Evaluation of the Economic Efficacy of Some Antimycotoxicosis Compounds on Production and Humoral Immunity in Broiler Chickens

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ABSTRACT

Mycotoxicosis is an important problem in poultry industry causing global economic losses constituting a great threat. The present study was designed to investigate the ameliorative effects of four different antimycotoxicosis compounds (AMCs) against production, economic performance and humoral immunity induced by intoxication of dietary aflatoxin (AF) and/or ochratoxin (OT) in broiler chicks. Experiment (I), AF (23 ppb) and OT (17 ppb) were fed alone or mixed and in association with antimycotoxin feed additives, product A or B (1 and 0.5 g/kg feed), respectively. In experiment (II), the intoxicated chicks treated with antimycotoxin drugs, product C or D in drinking water (1ml/liter/3 successive days and 0.5 ml/liter/one day), respectively. Results revealed that chicks intoxicated with AF and/or OT showed significant ($P<0.05$) increase in feed conversion ratio (FCR) and mortality percentages (%) while the antibody titers against Newcastle disease (ND) virus were significantly ($P<0.05$) reduced. It is interesting to note that the adverse effects on FCR and mortality % were significantly ($P<0.05$) reduced in the single AF or OT contaminated diets with dietary supplementation of product A but in the mixed AF and OT contaminated diet, FCR were significantly ($P<0.05$) decreased with addition of product B. Also, product B showed significant ($P<0.05$) increase in the antibody titers against ND in all AF and/or OT contaminated groups. Moreover, the treatment of AF and/or OT intoxicated chicks with product C or D in the drinking water was reported significant ($P<0.05$) decrease in the mortality% and non-significant ($P>0.05$) change in FCR and ND antibody titers.

Introduction

Mycotoxins are characterized in human and animals as food/feed related, non-contagious, non-transferable, non-infectious and non-traceable to microorganisms other than molds (Zain, 2011). Approximately 25% of world's grain supply are contaminated with mycotoxins both pre- and post-harvest that lead to millions of dollars annual losses worldwide, in condemned agricultural products and in animal and human health (FAO, 1983; CAST, 2003; Zain, 2011). "Silent killers", "unavoidable contaminants", and "natural toxicants" all these names have been given to, mycotoxins. Mycotoxins considered as unavoidable contaminants in foods and feeds all over the world (Surai and Mezes, 2005). Mycotoxin exposure affects health and performance, causing reduced weight gain (WG), decreased productivity, reduced immune response and even death (Resanovic *et al.*, 2009; Andretta *et al.*, 2011). Detoxification using mycotoxin binding agents (nutritionally inert adsorbents) is an effective method for decreasing or preventing intoxication of feed at risk for mycotoxin contamination. In ad-

dition, microbial feed additives show potential as an alternative detoxification method through the metabolism or degradation of mycotoxins. Yeast cell wall derivative is generally protective against naturally contaminated feed with multiple toxins. Interestingly, some products containing algae and plant extracts and other products such as combinations of enzymes, various binders, nutrient supplements, or traditional spices demonstrating binding effects and ameliorate mycotoxin toxicity (Hoerr, 2013). This study carried out as a semi-field trial in broiler chickens to evaluate the efficacy of some antimycotoxicosis compounds, which are used in Egypt, on the production, economic performance as well as humoral immunity.

Materials and methods

Experimental chicks, diet and designs

Five hundred and twenty eight, apparently healthy one day old Arbor-Acres broiler chicks were obtained from a commercial company for poultry in Egypt and randomly divided into 10 equal groups (3 replicates/group and 11 birds/replicate) in two experiments (I and II). In both experiments (I-II), group (1) served as a control negative group (0 ppb AF and/or OT), while groups 2, 5 and 8 were used as control positive

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Table 1. Design of the experiments

Group No.	Contaminated diet		Experiment (I)		Experiment (II)	
			Antimycotoxicosis compounds (♦)			
	AF	OT	Product A	Product B	Product C	Product D
Group 1	-	-	-	-	-	-
Group 2	+	-	-	-	-	-
Group 3	+	-	+	-	+	-
Group 4	+	-	-	+	-	+
Group 5	-	+	-	-	-	-
Group 6	-	+	+	-	+	-
Group 7	-	+	-	+	-	+
Group 8	+	+	-	-	-	-
Group 9	+	+	+	-	+	-
Group 10	+	+	-	+	-	+

(•) Experiment (I): antimycotoxicosis compounds/ feed * Prod. A: combination of *Eubacterium BBSH^797* and *Trichosporon mycotoxinivoran*, plant extract, algae extract. ** Prod. B: formed from nano-clay, seaweed extract, yeast cell wall, diatomaceous earth.

