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RESEARCH ARTICLE

Protective Effect of Yeast Sludge and Whey Powder against Ochratoxicosis in Broiler Chicks

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ABSTRACT

The aim of the present study was to determine the detoxification potential of the food industry by-products such as yeast sludge (YS) and whey powder (WP) against harmful effects of ochratoxin A (OTA) on broilers. One day old broilers chicks (n=1250) were randomly divided into five groups replicated five times with each replicate having 50 birds. The experimental feed in different groups was as; group A (basal feed), group B (200ppb OTA), group C (200ppb OTA and 0.2% Dried YS), group D (200ppb OTA and 0.2% WP) and group E (200ppb OTA and 0.2% protemyc). OTA adversely affected body weight gain, feed consumption and feed conversion ratio (FCR) of broiler chicks. Haematobiochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), and creatinine levels raised by OTA feeding were significantly (P>0.05) reduced in YS and WP supplemented group. Residues of OTA were detected in all the tissues studied, with highest levels observed in kidneys, YS and WP significantly reduced the tissue residues of OTA. In conclusion, present study suggested that addition of YS and WP in broilers feed reduce the harmful effects of OTA in broiler chicks as efficiently as protemyc a commercial mycotoxin binder.

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INTRODUCTION

Ochratoxins are mycotoxins produced by toxigenic strains of *Aspergillus ochraceus*, *Aspergillus westerdijkiae*, *Aspergillus niger* and some spps. of *Pencillium*, it has three main types, i-e., Ochratoxin A (OTA), ochratoxin B (OTB) and Ochratoxin C (OTC). Of these, OTA has potent immunotoxic, nephrotoxic, mutagenic and teratogenic effects (Paola and Marco, 2015). The presence of OTA in poultry feedstuffs appears to put a severe hazard for the native poultry industry (Hameed *et al*., 2017). It also induces alterations in the biochemical parameters (reduction in, hematocrit, hemoglobin, blood cells, serum total proteins; whereas the uric acid, creatinine, liver enzymes were increased). It disturbs protein synthesis, carbohydrate and lipid metabolism blood coagulation and hormones. Ochratoxin A is also characterized as severe immunosuppressive in avian species (Saleemi *et al*., 2017; Elaroussi *et al*., 2008).

Due to prevalence of high level toxicity of OTA, it is imperative to find procedures for elimination of OTA from poultry food and feedstuffs (Amézqueta *et al.*, 2009). An effective approach for detoxification of mycotoxin in animal feedstuff is the usage of nutritionally inert substances which can minimize the level of toxicity. Toxin binders, such as aluminosilicate, yeast, lactic acid producing bacteria and detoxifying agents such as vitamin E, are used in the feed to reduce the harmful effects of these mycotoxins on poultry birds (Khan *et al.*, 2010; 2014a; Chen *et al.*, 2016).

Distillery yeast sludge (DYS) a by-product of alcohol production industry contains high amount of glucomannans (Sharif *et al.*, 2012), which can adsorb different mycotoxins. An improvement in the blood biochemistry parameters and productive performance of broilers has been reported by the supplementation of DYS in mycotoxin contaminated feed (Mujahid *et al*., 2012; Hashmi *et al.*, 2006; Khatoon *et al*., 2017).

Dried concentrated whey is a by-product of cheese making industry, it enhances the immunity, improve survival rates, and stimulate the growth of beneficial intestinal bacteria in broilers because it is a natural prebiotic. Greater abundance of *L. salivarius* with improved feed conversion ratio (FCR) has been reported

by the supplementation of whey in Broiler diet. (Pineda-Quirogaa *et al*., 2017). Anti-ochratoxin effect of whey can be attributed to its capacity to increase the *lactobacillus* count in the gastrointestinal tract of the poultry (Zarei *et al.*, 2018) Moreover Mansour *et al.*, (2011) and (2015) proved that whey successfully neutralizes the drastic toxic effects of OTA in *Oreochromus niloticus*, No available studies conducted to investigate the effect of whey on ochratoxicosis in broilers. Therefore, the objective of this paper is to discuss the changes on broiler performance and biochemical parameters associated with ochratoxicosis induced in broiler chicks by feeding them with a diet containing known concentration of OTA at level of 200ppb. In addition, to determine the protective effect of YS and WP against harmful effects of OTA on broiler performance, biochemical parameters and tissue residues.

