EFFECTS OF OCHRATOXIN A AND PREVENTIVE ACTION OF A MYCOTOXIN-DEACTIVATION PRODUCT IN BROILER CHICKENS

Yang Don Joo[1], Chang Won Kang[1], Byoung Ki An[1], Jong Sung Ahn[2], Radka Borutova[3]
[1]*Animal Resources Research Center, College of Animal Bioscience and Technology, Konkuk University 143-701 Seoul, Korea*
[2]*National Agricultural Products Quality Management Service, Seoul, Korea*
[3]*Biomin Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria*

Corresponding author: Radka Borutova
E-mail: radka.borutova@biomin.net; Phone: +436 64 814 3805

Abstract. Effects of dietary contamination with various levels of ochratoxin A (OTA) and potential preventive action of mycotoxin-deactivation product were investigated on two hundred one-day-old male broiler chicken. The birds were divided into 20 groups (5 treatment x 4 replication x 10 bird each) and fed 5 different diets for 5 weeks. Group 1: control (OTA free); group 2: OTA (1 mg/kg) without mycotoxin deactivator; group 3: OTA (1 mg/kg) with addition of mycotoxin deactivator at 0.2 % of the diet; group 4: OTA (2 mg/kg) without mycotoxin deactivator; group 5: OTA (2 mg/kg) with mycotoxin deactivator at 0.2 % of the diet. As dietary OTA increased, feed intake and weight gain were gradually and significantly decreased. These negative effects were partially counteracted by feeding the mycotoxin deactivator. The relative weights of liver and kidney, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the groups fed diets containing OTA alone were significantly higher compared to the control group. The level of serum total-cholesterol was significantly reduced by feeding OTA contaminated diets. As dietary OTA increased, the levels of OTA in liver and kidney tissue were significantly higher. Presence of mycotoxin deactivator in contaminated diets significantly decreased the OTA accumulation in organs. Moreover the fecal excretion of OTA and its metabolite OTα were significantly increased by feeding the mycotoxin deactivator. These results demonstrated that feeding the mycotoxin deactivator reduced the organ accumulation of OTA and OTA - induced performance reduction. In conclusion the contents of OTA in liver and kidney tissue were found to be a suitable indicator of OTA presence in broiler feed.
Keywords: broiler, ochratoxin A, mycotoxin-deactivation product.

OCHRATOKSINO A POVEIKIS VIŠČIUKŲ BROILERIŲ ORGANIZMUI IR MIKOTOKSINUS NEUTRALIZUOJANČIO PRIEDO PREVENCINĖS SAVYBĖS

Yang Don Joo[1], Chang Won Kang[1], Byoung Ki An[1], Jong Sung Ahn[2], Radka Borutova[3]
[1]*Gyvūnų išteklių tyrimų centras, Gyvūnų biomokslų ir technologijų koledžas, Konkuko universitetas 143-701 Seulas, Korėja*
[2]*Nacionalinė žemės ūkio produktų kokybės kontrolės tarnyba, Seulas, Korėja*
[3]*Biomin GmbH holdingas, Industriestrasse 21, Hercogenburgas, Austrija*
el. paštas: radka.borutova@biomin.net; tel. +436 64 814 3805

