

DETOXIFICATION POTENTIAL OF OCHRATOXIN BY YEAST SLUDGE AND EVALUATION IN BROILER CHICKS

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ABSTRACT

Aspergillus ochraceus was cultured using Sabraud's Dextrose Agar (SDA) and confirmed on the basis of macroscopic and microscopic characters. Ochratoxin was produced on fermented wheat grains, was extracted from the samples by immunoaffinity column. Estimation of ochratoxin was performed by high-performance thin layer Chromatography (HPTLC) against known mycotoxin standard. Broiler chicks (n=150) were purchased and were reared on commercial Broiler feed for 7 days. At the end of first week birds (n=120) were selected on uniform weight basis. Four diets formulated were A (plain feed), B (Ochratoxin A 500ppb), C (Ochratoxin A 500ppb and 1% Yeast sludge) and D (Ochratoxin A 500ppb and 2% Yeast sludge). Completely randomized design (CRD) was adopted for the trial with a total of 12 experimental units. The chicks were fed their respective experimental diets *ad libitum* for 35 days during which the daily feed consumption, group weight changes and Feed conversion ratio (FCR) were measured. Feed conversion ratio (FCR) was improved in group D (1.97) followed by A (2.05), B (2.07) and C (2.04). The level of serum total protein and albumin was found to be highest in Group D followed by A, C and B activity of alanine transaminase (ALT) was highest in group B followed by C, D and A. Level of ochratoxin in liver samples collected from group A, B, C and D was 0, 87.5, 57.5 and 43.7 ppb, respectively. Level of ochratoxin in liver samples collected from group A, B, C and D was 0, 116, 89 and 35.5 ppb, respectively. Concludingly detoxification potential in (2) percent yeast sludge was better than (1) percent. Yeast sludge can efficiently used for detoxification of mycotoxins.

Key words: *Aspergillus ochraceus*, Ochratoxin, Yeast sludge, FCR, TLC.

INTRODUCTION

The occurrence of mycotoxins produced by fungus present in the raw material which is used in poultry feed is the key reason of mycotoxicosis. Consumption of mycotoxins at a very low level can cause severe mycotoxicosis but it can also result in damage to innate and acquired resistance to infections (Dalcero *et al.* 1998). The presence of aflatoxin B₂ and ochratoxin A in poultry feed ingredients and processed feed seems to pose a serious threat for local poultry industry (Anjum *et al.* 2011). On the basis of acute mean lethal dose and minimal growth inhibitory concentration ochratoxin A is the most dangerous mycotoxin studied up in chickens. The most effective indicator of ochratoxicosis in young broiler chickens is enlarged kidneys. Ochratoxicosis in young White Leghorn cockerels leads to acute nephrosis, hepatic degeneration and suppression of hematopoiesis. In laying hens low concentrations of ochratoxin results in delayed sexual maturity, egg production and reduced hatchability (Doupnik *et al.* 1970). Method mostly applied for protecting animals against mycotoxicosis is the use of adsorbents mixed with the feed which adsorb the mycotoxins effectively in the intestinal tract. Different mycotoxin binders are activated charcoal, aluminosilicate, diatomaceous earth, yeast and lactic acid

producing bacteria (Huwig *et al.* 2001). Another adsorbent is yeast sludge, which is produced as a by product of alcohol industry. It was found that 1% yeast sludge worked effectively at 100 and 200 ppb aflatoxins level (Hashmi *et al.* 2006)

Yeast sludge contains mannan oligosaccharides in its cell wall, which can detoxify Ochratoxin by adsorption. Detoxification potential of yeast sludge against ochratoxin in broiler chicks was evaluated.

MATERIALS AND METHODS

Ochratoxin purified from *A. ochraceus* was detoxified with yeast sludge and potential evaluated by feeding mixture to broiler chicks under experimental conditions.

Confirmation of *Aspergillus ochraceus*: *Aspergillus ochraceus*, procured from department of Pathology, University of Agriculture, Faisalabad was confirmed on the basis of colony (obverse and reverse) and microscopic characters by culturing on Sabrauds dextrose agar (SDA). Microscopic characters like nature of hyphae, vesicle and type of conidiospores were observed by slide culture to confirm genus. Species under *Aspergillus* was identified on the basis of colony texture and deposition of colour in central portion. (Trenk *et al.* 1971)

Ochratoxin production: Wheat grains (40g) were soaked in 40ml water and autoclaved at 121°C 15psi for 20min. Soaked wheat grains were inoculated *A. ochraceus* spores (1×10^6) and incubated at 27°C for one month. Flasks were shaken three times in a day till the end of experiment. (Trenk *et al.* 1971)

Extraction of Ochratoxin: Fermented wheat grains (20g) were mixed in 50ml acetonitrile water (75:25) and placed on shaking water bath for two hours. Mixture was filtered through whatmann filter paper (0.45 μ m) under sterile conditions. Filtrate was passed through immunoaffinity column and eluted with 1ml methanol. (Trenk *et al.* 1971)

Ochratoxin estimation: Extracted ochratoxin mixture was submitted to Romer laboratory for estimation. The purified extracts were analyzed by instrumenta high performance thin layer chromatography (HPTLC). The residues (30 μ l) were spotted on a commercially available silica gel plate. The plate was developed with toluene: ethyl acetate: formic acid (6:3:1) (Dragan *et al.* 2009).