MATERIALS AND METHODS

Collection of Industrial waste: Yeast sludge (YS) was collected from Shukkar Gunj Sugar Mills, Jhang and whey powder (WP) was purchased from local market. Protemyc (yeast cells and bentonite) Biorigin, Brazil was purchased from local distributors.

Production of OTA: OTA was produced from *Aspergillus ochraceus* (CECT 2948, Culture Collection Center, University De Valencia, Spain) by solid state fermentation on wheat grain Trenk *et al*. (1971). Briefly, 50g of wheat grains were soaked in 50 mL of distilled water for 2 hours in a 500 mL Erlenmeyer flask. The flask was autoclaved, inoculated with *A. ochraceus* spores and incubated for two weeks at 28°C in dark and shaken once daily. OTA was extracted from the fermented wheat by solvent extraction in acetonitrile-water and quantified by HPLC (Bayman *et al*., 2002).

Biological trial: A Biological trial of 35 days duration was conducted at Hi-Tech Research and Development Centre, Lahore. An approval for the study was granted by ethical review committee, University of Veterinary and Animal sciences, Lahore.

Birds and experimentation: A total of 1250 one day old broiler chicks of Arbreaker breed were divided randomly into five groups (A-E) replicated five times in such a way that each replicate contained 50 birds. Five diets formulated were A (basal poultry feed, 22% protein contents), B (OTA 200ppb), C (OTA 200ppb and 0.2% dried YS), D (OTA 200ppb and 0.2% WP) and E (200ppb OTA and 0.2% protemyc). Experiment was conducted under completely randomized design (CRD). The Broilers were assigned these rations *ad libitum* for 35 days. During the trial weekly weight gain (g) and feed consumption (g) was recorded. At the end of experimental trial feed conversion ratio (FCR) and mortality rate was calculated Khan *et al*. (2017).

Sample collection: At end of trail two birds were selected randomly per replicate. Blood (2 mL) was collected per bird by syringe from wing vein. Serum was separated and stored at -20°C. The thigh muscles, liver, kidney and heart samples of slaughtered birds were taken in sterilized plastic containers, labelled and stored at -20˚C.

Estimation of serum Biochemical parameters: Estimation of serum total protein, albumin, creatinine, Activity of ALT and AST was carried out by using commercially available kits (Human, Wiesbaden, Germany).

Hemagglutination inhibition (HI) test against Newcastle disease virus (NDV): The Humoral Immune response was evaluated by Hemagglutination Inhibition test against NDV (Anon, 1971). In brief the hemagglutination Inhibition pattern was detected of the highest dilution of the virus giving complete HA pattern. The values of the last dilutions which causes total inhibition of hemagglutination were calculated as the logarithm to the base 2.

Extraction of OTA from tissues: Two millilitres of serum sample was mixed with 2.5mL of phosphoric acid and twenty gram of tissues samples (muscles, kidneys, heart and livers) were homogenized with 7.5 mL of 1 M phosphoric acid in a homogenizer. Two millilitres of phosphoric acid treated samples were extracted thrice with ethyl acetate (5 mL), and centrifuged at 350 g for 5 min. The organic phase concentrated and extracted with 2 mL of 0.5 M NaHCO3. The aqueous extract was purified further by immune affinity column (OchraTest WB column, Vicam, USA) **(**Bozzo *et al.*, 2008).

Estimation of OTA in tissue samples: The purified extract was analyzed by high performance liquid chromatography (HPLC) Agilent 1260 Infinity II system. The mobile phase was acetonitrile: water: acetic acid (99:99:2) (Biro *et al.*, 2002).

Statistical analysis: The data was analyzed statistically by One Way Analysis of Variance and significant differences among means were compared using the Duncan Multiple Range Test using SPSS 16 software.