(•) Experiment (II): antimycotoxicosis compounds/ drinking water * Prod. C: combination of bacterial cell wall extract, oligosaccharides, L-form bacteria extract, group of mycotoxin biotransforming enzymes, organic acid and salts, hepatic and renal tonic, vitamins. ** Prod. D: formed from isomalto-oligosaccharide, micronized-mannanoligosacharide, lactobacillus extract, and cholestyramine.

groups, as illustrated in Table 1. Groups (2), (3-I), (3-II), (4-I) and (4-II) were fed on diets contaminated with 23 ppb AF, groups (5), (6-I), (6-II), (7-I) and (7-II) were fed on diets contaminated with 17 ppb OT, groups (8), (9-I), (9-II), (10-I) and (10-II) were fed on diets contaminated with 23 ppb AF and 17 ppb OT. Chicks were maintained under standard conditions of temperature and lighting regimen. Feed and water provided adlibitum throughout the 35-d time of the experiment. They fed a commercial starter ration (22.48% crude protein and 3051.5 kcal metabolizable energy (ME)/kg) from day 1 to day 21, then a grower ration (20.04% crude protein and 3201.9 kcal ME/kg) from day 21 to day 35 as recommended by National Research Council, (1994). Chicks were vaccinated against ND, avian influenza, and infectious bursal disease. Four AMCs were purchased from the imported companies in Egypt. Product A and product B incorporated into the starter contaminated diets at 1g/kg and 0.5 g/kg, respectively as preventive feed additives in experiment (I) for 21 days. Product C and product D used in the drinking water for controlling mycotoxicosis at a dose of (1ml/liter/3 successive days) and (0.5 ml/liter/one day), respectively. All study protocols and procedures were approved by the committee of graduate studies and research of faculty of veterinary medicine, Benha University (place where experiments were conducted).

Mycotoxin production and analysis

Aflatoxin was produced by growing standard aflatoxigenic strain (*A. flavus* ATCC 16875) on sterile grounded corn according to Shotwell *et al.* (1966). While A. ochraceous strain ATCC 63304 inoculated on sterile crushed corn to produce OT by using the method of Trenk *et al.* (1971). The mycotoxin contaminated corns were collected separately for each fungus and using affinity column chromatography to determine the AF and OT concentration (Aflatest and Ochratest, VICAM, USA) and fluorometric analysis (Series-4Ex Fluorometer, VICAM, USA). Finally, the contaminated corn with AF or OT incorporated into the experimental starter diets were at 23 ppb and/or 17 ppb, respectively (Nabarawy *et al.*, 2016).

Production and economic performance parameters

Daily feed intake (DFI), daily WG (DWG), FCR, mortality %, EBI, feed cost (FC), total cost (TC), total return (TR), net return (NR), economic efficiency (EE) and relative EE (REE) (Shehab, 2008; Marţi *et al.*, 2011) were evaluated and calculated by following formulas:

WG (g) = Body weight (BW) (g) at the ending of period - BW (g) at the beginning of period.

DWG (g/ chicks/ period) = WG (g/ chicks/ period) /number of days (period).

FCR= FI (g/chick/period) / WG (g/chick/period).

Mortality % = (No. of deaths /total population) ×100.

EBI= [DWG (g) × (100- mortality (%)]/ (FCR× 10).

TC (L.E/chick) = chick price (L.E) + management cost (L.E/chick) + feed cost (L.E/chick).

TR (L.E/chick) = final BW (kg) × selling price of live chicken (L.E/ kg).