Santrauka. Norint nustatyti su lesalais gaunamo ochratoksino A (OTA) kiekį ir galimą jo prevenciją naudojant mikotoksinus neutralizuojantį priedą, ištirta 200 vienos dienos viščiukų broilerių. Paukščiai suskirstyti į 20 grupių (keturias bandomąsias ir vieną kontrolinę, po 10 viščiukų kiekvienoje). Penkias savaites viščiukai buvo lesinami taikant skirtingas dietas. Kontrolinės grupės (I) viščiukai buvo lesinami lesalais be OTA; II grupės viščiukai gavo OTA (1 mg/kg) be mikotoksinus neutralizuojančio priedo; III grupės – OTA (1 mg/kg) kartu su mikotoksinus neutralizuojančiu priedu (jis lesale sudarė 0,2 proc.); IV grupės viščiukai gavo OTA (2 mg/kg) be mikotoksinus neutralizuojančio priedo ir V grupės – OTA (2 mg/kg) su mikotoksinus neutralizuojančiu priedu (0,2 proc.). Didinant OTA kiekį, sunaudoto lesalo kiekis ir viščiukų svorio prieaugis palaipsniui ženkliai mažėjo. Mikotoksinus neutralizuojantis priedas iš dalies panaikino neigiamą OTA poveikį. Tiriamųjų grupių viščiukų kepenų ir inkstų svoris, taip pat ALT ir AST aktyvumas buvo daug didesni negu kontrolinės grupės. Lesinant lesalais su OTA, bendras cholesterolio kiekis buvo gerokai mažesnis. Didinant OTA kiekį lesale, jo kiekis kepenų ir inkstų audiniuose taip pat ženkliai padidėjo. Lesinant lesalais, kuriuose, be OTA, buvo ir mikotoksinus neutralizuojančio priedo, OTA vidaus organuose kaupėsi mažiau. Be to, mikotoksinus neutralizuojantis priedas pastebimai pagerino OTA ir metabolito OTA šalinimą su ekskrementais. Tyrimų rezultatai parodė, kad mikotoksinus neutralizuojantis priedas sumažino OTA kaupimąsi organuose ir susilpnino jo neigiamą poveikį svorio prieaugiui. Taigi OTA kepenų ir inkstų audiniuose gali būti laikomas ochratoksinu A užterštų lesalų indikatoriumi.
Raktažodžiai: broileris, ochratoksinas A, mikotoksinus neutralizuojantis priedas.

Introduction. Ochratoxin A (OTA), a dihydroisocoumarin derivative linked over a 7-carboxy group to L-phenylalanine by an amide bond, is a secondary metabolite produced by various species of the fungal genera *Aspergillus* and *Penicillium*. OTA has been found worldwide as a natural contaminant of cereal grains such as barley, wheat, oats, rye, and maize. It was proved to exhibit nephrotoxic, immunosuppressive, teratogenic, and carcinogenic properties (Lea *et al.*, 1989). OTA is the most toxic mycotoxin for domestic fowl (Leeson *et al*., 1995). At the molecular level, OTA interferes with DNA, RNA, and protein synthesis by inhibiting the enzyme phenylalanine - tRNA synthetase. This mycotoxin is able to induce damage of the epithelium of the renal proximal tubules, decrease electrolyte absorption and increase water consumption. In broilers, major clinical signs of ochratoxicosis are poor growth, reduced feed efficiency and increased water consumption which is a consequence of damaged kidneys. At post mortem examination of broilers fed dietary levels 0.3–16 mg/kg of OTA, swollen, enlarged and pale kidneys were found (Leeson *et al.*, 1995). Younger birds appear to be more susceptible to OTA than older birds as 3 week old broilers have higher LD_{50} values than one-day old chicks (Huff *et al*., 1974).

OTA is easily absorbed through biological membranes. It is absorbed primarily from the stomach and proximal jejunum and enters the blood circulation through the portal vein. The rate of absorption is faster in those parts of the gastrointestinal tract (GIT) that have a low rather than a high pH (Leeson *et al*., 1995). OTA is also reabsorbed in the proximal and distal tubules of kidneys and Di Paolo *et al*., (1993) discovered that it can be absorbed through the lungs into the systemic circulation as well. The overall rate of absorption of OTA in chickens is approximately 40 % (Galtier *et al.,* 1981). The serum half-life of OTA for poultry is about 4.1 hours (Galtier *et al.,* 1981) i.e. much shorter than in pigs and other mammals. As in other animal species, the highest residual amounts of ochratoxin A are found in the kidney and liver tissue.

Preventive measures to avoid the formation of OTA were not successful in the past and therefore new strategies to counteract mycotoxins in feed are needed. A very promising method is the use of microorganisms that biotransform mycotoxins to non- or less toxic metabolites. More than 20 new strains were classified based on OTA- degradation velocity, influence of different culture media on OTA cleavage, function in environments with low pH- values and redox potential and antibiotic resistance pattern. Based on the obtained results *Trichosporon mycotoxinivorans* (Biomin MTV) was chosen to be used in a feed additive and tested in feeding trials.