RESULTS

Body weights of OTA treated group was slightly but not significantly decreased (Table 1) from the control (A) at any of the 5 weeks, weight gain in group D fed on WP at week 2 and 4, was significantly higher than Group B. Group E treated with protemyc showed significantly ($P<0.05$) higher weight gain then other groups at week 4 and 5 ($P<0.05$). On day35, the ameliorating effect of protemyc was clear: though group D was not significantly different at 1, 3 and 5 weeks but the body weights of group D were significantly greater ($P<0.05$) at weeks 2 and 4 when compared with group B.

As evident from Table 2, no significant difference was observed in feed consumption in all groups. The quantity of feed consumed in groups D and E fed on WP and protemyc showed significantly higher feed consumption in week 2, the feed consumption of YS, WP and protemyc was slightly, but not significantly, improved.

No significant differences between groups were found when FCR was calculated (Table 3). Only in week 3 was the FCR of group E increased ($P<0.05$). At week 5, the FCR of YS, WP and protemyc treated group was slightly, but not significantly improved than group B.

Table 1: Effects of Feeding OTA, YS, WP and protemyc supplementation on weight gain of broiler chicks (Means±SD)

Treatment	Weeks					
	Bodyweight (g)					
	1st Day	1	2	3	4	5
A (basal feed)	42±1.5	171.6±5.7^{ab}	372.6±12.6^{ab}	754.8±34.3^{a}	1003±98.6^{a}	1382.4±74.2^{ab}
B (200ppb OTA)	42±1.5	166.2±17.4^{ab}	338.8±9.4^{a}	747.8±39.3^{a}	993±56.05^{a}	1361.8±37.0^{a}
C (200ppb OTA+YS)	42±1.5	172.9±5.1^{b}	395.6±34.3^{ab}	765.5±69.0^{a}	1111.4±86.9^{ab}	1428.6±4.1^{ab}
D (200ppb OTA+ WP)	42±1.5	161.8±6.7^{ab}	410.8±39.5^{b}	816.8±70.1^{a}	1166.2±71.9^{b}	1461±54 .6^{ab}
E (200ppb OTA+ protemyc)	42±1.5	159.3±9.3^{a}	394.6±30.9^{ab}	770.6±63.9^{a}	1191.8±104.6^{b}	1476.2±84.9^{b}

Different superscripts on means in a column show significant difference among groups (P<0.05). OTA (Ochratoxin A); YS (yeast sludge); WP (whey powder).

Table 2: Effects of Feeding OTA, YS, WP and protemyc supplementation on Feed Consumption of broiler chicks (Means±SD)

Treatment	Weeks				
	Feed Consumption (g)				
	1	2	3	4	5
A (basal feed)	161.6±11.8^{a}	445.8±15.1^{ab}	694.5±15.6^{a}	1669.2±42.0^{a}	2312±73.9^{a}
B (200ppb OTA)	156.1±14.3^{a}	396.9±38.8^{a}	690.4±6.1^{a}	1668.4±167.5^{a}	2303.7±178.8^{a}
C (200ppb OTA+YS)	167.6±10.46^{a}	450.7±35.3^{ab}	701.0±71.7^{a}	1747.8±157.1^{a}	2391.8±175.0^{a}
D (200ppb OTA+ WP)	162.8±12.2^{a}	462±37.1^{b}	698.9±43.3^{a}	1767.6±116.8^{a}	2416.3±183.2^{a}
E (200ppb OTA+ protemyc)	166.2±11.9^{a}	469.96±26.6^{b}	735.6±93.5^{a}	1769.8±146.9^{a}	2460.4±147.16^{a}

Different superscripts on means in a column show significant difference among groups (P<0.05). OTA (Ochratoxin A); YS (yeast sludge); WP (whey powder).