NR (L.E/chick) = TR (L.E/chick) - TC (L.E/chick).

EE (tested group) = NT (L.E/chick/group)/ TC (L.E/chick/group).

REE (tested group) = [EE (tested group) / EE (control group)] ×100.

Estimation of humoral immune response

Blood samples were collected in non-heparinized tubes by jugular vein puncture at 35th day of chick's age. Sera were separated and stored at -20°C for subsequent assessment of serum antibody titers of ND using HI test (OIE, 2012).

Statistical analysis

Differences between groups were analyzed by using One-Way ANOVA and Duncan's multiple comparison Post Hoc tests (Duncan, 1955). Statistical analysis was performed using the statistical software package SPSS for Windows (version 20.0, SPSS Inc., Chicago, IL, USA). Statistical significance between mean values was set at ($p < 0.05$).

Results

In the present study, chicks intoxicated with AF and/or OT in the diet with and without treatment showed significantly (P<0.05) decrease in DWG and significantly (P<0.05) increased in FCR and mortality % as illustrated in Table (2). DFI and DWG in the intoxicated groups with AF and/or OT (2, 5 and 8) significantly (P<0.05) decreased in comparison with control group and reported DFI as, 67, 67.4 and 66.5 g/chick, and DWG as, 33.3, 31.5 and 30.9 g/chick, respectively along the course of the experiment. On the other hand, FCR in those groups showed significant (P<0.05) increase along the period 1-35d of both experiments when compared with control group that reported as 2.1 in groups 2 and 5 and 2.2 in group 8. Non-significant (P>0.05) difference was observed between groups intoxicated with AF and / or OT. Moreover, AF and/or OT contaminated diets in both experiments resulted in a significant (P<0.05) increase in the mortality% as 9.1, 15.15 and 24.24 in group (2), (5) and (8), respectively.

The adverse effects on performance were overcome and the mortality rates were significantly (P<0.05) decreased by the dietary supplementation of products (A or B) in experiment (I). Addition of product A to single contaminated diets with AF or OT showed significant (P<0.05) decrease in FCR and mortality% than product B while product B in contaminated diet with both AF and OT showed significant (P<0.05) improvement of FCR (Table 2). Another strategy used to control mycotoxins in the chicken was the administration of AMCs (C/D) through drinking water in experiment (II), represented in Table (2), reported significantly (P<0.05) decrease in the mortality%.

Based on the local market prices of feed ingredients and selling price of live broiler chickens, FC, TC, TR and NR were calculated and summarized in Table (3). The highest value of TR found as 22.16 L.E/chicken for control group, while the lowest values found as 15.59 L.E/chicken for group (4) in experiment (I) and 14.35 L.E/chicken for group (10) in experiment (II). Control negative group gave the best NR (3.95 L.E/chicken). NR of groups 3, 6, 7 and 10 in experiment (I) reported as 0.76, 2.34, 0.50 and 0.24 L.E/chicken.

Table 2. Effects of the investigated antimycotoxicosis compounds on growth performance of chicks fed AF and/or OT contaminated diet.

