 mg % on 14th, 28th and 42nd days, respectively. The mean serum triglycerides levels were significantly ($p$<0.01) different among the groups. The serum triglyceride levels (Table 1) decreased significantly ($p$<0.01) in group 2 compared to other groups. Marked depression in serum triglycerides in group 2 may be due to inhibitory effect on triglyceride synthesis. This might be due to hepatic damage observed in broilers due to combined toxocosis. Similar results were reported by Kalorey *et al.,* (2005) [17] and Rao *et al.,* 2018 [33].

**Serum calcium:** The mean serum calcium values were 12.74, 8.75, 10.55 and 11.43 (mg %) for the groups 1, 2, 3 and 4, respectively. The mean serum calcium values were 10.47, 10.88 and l 1.24 (mg %) on 14th, 28th and 42nd days, respectively. Serum calcium levels were significantly ($p$<0.01) lower in group 2. This may be attributed to synergistic effect of the two toxins. The decreased serum calcium levels in group 2 may be due to damage to liver and kidney, the organs that play a major role in vitamin $D_3$ metabolism which regulates the serum calcium levels. The present observations were in agreement with the decrease in serum calcium in aflatoxicosis and ochratoxicosis reported by Rao *et al.,* 2018 [33] and Raj *et al.,* (2021) [42].

**Serum phosphorous:** The mean serum phosphorous values were 8.70, 6.97, 7.54 and 8.02 (mg %) for the groups 1, 2, 3 and 4, respectively. The mean serum phosphorous values were 7.13, 7.78 and 8.51 (mg %) on 14th, 28th and 42nd days, respectively. The serum phosphorous levels decreased significantly on group 2. This may be attributed to synergistic effect on the toxins which might have caused damage to liver and kidney leading to alteration in renal and intestinal transport of phosphorous. The present observations were in agreement with the decrease in serum phosphorous levels reported by Jindal *et al.,* (1994) [15], Shukla and Pachauri (1995) [39] and Santurio *et al.* (1999) [37] in alflatoxicosis and in ochratoxicosis reported by Huff *et al.,* (1975) [13], Kubena *et al.,* (1983) [19], Manning and Wyatt, (1984) [26] and Rao *et al.,* 2018 [33].

Increased levels of serum glucose levels, serum total protein, serum albumin, mean serum cholesterol levels, serum triglycerides levels, serum calcium, serum phosphorous and decreased levels of serum bilirubin in group 3 compared to group 2 might be due to ameliorating effect of AC. Al-Masad reported an improvement in serum glucose levels, serum total protein, serum albumin, mean serum cholesterol levels, serum triglycerides levels in broilers on inclusion of AC in aflatoxin contaminated diet. The ameliorative effect of serum biochemical parameters in group 3 was less than that of group 4 indicating that Yc had a complementary effect in combination with AC in ameliorating the combined effect of aflatoxin and ochratoxin A in broilers Ameliorative nature of Yc on serum biochemical parameters was reported by Modirsanei *et al.,* (2004) [28] and Rao *et al.,* (2018) [33]. However, these parameters in broiler group fed with diet 4 recorded significantly lower values than that of control diet indicating that AC and Yc could not ameliorate the toxic effect completely.

**Table 1:** Mean values of serum biochemical profile in broilers as affected by different experimental groups

| Serum parameters | Groups | Period (days) | | | Overall mean ± S.E |
|---|---|---|---|---|---|
| | | 14th | 28th | 42nd | |
| Glucose (mg %) | 1 | 112.42±3.39 | 131.27±1.76 | 144.52±2.69 | 129.40±4.21$^{d}$ |
| | 2 | 98.21±2.24 | 102.63±2.99 | 112.77±2.01 | 104.54±2.24$^{a}$ |
| | 3 | 104.27±2.78 | 118.52±1.52 | 124.14±1.74 | 115.64±2.75$^{b}$ |
| | 4 | 106.85±1.86 | 121.48±2.68 | 132.32±1.48 | 120.22±3.38$^{c}$ |
| Overall mean ± S.E | | 105.44±1.77$^{a}$ | 118.48±2.85$^{b}$ | 128.44±3.13$^{c}$ | |