Table 3: Effects of Feeding OTA, YS, WP and protemyc supplementation on FCR of broiler chicks (Means±SD)

Treatment	Weeks				
	FCR				
	1	2	3	4	5
A (basal feed)	0.941±0^{a}	1.19±0^{ab}	0.92±0^{a}	1.67±0.1^{a}	1.67±0^{a}
B (200ppb OTA)	0.933±0^{a}	1.13±0.1^{a}	0.93±0^{a}	1.56±0.1^{a}	1.68±0^{a}
C (200ppb OTA+YS)	0.966±0^{a}	1.14±0^{a}	0.91±0^{a}	1.57±0^{a}	1.67±0^{a}
D (200ppb OTA+ WP)	1.007±0^{a}	1.19±0^{ab}	0.86±0^{a}	1.51±0.1^{a}	1.65±0^{a}
E (200ppb OTA+ protemyc)	1.024±0^{a}	1.33±0.1^{b}	0.95±0.1^{a}	1.48±0^{a}	1.67±0^{a}

Different superscripts on means in a column show significant difference among groups (P<0.05). OTA (Ochratoxin A); YS (yeast sludge); WP (whey powder).

Table 4: Effects of Feeding OTA, YS, WP and protemyc supplementation on serum Biochemical parameters of broiler chicks (Means±SD)

Treatments	Serum Total Protein (g/dL)	Serum Albumin (g/dL)	Activity of ALT (IU/dL)	Activity of AST (IU/dL)	Seum Creatinine Levels (g/dL)	HI Titer against NDV
A (basal feed)	3.02±0.1^{a}	1.74±0.1^{d}	7.1±0.7^{a}	159±1.5^{a}	0.92±0.1^{ab}	5.4±0.8^{ab}
B (200ppb OTA)	2.88±0.3^{a}	1±0.16^{a}	42±2.2^{e}	401.6±2.0^{e}	1.24±0.1^{c}	4.46±0.5^{a}
C (200ppb OTA+ YS)	3.64±0.3^{b}	1.5±0.21^{c}	32.6±1.8^{d}	373±10.5^{d}	0.78±0^{a}	5.6±1.1^{ab}
D (200ppb OTA+ WP)	3.72±0.1^{b}	1.16±0.11^{ab}	18±1.5^{c}	251.6±6.3^{c}	1.10±0.1^{bc}	6.2±0.4^{ab}
E (200ppb OTA+ protemyc)	4.78±0.7^{c}	1.34±0.15^{bc}	12.8±1.4^{b}	211.8±1.0^{b}	1.06±0.2^{bc}	6.4±2.1^{b}

Different superscripts on means in a column show significant difference among groups (P<0.05). OTA (Ochratoxin A); YS (yeast sludge); WP (whey powder).

Table 5: Effects of Feeding OTA, YS, WP and protemyc supplementation on Tissue residues of Ochratoxin A in broiler chicks (Means±SD)

Treatments	Tissue Levels of ochratoxin A µg/Kg				
	Heart	Thigh muscle	Liver	kidney	Serum
A (basal feed)	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
B (200ppb OTA)	0.073±0^{b}	0.016±0^{c}	0.93±0.3^{b}	1.14±0.2^{b}	3.4±0.2^{d}
C (200ppb OTA+YS)	0.068±0^{b}	0.004±0^{ab}	0.03±0^{a}	0.08±0^{a}	1.4±0^{c}
D (200ppb OTA+WP)	0.024±0^{a}	0.003±0^{ab}	0.0022±0^{a}	0.06±0^{a}	0.34±0^{b}
E (200ppb OTA+ protemyc)	0.024±0^{a}	0.006±0^{b}	0.07±0^{a}	0.08±0^{a}	0.53±0.1^{b}

Different superscripts on means in a column show significant difference among groups (P<0.05). OTA (Ochratoxin A); YS (yeast sludge); WP (whey powder).

Highest mortality (9.2%) was recorded in birds received diet contaminated with 200ppb OTA followed by Group E (7.6%) fed with protemyc. The Mortality rate in Group C supplemented with YS is 5.6 %, Lowest mortality (4%) was recorded in group D treated with WP in the presence of OTA. Supplementing broiler diets with YS, WP and protemyc in presence of OTA alleviate the toxic effect of OTA on broiler mortality.

The results in Table 4 indicate that serum total protein was slightly reduced in OTA treated group but serum albumin levels were significantly reduced (P<0.05) in OTA treated group as compared to control. Significant increase in serum total protein and albumin was observed in YS, WP and protemyc treated groups. Activity of ALT and AST was significantly increased (P<0.05) in OTA treated group as compared to control group. YS, WP and protemyc significantly improved the level of these enzymes. Serum creatinine level was also significantly increased (P<0.05) in OTA treated group as compared to control group and significant improvement was observed in YS, WP and protemyc supplemented groups. HI titre was slightly but not significantly (P<0.05) reduced in OTA treated group as compared to control group and it was significantly increased by protemyc treatment.