Materials and methods

Experimental design

A total of two hundred 1-day-old Ross 308 male broiler chicks were used in this experiment. The birds were randomly divided at the day of hatching into 20 groups (5 treatments; 4 replicates per treatment group; n=10 in each group). For the subsequent 5 weeks birds were fed diets differing only in OTA contamination and/or mycotoxin deactivation product (MDP) supplementation. Group 1: control (OTA free); group 2: OTA (1 mg/kg) without mycotoxin-deactivation product; group 3: OTA (1 mg/kg) with mycotoxin-deactivation product at 0.2% of the diet; group 4: OTA (2 mg/kg) without mycotoxin-deactivation product; group 5: OTA (2 mg/kg) with mycotoxin-deactivation product at 0.2% of the diet. Contamination of diets was done artificially by Heung Sung Feed Co., Ltd., Korea by mixing uncontaminated diet with dried wheat-mycelia mixture with known OTA concentration until the desired OTA contamination was reached. Ochratoxin A was produced by inoculation of cracked wheat by *Petromyces albertensis* (Schatzmayr *et al.*, 2001). The cracked wheat was incubated for at least 4 weeks at 25 °C and 50% humidity and was dried at 60 °C afterwards. The dried wheat-mycelia mixture was ground and the OTA concentration was determined (Entwisle *et al.*, 2000).

Experimental diets and water were provided *ad libitum*. Rearing of the chickens was done with a lighting regimen of 24 h light and lasted for five weeks. The initial room temperature of 32–33°C was reduced weekly by 1°C to a final temperature of 28°C. The experiment was carried out in accordance with International Guiding Principles for Biomedical Research Involving Animals as Issues by the Council for the International Organizations of Medical Sciences and EU Directive 86/609/EEC and EC recommendations 2007/526 EC.

At the end of the experimental period, all the chicks were weighed and used for further analyses. The mycotoxin deactivation product used was Mycofix® Select MTV inside (Biomin Holding GmbH).

Sample collection and analysis

At the end of experimental period, 10 chicks from each group were randomly selected. Blood was drawn from wing vein using sterilized syringes for determination of the various blood profiles. The blood serum was separated from each blood sample by centrifugation and stored at -30º C until use. Growth parameters as feed intake and weight gain were measured three times during the experiment, at days 1, 22 and 35. The liver, spleen, bursa of Fabricius and kidney were removed and weighed immediately after euthanasia and necropsy of the birds. The concentration of total cholesterol was estimated according to the colorimetric method using cholesterol diagnostic kit (Total cholesterol kit, Asan Pharmaceutical Co. Ltd.). The concentration of glucose, total protein, uric acid and creatine were measured using blood chemistry analyzer (COBAS MIRA Select, Roche Diagnostics, Germany). The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were measured according to the colorimetric method using GOT-GPT assay kit (BCS GOT-GPT assay kit, Bio Clinical System Corporation), following the manufacturer's direction. Samples of liver, kidneys, blood and fresh feces (collected 24h before trial ended) were collected after euthanasia for quantification of OTA and OTα. These samples were frozen at -20º C until analysis. The OTA standard was purchased from Biopure GmbH

(Tulln, Austria). The OTα standard was prepared according to the method of Stander et al., 2001. Bovine pancreatic carboxypeptidase A and phosphate-buffered saline, pH 7.4, (PBS) were purchased from Sigma-Aldrich (Seoul, Korea). Strata SCX (Phenomenex, Torrance, CA) were used for solid phase extraction (SPE). All solvents and reagents were analytical grade.