Group	Treatment	Experiment (I)				Experiment (II)			
		DFI	DWG	FCR	Mortality%	DFI	DWG	FCR	Mortality%
1	Control	74.8±0.6[a]	43.9±1.2[a]	1.7±0.04[f]	0±0[f]	74.8±0.6[a]	43.9±1.2[a]	1.7±0.04[b]	0±0[h]
2	AF	67.0±1.2[b]	33.3±0.5[def]	2.01±0.01[bc]	9.1±0[c]	67.0±1.12[d]	33.3±0.5[bc]	2.01±0.01[a]	9.1±0[e]
3	AF+ AMC*	65.0±1.1[bc]	35.2±0.2[cd]	1.9±0.02[de]	3.03±0[e]	63.4±0.2[f]	32.1±0.1[cde]	1.9±0.01[a]	3.03±0[g]
4	AF+ AMC**	62.3±0.9[c]	30.5±0.7[g]	2.04±0.04[bc]	6.06±0[d]	69.3±0.4[bc]	33.7±0.4[bc]	2.1±0.01[a]	9.1±0[e]
5	OT	67.4±0.4[b]	31.5±0.1[fg]	2.1±0.01[ab]	15.15±0[b]	67.4±0.4[cd]	31.5±0.1[de]	2.1±0.01[a]	15.15±0[c]
6	OT+ AMC*	73.1±0.9[a]	40.67±0.9[b]	1.8±0.1[ef]	0.0±0[f]	67±0.02[d]	34.1±0.6[b]	1.9±0.03[a]	12.12±0[d]
7	OT+ AMC**	71.8±2.6[a]	36.6±0.7[c]	1.9±0.1[cd]	3.03±0[f]	70.2±0.9[b]	33.01±0.1[bcd]	2.1±0.03[a]	6.07±0[f]
8	AF+OT	66.5±0.8[b]	30.9±0.4[fg]	2.2±0.03[ab]	24.24±0[a]	66.5±0.8[de]	30.9±0.4[e]	2.2±0.03[a]	24.24±0[a]
9	AF+OT+ AMC*	71.9±1.6[a]	32.5±1.4[efg]	2.2±0.1[a]	9.1±0[c]	63.2±0.4[f]	30.8±0.3[e]	2.2±0.01[a]	18.18±0[b]
10	AF+OT+ AMC**	66.2±0.8[b]	34.5±0.2[cde]	1.9±0.03[cde]	9.1±0[c]	64.6±0.9[ef]	27.9±0.4[f]	2.1±0.2[a]	18.18±0[b]

"a, b and c": there is significant difference (P< 0.05) between any two means, within the same column have the different superscript letter.
• DFI: daily Feed intake, DWG: daily weight gain, FCR: feed conversion ratio, Mortality%: mortality percentages.
AMCs in experiment (I): antimycotoxicosis compounds/ feed * Prod. A: combination of *Eubacterium BBSH*[797] and *Trichosporon mycotoxinivoran*, plant extract, algae extract. ** Prod. B: formed from nano-clay, seaweed extract, yeast cell wall, diatomaceous earth.
AMCs in experiment (II): antimycotoxicosis compounds/ drinking water * Prod. C: combination of bacterial cell wall extract, oligosaccharides, L-form bacteria extract, group of mycotoxin biotransforming enzymes, organic acid and salts, hepatic and renal tonic, vitamins.
** Prod. D: formed from isomalto-oligosaccharide, micronized-mannanoligosacharide, lactobacillus extract, and cholestyramine.

Table 3. Effect of the investigated antimycotoxicosis compounds on the production costs and returns of the broiler chickens fed AF and/or OT contaminated diet.

Group	Treatment	Experiment (I)				Experiment (II)			
		FC♦	TC♦	TR♦	NR♦	FC♦	TC♦	TR♦	NR♦
1	Control	10.46±0.08[a]	18.22±0.08[a]	22.16±0.6[a]	3.95±0.55[a]	10.46±0.08[a]	18.22±0.08[a]	22.16±0.6[a]	3.95±0.55[a]
2	AF	9.38±0.17[b]	17.13±0.17[b]	16.95±0.3[def]	-0.18±0.1[cd]	9.38±0.17[ab]	17.13±0.17[bc]	16.95±0.26[bc]	-0.18±0.09[bc]
3	AF+ AMC*	9.1±0.16[bc]	17.11±0.16[b]	17.87±0.1[cd]	0.76±0.06[c]	8.87±0.02[bc]	16.89±0.02[bc]	16.39±0.07[cde]	-0.50±0.06[bc]
4	AF+ AMC**	8.72±0.14[c]	16.78±0.14[b]	15.59±0.36[g]	-1.2±0.28[de]	9.70±0.06[ab]	17.56±0.06[ab]	17.19±0.19[bc]	-0.37±0.15[bc]
5	OT	9.44±0.05[b]	17.19±0.05[b]	16.10±0.04[fg]	-1.1±0.03[de]	9.44±0.05[ab]	17.19±0.05[ab]	16.10±0.04[cd]	-1.1±0.03[bcd]
6	OT+ AMC*	10.23±0.12[a]	18.24±0.12[a]	20.58±0.49[b]	2.34±0.53[b]	9.40±0[ab]	17.42±0[ab]	17.38±0.25[b]	-0.03±0.25[b]
7	OT+ AMC**	10.05±06[a]	18.11±0.36[a]	18.60±0.35[c]	0.5±0.6[c]	9.83±0.12[ab]	17.69±0.12[ab]	16.84±0.07[bcd]	-0.85±0.19[bcd]
8	AF+OT	9.31±0.10[b]	17.06±0.1[b]	15.77±0.18[fg]	-1.3±0.18[de]	9.31±0.1[b]	17.06±0.1[bc]	15.77±0.18[e]	-1.29±0.18[cd]
9	AF+OT+ AMC*	10.06±0.22[a]	18.07±0.22[a]	16.61±0.7[efg]	-1.46±0.6[e]	8.85±0.06[bc]	16.86±0.06[bc]	15.75±0.16[e]	-1.12±0.12[bcd]
10	AF+OT+ AMC**	9.26±0.11[b]	17.32±0.1[b]	17.55±0.1[cd]	0.24±0.18[c]	8.15±1.02[c]	16.01±1.02[c]	14.35±0.21[f]	-1.66±0.84[d]