| | | | | | |
|---|---|---|---|---|---|
| Total proteins (g %) | 1 | 3.78±0.07 | 4.06±0.03 | 4.43±0.03 | 4.069±0.08$^{d}$ |
| | 2 | 2.37±0.06 | 2.52±0.09 | 1.85±0.07 | 2.24±0.10$^{a}$ |
| | 3 | 3.19±0.06 | 3.59±0.06 | 3.09±0.13 | 3.29±0.08$^{b}$ |
| | 4 | 3.46±0.07 | 3.82±0.05 | 3.34±0.04 | 3.54±0.07$^{c}$ |
| Overall mean ± S.E | | 3.20±0.14$^{a}$ | 3.50±0.15$^{b}$ | 3.18±0.24$^{a}$ | |
| Albumin (g %) | 1 | 2.39±0.02 | 2.60±0.02 | 2.97±0.03 | 2.65±0.07$^{d}$ |
| | 2 | 1.17±0.01 | 0.91±0.02 | 0.59±0.01 | 0.89±0.07$^{a}$ |
| | 3 | 1.57±0.04 | 1.16±0.04 | 1.05±0.02 | 1.26±0.07$^{b}$ |
| | 4 | 1.74±0.02 | 1.42±0.02 | 1.09±0.02 | 1.42±0.08$^{c}$ |
| Overall mean ± S.E | | 1.72±0.11$^{c}$ | 1.52±0.17$^{b}$ | 1.43±0.24$^{a}$ | |
| Globulins (g %) | 1 | 1.40±0.07 | 1.46±0.03 | 1.46±0.04 | 1.44±0.03$^{b}$ |
| | 2 | 1.20±0.05 | 1.61±0.08 | 1.26±0.07 | 1.35±0.07$^{a}$ |
| | 3 | 1.62±0.08 | 2.43±0.08 | 2.04±0.11 | 2.03±0.11$^{c}$ |
| | 4 | 1.72±0.08 | 2.40±0.03 | 2.25±0.05 | 2.12±0.09$^{d}$ |
| Overall mean ± S.E | | 1.48±0.06$^{a}$ | 1.97±0.12$^{c}$ | 1.75±0.77$^{b}$ | |
| Bilirubin (mg %) | 1 | 3.16±0.07 | 3.32±0.06 | 3.67±0.02 | 3.38±0.07$^{a}$ |
| | 2 | 3.43±0.02 | 3.92±0.04 | 4.18±0.02 | 3.84±0.09$^{d}$ |
| | 3 | 3.29±0.01 | 3.54±0.02 | 3.86±0.02 | 3.56±0.07$^{c}$ |
| | 4 | 3.21±0.03 | 3.40±0.04 | 3.79±0.03 | 3.47±0.07$^{b}$ |
| Overall mean ± S.E | | 3.27±0.03$^{a}$ | 3.54±0.06$^{b}$ | 3.88±0.05$^{c}$ | |
| Cholesterol (mg %) | 1 | 9.42±1.52 | 117.40±2.11 | 142.62±2.77 | 118.15±6.04$^{d}$ |
| | 2 | 70.80±2.15 | 79.48±3.76 | 87.95±1.53 | 79.41±2.53$^{a}$ |
| | 3 | 84.61±2.34 | 96.32±1.90 | 115.44±2.47 | 98.79±4.01$^{b}$ |
| | 4 | 90.35±1.10 | 103.64±2.03 | 126.93±1.88 | 106.97±4.65$^{c}$ |
| Overall mean ± S.E | | 85.04±2.45$^{a}$ | 99.21±3.71$^{b}$ | 118±5.25$^{c}$ | |
| Triglycerides (mg %) | 1 | 107.33±3.42 | 117.19±8.07 | 134.79±2.34 | 119.77±4.38$^{d}$ |
| | 2 | 74.63±1.13 | 66.67±1.81 | 59.19±1.58 | 66.83±2.06$^{a}$ |
| | 3 | 87.43±2.04 | 78.31±0.89 | 70.92±0.59 | 78.89±2.15$^{b}$ |
| | 4 | 95.72±0.86 | 87.13±0.78 | 76.28±0.96 | 86.38±2.44$^{c}$ |
| Overall mean ± S.E | | 91.28±3.22$^{a}$ | 87.33±5.18$^{a}$ | 85.29±7.58$^{a}$ | |
| Calcium (mg %) | 1 | 11.47±0.85 | 12.94±0.19 | 13.82±0.45 | 12.74±0.42$^{d}$ |
| | 2 | 9.14±0.32 | 8.62±0.25 | 8.48±0.70 | 8.75±0.26$^{a}$ |
| | 3 | 10.43±0.93 | 10.55±0.35 | 10.67±0.28 | 10.55±0.31$^{b}$ |
| | 4 | 10.85±0.49 | 11.43±0.63 | 12.02±0.21 | 11.43±0.29$^{c}$ |
| Overall mean ± S.E | | 10.47±0.38$^{a}$ | 10.88±0.44$^{a}$ | 11.24±0.54$^{b}$ | |
| Phosphorus (mg %) | 1 | 7.35±0.43 | 8.73±0.74 | 10.02±0.45 | 8.70±0.44$^{d}$ |
| | 2 | 6.90±0.06 | 6.77±0.35 | 7.22±0.11 | 6.97±0.13$^{a}$ |
| | 3 | 7.02±0.25 | 7.64±0.16 | 7.95±0.10 | 7.54±0.15$^{b}$ |
| | 4 | 7.23±0.17 | 7.97±0.32 | 8.86±0.08 | 8.02±0.23$^{c}$ |
| Overall mean ± S.E | | 7.13±0.13$^{a}$ | 7.78±0.27$^{b}$ | 8.51±0.29$^{c}$ | |

Values bearing different superscripts within rows as well as columns differ significantly ($p$<0.01)

## Conclusion

Mycotoxins are extremely toxic to poultry and has the potential to compromise poultry health and production. From the present study, it is evident that aflatoxin and ochratoxin A together exhibited a negative impact on the serum biochemical profile in broilers. Non-nutritive adsorbent materials when mixed with the diet help in the reduction of absorption of mycotoxins by gastrointestinal tract. Based on the results obtained, AC showed partial ameliorative nature on combined toxicity in broilers. Combination of AC and Yc showed greater ameliorative effect than AC alone due to their complementary action on combined toxicity. However, this combination did not completely ameliorate the toxic effects of the mycotoxins when compared with the control group.

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