SPE Cleanup of chicken tissue and feces

After the evaluation of various SPE media, including C18, NH, and SAX (Monaci et al., 2004; De Saeger et al., 2004), strong cation exchange (SCX) was chosen to clean up the complex sample matrix based on its efficient removal of interfering materials from complex feces and ease of use. SCX does not require a wash and elution step after sample loading, which could result in the loss of OTA or its derivatives. Twenty-five grams of sample was washed two times with 100 mL hexane to remove free fatty acids and lipids and then partitioned between 60 mL ethyl acetate and 40 mL water for 30 min with shaking. By adjusting the extraction solution to pH 2 with 6 N hydrochloric acid the weakly acidic OTA was efficiently partitioned to the organic phase. Five milliliters of the upper organic layer were reduced to dryness in a test tube under a mild stream of nitrogen gas and the residue on the surface of tube was carefully dissolved in 2 mL MeCN and applied to a preconditioned SCX (500 mg/3 mL) cartridge. The initial fraction, which was collected directly from the cartridge immediately after loading the sample on the SPE column, contained acidic OTA and OTA derivatives, OTα. Residual OTA in the cartridge was collected by washing again with 1 mL MeCN and the final 3 mL of sample solution was transferred to a vial for analysis and quantification.

Quantification of OTA by High Performance Liquid Chromatography

Fifty microliters of sample solution were injected onto an Agilent HPLC 1100 series (Waldbronn, Germany) consisting of degasser, binary pump, autosampler, column thermostat, and fluorescence detector (FLD) and separated over a Eclipse XDB-C18 column (5 µm, 4.6 × 250 mm) at 35 °C. Two different elution processes were used in this study. Initially, an injected sample was eluted isocratically with a mixture of acetonitrile, deionized water, and acetic acid (51:47:2, v/v/v). But in order to facilitate separation of OTA and its derivatives, 2% acetic acid in water (mobile phase A) and 2% acetic acid in acetonitrile (mobile phase B) were programmed to elute in a gradient in the pattern: 2 min, 90% A; 5 min, 70% A; 7 min, 20%A; 4 min, 20%A; 2 min, 90%A; 4 min, 90%A for a run time of 24 min at a flow rate of 1mL/min. The fluorescence detector was set at an excitation wavelength of 333 nm and an emission wavelength of 460 nm. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as signal/noise of 3:1 and 10:1 respectively (Boudra and Morgavi 2006).

LOD and LOQ of the fluorescence determination after the SPE concentration were 0.2 and 0.7 ng/g chicken tissue (feces) respectively.

Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) with the Duncan's multiple range test using General Linear Model (GLM) program of SAS (SAS, 2002). Results are given as means ± SEM.

Results

The feeding of diets contaminated with OTA significantly decreased final body weight (1904.4 ± 38.38 g/bird in the control vs. 1710.55 ± 15.47 g/bird in the OTA (1 mg/kg) -treated group, $P < 0.05$), body weight gain and feed intake of birds compared with birds from the control group. The MDP was able to significantly increase the final body weight of broilers in comparison with groups of birds fed contaminated diets without this feed additive (Table 2). OTA significantly increased the weight of liver and kidney after 35 days. Addition of the MDP into the contaminated diet significantly reduced liver and kidney weight in comparison to animals fed the contaminated non-supplemented diet (Table 3). Birds fed on diets contaminated with both OTA concentrations had significantly increased activities of liver enzymes ALT (193.68 ± 6.01 U/L in the control vs. 247.2 ± 11.76 U/L in the OTA (1 mg/kg) -treated group, $P < 0.05$) and AST (1.73 ± 0.12 U/L in the control vs. 2.42 ± 0.09 U/L in the OTA (1 mg/kg) -treated group, $p < 0.05$) in blood serum while the addition of the MDP into such contaminated diets significantly reduced activities of these enzymes. Moreover, OTA significantly reduced the concentration of total-Cholesterol (total-C) in blood of broilers (Table 5). Diets contaminated with both concentrations of OTA significantly increased the glucose levels in blood of birds. MDP addition into the contaminated diet did not have any effect on glucose blood concentration (Table 4). As can be observed in table 6, presence of both OTA concentrations in feed of broilers significantly increased OTA content in blood, liver and kidney tissue. OTA was not detected in blood and organs of birds which were fed OTA free diet. Except in blood, MDP significantly reduced OTA concentrations in liver and kidney tissue if compared with birds fed on contaminated diet but without feed additive. From table 7 it is clear that the MDP significantly increased the excretion of OTA and OTα in feces when compared to the group of broilers which got contaminated diet without the MDP (197.53 ± 29.43 ng/g in the control vs. 316,92 ± 32.42 ng/g in the OTA (1 mg/kg) -treated group, $P < 0.05$).