"a, b and c": There is significant difference (P<0.05) between any two means, within the same column have the different superscript letter.
• Feed cost (FC), total cost (TC), total return (TR), net return (NR).
AMCs in experiment (I): antimycotoxicosis compounds/ feed * Prod. A: combination of *Eubacterium BBSH*[797] and *Trichosporon mycotoxinivoran*, plant extract, algae extract. ** Prod. B: formed from nano-clay, seaweed extract, yeast cell wall, diatomaceous earth.
AMCs in experiment (II): antimycotoxicosis compounds/ drinking water * Prod. C: combination of bacterial cell wall extract, oligosaccharides, L-form bacteria extract, group of mycotoxin biotransforming enzymes, organic acid and salts, hepatic and renal tonic, vitamins. ** Prod. D: formed from isomalto-oligosaccharide, micronized-mannanoligosacharide, lactobacillus extract, and cholestyramine.

In experiment (I), the addition of product A to the contaminated diets with AF or OT in group (3) and (4) gave significant (P<0.05) improvement to TR and NR than product B while in the chicks intoxicated with AF and OT, product B was more efficient than product A as shown in Table (3). Moreover, non-significant (P>0.05) difference was reported in treatment of the chicks intoxicated with AF and/or OT by product C or D.

Concerning production costs and returns, there was a significant (P<0.05) decrease in EE and REE in all intoxicated groups with AF and/or OT when compared with control (-ve) group. The addition of product A to the single contaminated diet with AF or OT in-group 3 and 4, respectively, gave significant (P<0.05) increase in the EE than product B. Although supplementation of product B to the diet contaminated with both AF and OT in group (10) was more efficient than product A. Interestingly, no significant (P>0.05) difference in EE and REE was found when using product C or D in the drinking water for treatment of the intoxicated chicks with AF and/or OT (Table 4; Figs. 1and 2).

Also, Table (4) shows that the lower values of EBI recorded as 108.5 in the intoxicated chicks with AF and OT (group 8) followed by 124.85 in group 5 (OT contaminated diet) then 150.15 in group 2 (AF contaminated diet). In experiment (I), chickens supplemented with product A in group 3 (contaminated diet with AF) and group 6 (OT contaminated diet) recorded significant (P<0.05) higher EBI up to 184.49 and 226.54, respectively. On the other hand the addition of product B in diet contaminated with AF and OT (group 10) revealed significant (P<0.05) higher EBI as 163.39 than product A. Moreover, treatment by product C and D showed significant (P<0.05) improvement in EBI for chickens fed contaminated diet with OT as 152.44 and 145.85 in group 6 and 7 respectively when compared with group 5 (untreated contaminated with OT). EBI for control group in both experiments was 258.54 which is significantly (P<0.05) better when compared with all groups contaminated with AF and/or OT.