Toxin residues were detected in all the tissue samples of group B (200ppb OTA fed group), and the concentration of OTA in serum, liver and kidney was significantly higher in group B (P<0.05) as compared to

other groups as shown in Table 5. Heart and muscle contain very low level of OTA, WP and protemyc significantly reduced the tissue residues in these organs. Tissue level of ochratoxin A was found to be Serum>Kidney>Liver>Heart>Muscle. Whereas in groups fed with diet supplemented with YS, WP and protemyc a considerable decrease in serum and tissue levels was detected.

DISCUSSION

Results obtained in the present study shows that body weight, feed consumed and FCR was slightly but not significantly impaired by OTA at a level of 200ppb when compared to control group. At the first week of experiment, mortality was observed in all groups due to stress of travelling, weak immune system and adjustment to the new environment, thereafter mortality was increased in OTA group as compared with the other groups. YS, WP and protemyc improved the performance parameters in OTA treated broiler chicks. The slight alteration in body weight, FC and FCR due to OTA in the present study was in accordance with several previous investigations using dietary OTA inclusion rates of 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg/Kg feed (Zahoor *et al.*, 2010). Dönmez *et al.*, 2012 indicated that addition of yeast sludge to broiler diets providing the partial protection against the harmful effects of OTA. Similar results of L-carnitine are also reported by Bhatti *et al*. (2018). The addition of whey as a prebiotic to diets may influence broiler weight gain. The improvement in growth rate of birds fed with diets containing the tested prebiotic shows that the use of these products is a feasible alternative to antibiotics and antimycotoxins used as growth promoters (Nagarachi *et al*., 2007).

Antiochratoxin effect of whey can be attributed to its capacity to increase the *lactobacillus* count in the gastrointestinal tract of the poultry and *Lactobacillus* strains were conducive to detoxification of OTA when added to a feed mixture for chickens (Śliżewska *et al.*, 2014).

Present results are in agreement with those reported by Mansour *et al*. (2011) and (2015) in *Oreochromus niloticus* Addition of whey to fish diets with OTA improved the productive performance, histopathological alterations and Biochemical parameters.

OTA administrations to broilers also alter different serum biochemical parameters adversely. Activity of ALT, AST and creatinine levels were significantly increased in OTA fed group as compared to all other groups. Feeding of OTA increased the serum levels of ALT (Khan *et al*., 2017), urea and creatinine. Zahoor *et al*., (2010) also reported that Serum levels of total proteins and albumin were significantly decreased in the groups fed OTA.

The findings of present study revealed that supplementation of feed with YS and WP reduce the OTA levels in serum, kidneys and liver significantly. We previously reported that level of OTA is highest in kidney as compared to liver in chicks fed with 500 ppb OTA and 2% YS effectively reduced the level of ochratoxin A in tissues (Mujahid *et al.*, 2012). Supplementation of WP also reduces the tissue residues of OTA indicating the gastrointestinal removal of OTA probably due to increase in *Lactobacillus* count in gastrointestinal tract of poultry. Lactic acid bacteria are actively involved in the adsorption of OTA (Śliżewska *et al.*, 2014).

Conclusions: The present study described performance parameters and biochemical alterations in OTA fed broilers. Improvement in FCR, serum biochemical changes, tissue residues and decreased mortality in YS and WP supplemented groups suggested a decrease in severity of the ochratoxicosis with supplementation of these industrial by products in feed.

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Authors contribution: HM: Designed and performed the experiments, analysed the data and wrote the manuscript. ASH: Proposed the idea of the research work, designed the study and also help in planning and arrangement of trials. MZK: Provided the fungal strain and methodology for toxin production and also helps to prepare the final manuscript. MT: Worked out almost all of the technical details, and analyzed the data obtained from the experiments. WS: Provided the chemicals and raw materials required in the study, contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All the authors read and approved the manuscript.

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