Discussion

Ochratoxin A is a mycotoxin that causes many adverse effects in domestic livestock and humans. This mycotoxin can cause subacute and chronic production losses in poultry. Ochratoxin A seems to produce its toxic effect by one of three mechanisms: inhibition of phenylalanine metabolizing enzymes, promotion of lipid peroxidation, or inhibition of mitochondrial ATP production. Ochratoxin A is of concern because there is a high potential for its production in stored foods and feeds under appropriate temperature and moisture conditions. OTA has been known to occur naturally in grains and food, it has been detected in the blood of poultry and it is a potent carcinogen (group 2B) and nephrotoxin (IARC, 1993).

Table 1. **Composition of the experimental diet** (%)

Ingredients	Starter	Finisher
Yellow corn, ground	57.00	58.60
Soybean meal	26.80	26.60
Corn gluten meal	3.00	2.00
Full fat Soybean	3.00	2.00
Fish meal	2.00	2.00
Tallow	4.30	5.10
Vit.+Min. mixture[1]	0.15	0.15
Limestone	1.14	1.19
Dicalcium phosphate	1.75	1.60
Choline-Cl (50%)	0.05	0.05
Lysine-Hcl (78%)	0.30	0.21
DL-methionine (98%)	0.11	0.10
Threonine (99%)	0.08	0.08
Tryptophan (10%)	0.05	0.05
Salt	0.22	0.22
Lasalocid 150g	0.05	0.05
Total	100	100
Calculated values		
Crude protein	21.00	20.00
Methionine	0.50	0.46
Lysine	1.26	1.16
Ca	1.00	1.00
P	0.70	0.67
TMEn, kcal/kg	3,150	3,200

[1] Vit.+Min.mixture provided the following nutrients per kg of diet: vitamin A, 12,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 20,000 IU; vitamin K_3, 1,800 mg; vitamin B_1, 2,000 mg; vitamin B_2, 6,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 20,000 µg; niacin, 25,000 mg; pantothenic acid, 10,000 mg; folic acid, 1,000mg; biotin, 50 mg; Fe, 50,000 mg; Zn, 65,000 mg; Mn, 65,000 mg; Co, 250 mg; Cu, 5,000 mg; I, 1,000 mg; Se, 150 mg

The results of this study demonstrate that OTA significantly decreased the feed intake which after 35 days led to a significant reduction of final body weight of broilers. These results are consistent with findings of Politis *et al.*, (2005) who found that 42 days feeding of broilers on diets contaminated with OTA led to tentatively lower live weight of birds compared to the control group. Conversely, these results are in disagreement with Huff *et al.*, (1974, 1975) who fed graded levels of OTA up to 8.0 mg/kg to 1-day-old broilers for 3 weeks and found that the minimum dietary level of OTA capable of reducing weight gain was 2 mg/kg. According to our results 1 mg/kg of OTA was already able to significantly decrease feed intake, final body weight and body weight gain of broilers. This discrepancy in our case could be possibly caused by the presence of some other mycotoxins in feed or masked mycotoxins which could eventually have led to synergistic or additive effects with OTA. It is no doubt that synergistic effects between mycotoxins exist and enhance their toxicity. Data of Huff and Doerr (1980) when searching for a synergism between aflatoxin and ochratoxin A in broiler chickens demonstrate that symptoms patterns can be altered, confusing preliminary diagnosis during multiple mycotoxicoses.

The adverse effect of OTA contaminated diet on the chicken liver is demonstrated also by findings of a significantly increased weight of liver and in addition significantly increased ALT and AST activities in blood serum of birds. Dietary OTA (4 mg/kg) significantly increased the relative weights of the liver and caused significant decrease of blood cholesterol concentration in 21 days old broiler chicken (Huff *et al.,* 1988). This decrease in total-cholesterol in blood of broilers could be also one of the OTA hepatotoxicity indicators. According to Stoev *et al.,* (2000) OTA was able to induce slight degenerative changes in hepatocytes which consequently led to decreased cholesterol blood concentrations.