Regarding the results of humoral immune response of chickens to ND vaccination using HI test, the antibody titers
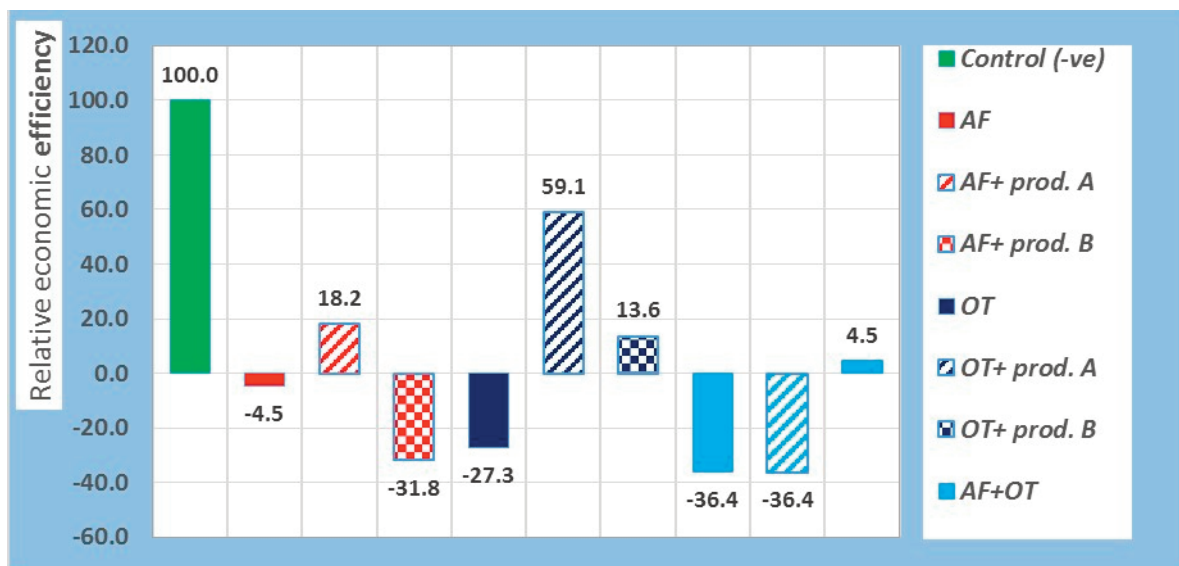

Fig. 1. Effects of the antimycotoxicosis compounds used as feed additives on relative economic efficiency of chicks fed AF and/or OT contaminated diet
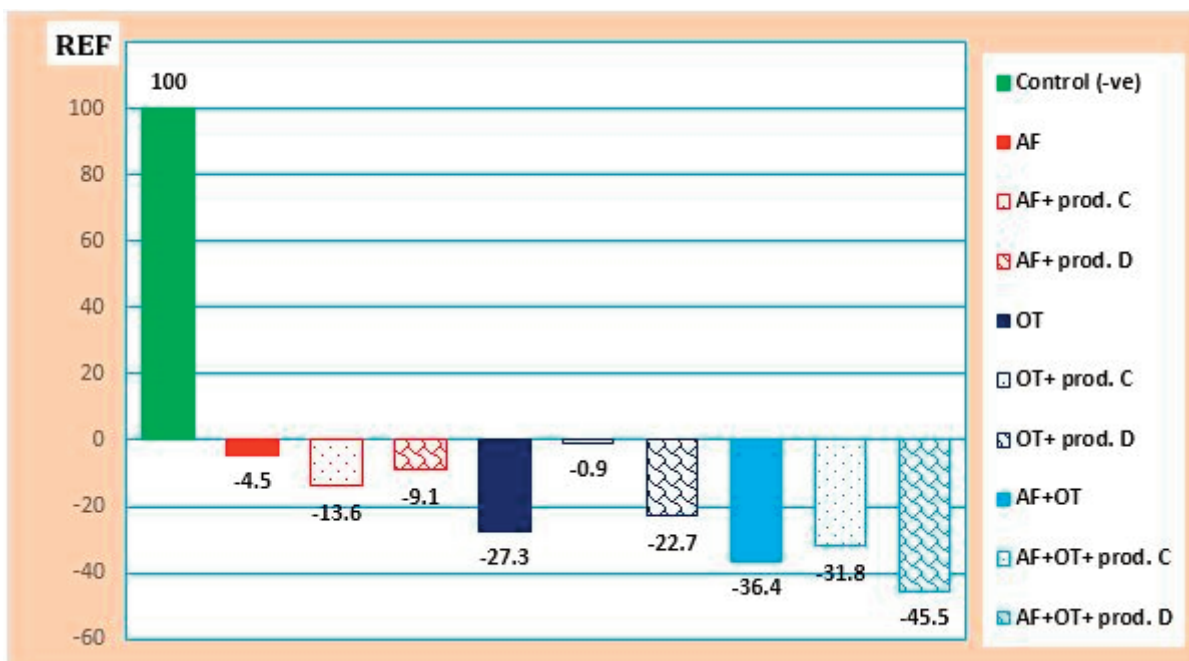