Biological trial: Day old broiler chicks (n=150) were purchased along with their feed from Big Bird hatchery. Yeast sludge was procured from Shukkur Gunj sugar mill Jhang. The chicks were reared on commercial Broiler feed for 7 days. At the end of first week birds (n=120) were selected on uniform weight basis. Four diets formulated were A (plain feed), B (Ochratoxin A 500ppb), C (Ochratoxin A 500ppb and 1% Yeast sludge) and D (Ochratoxin A 500ppb and 2% Yeast sludge). These four diets were fed randomly to the chicks, such that there were three replicates on each ration. Completely randomized design (CRD) was adopted for the trial with a total of 12 experimental units. The chicks were fed their respective experimental diets *ad libitum* for 35 days. Each replicate of chicks was weighed initially and there after weekly. The feed which was provided weighed at the start of the experiment and then at the end of five weeks feeding trial. During the biological trial weekly Weight gain (gm) was recorded. At the end of experiment Feed conversion ratio (F.C.R) and mortality rate was determined (Santin *et al.* 2002. Blood, liver and kidney samples were collected from each group in three replicates.

Analysis of Sera: Collected blood samples were placed in slanting position for overnight. Sera separated and cleared by centrifugation at 2000rpm for 20minutes. Estimation of serum total protein (Spectrum), serum albumin (Biocon) and activity of Alanine transaminase (ALT) (Crescent) were determined by kits on chemistry analyzer. (Zahoor ul Hasan *et al.* 2010)

Estimation of ochratoxin in liver and kidney: The detection of OTA in liver and kidney samples were carried out by thin-layer chromatography (TLC). The

samples were spotted on precoated TLC plates along with standard 30 μ l each and allowed to dry. Plates were then placed in mobile phase containing Benzene: methanol: acetic acid (90:5:5). Results were noted by visualizing plates under ultraviolet light (Dragan *et al.* 2009).

Statistical analysis Data were analyzed through one way analysis of variance (ANOVA) and Duncan's Multiple range test using CoStat Version 6.4

RESULTS AND DISCUSSION

Aspergillus ochraceus was confirmed on the basis of microscopic and macroscopic characters. Microscopic features observed were radiate conidia, thin walled hyaline vesicle, metulae covered entire vesicle, brownish conidiophore and pink to venaceous purple sclerotia. Mould colony was white cottony initially and on maturation centre converted to yellowish orange due to conidiospores. Presence of ochratoxin A was detected by TLC on the basis of greenish blue fluorescence under ultraviolet light. Confirmation and estimation was carried out at Romer laboratory using high performance thin layer chromatography (HPTLC) method which was 1407ppb per 2g wheat biomass.

It was observed that chicks fed on diet B containing 500ppb OTA had significantly ($p < 0.05$) lower weight as compared to the chicks fed on all other diets. Prior *et al.* (1980) reported reduction in weight of birds feeding the same level of OTA in broiler chicks. It was observed that birds fed on B ration consumed significantly ($p < 0.05$) less feed as compared to other rations. These results are in accord with Santin *et al.* (2006). In corroboration results were observed by addition of yeast cell wall in the broiler diet on feed conversion ratio. The mortality rate was lowered in the presence of yeast sludge. Severity of ochratoxicosis with increase in dietary yeast sludge in OTA contaminated diet was reduced.

Serum biochemical parameters of birds fed different levels of OTA studied by Zahoor-ul-Hassan *et al.* (2010) revealed that serum total proteins and albumin decreased significantly in ochratoxin fed group whereas activity of ALT increases as compared with control group. Findings of present study were comparable.

In birds fed on 500ppb OTA significantly higher levels of OTA in their liver and kidneys were detected as compared to other groups. Higher level of OTA was observed in kidneys than liver sample. Recent study from Dragan *et al.* (2009) showed that the most contaminated tissue with OTA was kidney. In kidneys the level of OTA was considerably higher and exceeded the permissible levels (10ng/g) of this toxin proposed by the European Commission.

From all the observations it can be concluded that ochratoxin is a nephrotoxic and hepatotoxic agent

and 2% yeast sludge is more effectively reduced the toxicity of OTA as compared to 1% yeast sludge to

detoxify 500ppb OTA. Compiled results of present work plan are present at table (01).

Table 01: Detoxification potential of yeast sludge in broiler chicks fed with diet contaminated with 500ppb ochratoxin at 7 to 35 days of age.