Table 2. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on growth performance in broilers.** Means ± SEM. n=10 in each group

Parameter	Control	OTA 1 (mg/kg)	OTA 1 (mg/kg) + MDP 0.2 %	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2 %
Initial BW, g/bird	47.6±0.04	47.6±0.04	47.6±0.05	47.6±0.06	47.6±0.04
Final BW, g/bird	1904.40±38.38^{a}	1710.55±15.47^{c}	1797.23±13.97^{b}	1634.10±26.59^{d}	1748.73±18.36^{bc}
Feed intake,g/d/bird (1-21d)	48.30±0.22^{a}	45.18±1.40^{b}	47.08±0.32^{ab}	41.58±1.14^{c}	44.98±0.23^{b}
Feed intake, g/d/bird (22-35d)	121.50±1.54^{a}	116.25±1.87^{ab}	119.90±0.86^{ab}	111.58±2.30^{c}	115.40±1.64^{bc}
Feed intake, g/d/bird (1-35d)	77.56±0.72^{a}	73.60±0.73^{b}	76.17±0.33^{a}	69.60±0.75^{c}	73.15±0.61^{b}
BW gain, g/d/bird (1-21d)	34.35±0.66^{a}	31.05±0.85^{bc}	33.73±0.23^{a}	29.0±1.06^{c}	32.58±0.78^{ab}
BW gain, g/d/bird (22-35d)	81.10±2.39^{a}	72.23±1.15^{bc}	74.35±0.78^{b}	69.88±0.72^{c}	72.65±0.99^{bc}
BW gain, g/d/bird (1-35d)	53.05±1.11^{a}	47.53±0.46^{c}	49.98±0.40^{b}	45.33±0.76^{d}	48.60±0.52^{bc}

[a-d] Different superscripts within a row indicate significant differences (P<0.05)

Table 3. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on carcass characteristics in broilers.** Means ± SEM. n=10 in each group

Tissue	Control	OTA 1 (mg/kg)	OTA 1 (mg/kg) + MDP 0.2 %	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2 %
Liver, g/100g BW	1.78±0.07^{c}	2.46±0.08^{a}	2.06±0.03^{b}	2.53±0.04^{a}	2.22±0.08^{b}
Spleen, mg/100g BW	114.0±5.21	104.33±17.13	108.17±12.44	106.50±8.35	113.33±13.30
Bursa of Fabricius, g/100g BW	0.25±0.02	0.25±0.01	0.28±0.03	0.26±0.01	0.25±0.03
Kidney, g/100g BW	0.62±0.03^{c}	0.82±0.04^{a}	0.71±0.03^{bc}	0.84±0.02^{a}	0.79±0.03^{ab}

$^{a-c}$ Different superscripts within a row indicate significant differences (P<0.05)

Table 4. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on blood levels of glucose, protein, uric acid and creatine in broilers.** Means ± SEM. n=10 in each group

Parameter	Control	OTA 1 (mg/kg)	OTA 1 (mg/kg) + MDP 0.2 %	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2 %
Glucose, mg/dL	219.0±8.74^{b}	241.73±6.38^{a}	236.95±3.96^{a}	246.38±2.48^{a}	235.70±1.27^{a}
Total protein, g/dL	2.57±0.16	2.80±0.18	2.65±0.21	2.82±0.17	2.68±0.20
Uric acid, mg/dL	3.17±0.32	4.0±0.17	3.23±0.40	4.0±0.35	3.4±0.08
Creatine, mg/dL	0.38±0.06	0.47±0.02	0.40±0.0	0.45±0.02	0.40±0.04

$^{a-b}$ Different superscripts within a row indicate significant differences (P<0.05)

Table 5. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on blood levels of total cholesterol** (Total-C)**, activities of aspartate aminotransferase** (AST) **and alanine aminotransferase** (ALT) **in broilers.** Means ± SEM, n=10 in each group

Parameter	Control	OTA 1 (mg/kg)	OTA 1 (mg/kg) + MDP 0.2%	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2%
total-C, mg/dL	106.68±2.84^{a}	93.77±2.80^{bc}	100.70±3.09^{ab}	91.22±0.79^{c}	94.30±1.57^{bc}
AST, U/L	193.68±6.01^{b}	247.20±11.76^{a}	202.0±2.79^{b}	283.42±12.74^{a}	210.95±15.21^{b}
ALT, U/L	1.73±0.12^{c}	2.42±0.09^{a}	2.13±0.07^{b}	2.60±0.07^{a}	2.05±0.02^{b}

$^{a-c}$ Different superscripts within a row indicate significant differences (P<0.05)

Table 6. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on ochratoxin A concentrations in blood, liver and kidney in broilers.** Means ± SEM, n=10 in each group

Parameter	Control	OTA 1 (mg/kg)	OTA 1(mg/kg) + MDP 0.2%	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2%
Blood, ng/ml	ND	2.58±0.50^{bc}	1.92±0.09^{c}	6.25±0.77^{a}	4.80±0.48^{ab}
Liver, ng/g	ND	20.28±2.74^{c}	10.64±1.26^{d}	31.02±0.82^{a}	26.21±1.49^{b}
Kidney, ng/g	ND	30.18±4.27^{b}	11.96±2.27^{c}	40.48±1.67^{a}	23.09±2.04^{b}

$^{a-d}$ Different superscripts within a row indicate significant differences (P<0.05)

Table 7. **Dietary effects of ochratoxin A contaminated feed with or without mycotoxin deactivating product on the concentrations of ochratoxin A and ochratoxin α in feces of broilers.** Means ± SEM, n=10 in each group

Parameter	Control	OTA 1 (mg/kg)	OTA 1 (mg/kg) + MDP 0.2 %	OTA 2 (mg/kg)	OTA 2 (mg/kg) + MDP 0.2 %
Ochratoxin A, ng/g	ND	310.03±8.20^{c}	416.16±22.73^{b}	482.79±52.90^{b}	629.56±22.99^{a}
Ochratoxin α, ng/g	ND	197.53±29.43^{c}	353.28±46.0^{ab}	316.92±32.42^{b}	439.52±9.95^{a}

Different superscripts within a row indicate significant differences (P<0.05).

Significantly increased blood glucose concentrations in broilers fed on diets contaminated with OTA could eventually result from damage of liver tissue. The liver function is a very important blood-glucose buffer system. In patients with severe liver disease, it becomes almost impossible to maintain stable blood glucose concentrations for proper functioning. Moreover OTA affects carbohydrate metabolism, particularly gluconeogenesis. It reduces the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK), which is a key enzyme in gluconeogenesis. PEPCK is the link between the citric acid cycle intermediates and their precursors into glucose and glycogen (Leeson *et al.,* 1995). Interference with this rate-limiting step in gluconeogenesis plays a key role in the development of functional damage to the kidney cortex (Ueno, 1991). Our findings are supported by Verma and Shalini (1998) who have discovered that OTA was able to induce hyperglycemia in rabbits.

Increased activities of ALT in blood plasma could be a sign of hepatitis, liver cancer, inflammation of the gall bladder and bile duct course and toxic liver damage (intoxications). Elevated AST activities are not specific for liver damage, and AST has also been used as a cardiac marker. Our results are in agreement with Wang *et al.,* (2009) who found that combination of OTA and T-2 toxin in diets of 21-d-old broiler chicken was able to significantly elevate activities of ALT and AST in blood serum. Mechanisms of increased activity of ALT in serum include enzyme release from damaged cells or induction of enzyme activity (increased enzyme synthesis) from toxin administration. Release of ALT from the cytosol can occur secondarily to cellular necrosis or as a result of cellular injury with membrane damage and bleb formation (Stockham and Scott, 2002). The degree of increase in ALT activity correlates with the number of hepatocytes damaged, which may be useful in helping to evaluate the extent of hepatic damage. ALT activity may also increase following release from hepatocytes during liver repair (Bain, 2003).