Groups	Avg. Feed intake (g)	Avg. weight gain (g)	Avg. FCR	Serum total protein (g/dL)	Serum albumin (g/dL)	Activity of ALT (U/L)	Level of ochratoxin in liver (µg/Kg)	Level of ochratoxin in kidneys (µg/Kg)	Percent Mortality
A	2097.5 ^c	1021.025 ^c	2.05 ^a	3 ^a	0.5 ^a	67 ^b	0 ^a	0 ^a	10
B	2068.5 ^b	1009.97 ^b	2.07 ^a	2.6 ^a	0.3 ^a	69 ^c	87.5 ^d	116 ^d	20
C	2068 ^b	989.75 ^a	2.04 ^a	2.2 ^a	0.2 ^a	76 ^d	57.5 ^c	89 ^c	3
D	2035.5 ^a	1032.05 ^d	1.97 ^a	2.8 ^a	0.5 ^a	62 ^a	43.7 ^b	35.5 ^b	6

Different superscripts on means in a column shows significant difference among groups (P < 0.05).

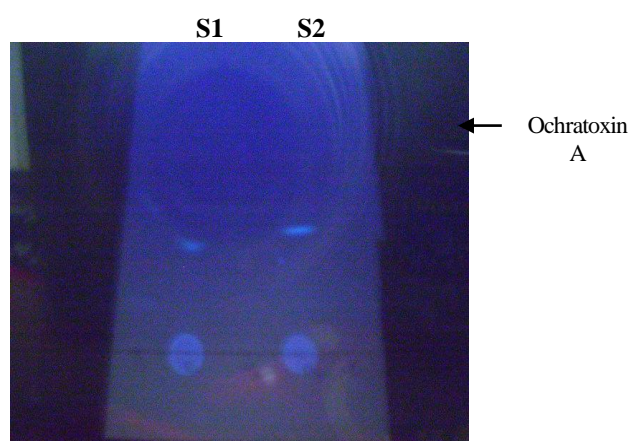


Figure 01 Detection of ochratoxin A by thin layer chromatography S1 and S2: sample extracted by immunoaffinity column.

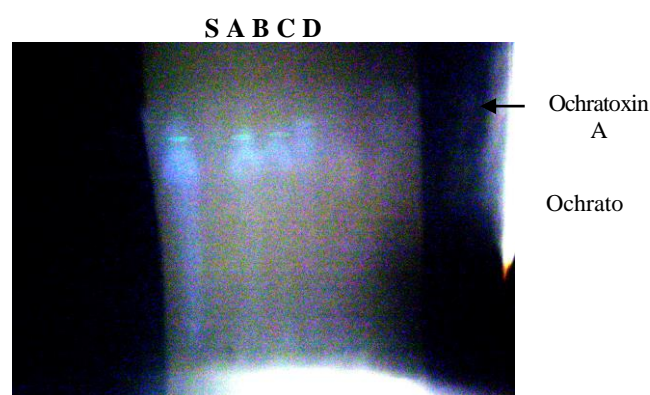


Figure 03 Detection of ochratoxin A deposition in kidneys of Broiler chicks Spot S: standard, A: control, B:500ppb ochratoxin, C: 500ppb ochratoxin A and 1% yeast sludge and D:500ppb ochratoxin A and 2% yeast sludge

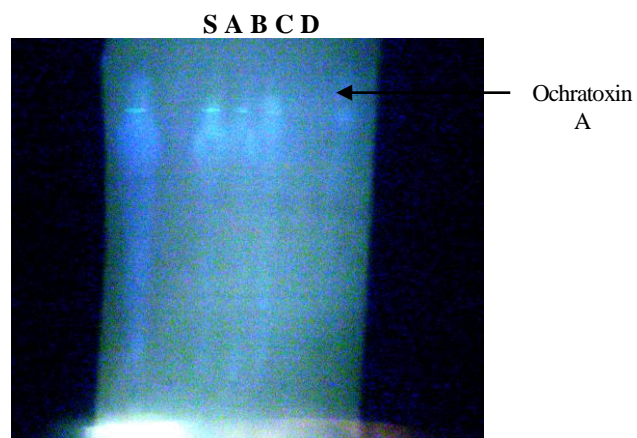


Figure 02 Detection of ochratoxin A deposition in liver of Broiler chicks Spot S: standard, A: control, B:500ppb ochratoxin, C:500ppb ochratoxin A and 1% yeast sludge and D:500ppb ochratoxin A and 2% yeast sludge.

Acknowledgment: The authors are thankful to Prof. Dr Khushi Muhammad Chairman Department of microbiology. University of Veterinary and Animal sciences, Lahore for their co/operation during research work. Thanks to Romer Labs for estimation of ochratoxin A produced through fermentation of wheat by *Aspergillus ochraceus*.

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