This 35 day feeding trial was not long enough for OTA to induce nephrotoxic effect in broilers. Feeding of broiler chickens with diet containing ochratoxin A (2.5 mg/kg) resulted in a significant reduction in weight gain and in an increase in relative kidney weight associated with increases in serum uric acid and triglycerides, and in decreases in total protein and albumin (Gentles *et al.,* 1999). Our suggestion that the kidney function was not impaired is supported by serum concentrations of uric acid and creatin which were not significantly changed. Liver is the primary organ involved in mycotoxin detoxification, which is probably the reason why it was affected much more than the kidneys.

Residue analyses after feeding chickens various diets were performed by Prior *et al.* (1980), Niemiec *et al.* (1988) and Micco *et al.* (1987). Most residue analyses have been performed after feeding animals with more than 1000 µg/kg ochratoxin A in the feeding stuff. In these experiments, the residues in the liver varied between 2 and 11 ng/g, and in muscles 2 ng/g OTA were found. Our results support these findings as significantly increased concentrations of OTA were found in blood, liver and kidney tissue. Measurements of ochratoxin A in human blood have been widely used as an indicator of exposure to this mycotoxin (Studer-Rohr, 2000; Palli *et al.,* 1999; Peraica and Domijan, 2001). The contents of OTA in liver and kidney were found to be a good indicator and probably suitable bio-marker which could show the exposure of broilers to this particular mycotoxin.

The fecal OTA and ochratoxin α (OTα) in chickens fed diet contaminated with OTA were linearly increased in proportion to the amount of dietary OTA. Poultry species appear to eliminate OTA faster than mammals. In chickens receiving a single oral dose of radiolabeled OTA, over 90% of the radioactivity was eliminated 48 h after intubation (Frye and Chu, 1977). Fecal OTA and OTα were significantly increased in both groups of birds fed the MDP product. It is believed that the reason for the increased fecal excretion of OTA was the effect of bentonite that MDP contains and the reason for the increase of fecal excretion of OTα was the effect of *Trichosporon mycotoxinivorans* (Biomin MTV) that is the main ingredient of MDP, able to biotransform OTA into OTα. While the use of adsorbents such as aluminosilicates have been shown to be efficacious against aflatoxins (Huff *et al.,* 1992) the adsorption of others mycotoxins like OTA and diacetoxyscirpenol could not be achieved to a similar extent (Kubena *et al.,* 1993).

The yeast *Trichosporon mycotoxinivorans* was selected as the strain to be used as OTA-detoxifying feed additive (Schatzmayr *et al.,* 2003). In addition, preliminary feeding trials with pigs and poultry have shown that *Trichosporon mycotoxinivorans* degrades OTA in these animal species (Politis *et al.,* 2005). The negative effect of OTA on performance parameters could be compensated by the addition of *Trichosporon mycotoxinivorans*. Results of the analysis showed a reduction of the OTA-concentration in plasma in the *Trichosporon* - group compared to the positive control group of birds (Politis *et al.,* 2005). Our results completely support the findings of previous authors where the MDP was able to quickly compensate the harmful effects of OTA in broilers. The MDP was not effective only by significantly improving performance parameters as final body weight, feed intake and body weight gain, but also by significantly reducing OTA bioaccumulation in the liver and kidney tissue. The yeast can detoxify OTA by cleavage of the phenylalanine moiety from the isocumarin derivate ochratoxin alpha (OTα). This metabolite has been described to be nontoxic or at least 500 times less toxic than the parent compound. *Trichosporon mycotoxinivorans* was selected as the strain with the highest potential to be used as OTA-deactivating feed additive (Schatzmayr *et al.,* 2006).

Conclusions

The results of this experiment demonstrate the adverse effects of the mycotoxin ochratoxin A on performance and blood biochemistry parameters in broiler chicken and the very good preventive action of a mycotoxin

deactivation product. It is shown that the content of OTA in liver and kidney is a good indicator and probably a suitable bio-marker which could demonstrate the exposure of broilers to this particular mycotoxin.

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