Fig. 2. Effects of the antimycotoxicosis compounds used in the drinking water on relative economic efficiency of chicks fed AF and/or OT contaminated diet

Table 4. Effects of the investigated antimycotoxicosis compounds on economic efficacy of chicks fed AF and/or OT contaminated diet

Group	Treatment	Experiment (I)			Experiment (II)		
		EE*	REE*	EBI*	EE*	REE*	EBI*
1	Control	0.22±0.03[a]	100	258.54±13.23[a]	0.22±0.03[a]	100	258.54±13.23[a]
2	AF	-0.01±0.01[cd]	-4.55	150.15±2.21[de]	-0.01±0.01[bc]	-4.5	150.15±2.21[b]
3	AF+ AMC*	0.04±0[c]	18.18	184.49±1.41[c]	-0.03±0[bc]	-13.6	157.45±1.22[b]
4	AF+ AMC**	-0.07±0.02[de]	-31.81	140.26±5.37[def]	-0.02±0.01[bc]	-9.1	149.35±2.85[b]
5	OT	-0.06±0[de]	-27.27	124.85±0.42[fg]	-0.06±0[bcd]	-27.3	124.85±0.42[c]
6	OT+ AMC*	0.13±0.03[b]	59.09	226.54±12.03[b]	-0.02±0.01[bc]	-0.9	152.44±4.85[b]
7	OT+ AMC**	0.03±0.03[c]	13.64	181.90±11.54[c]	-0.05±0.01[bcd]	-22.7	145.85±2.87[b]
8	AF+OT	-0.08±0.01[de]	-36.36	108.50±2.48[g]	-0.08±0.01[cd]	-36.4	108.5±2.48[c]
9	AF+OT+ AMC*	-0.08±0.03[e]	-36.36	134.13±9.87[ef]	-0.07±0.01[bcd]	-31.8	122.74±2.01[c]
10	AF+OT+ AMC**	0.01±0.0[c]	4.55	163.39±3.41[cd]	-0.10±0.05[d]	-45.5	113.14±13.02[c]

"a, b and c": There is significant difference (P< 0.05) between any two means, within the same column have the different superscript letter.
• Economic efficiency (EE), relative EE (REE), European broiler index (EBI).
AMCs in experiment (I): antimycotoxicosis compounds/ feed * Prod. A: combination of *Eubacterium BBSH797* and *Trichosporon mycotoxinivoran*, plant extract, algae extract. ** Prod. B: formed from nano-clay, seaweed extract, yeast cell wall, diatomaceous earth.
AMCs in experiment (II): antimycotoxicosis compounds/ drinking water * Prod. C: combination of bacterial cell wallextract, oligosaccharides, L-form bacteria extract, group of mycotoxin biotransforming enzymes, organic acid and salts, hepatic and renal tonic, vitamins. ** Prod. D: formed from isomalto-oligosaccharide, micronized-mannanoligosacharide, lactobacillus extract, and cholestyramine.

Table 5. Effects of the investigated antimycotoxicosis compounds on humoral immunity of chicks fed AF and/or OT contaminated diet against Newcastle disease (ND)

Group	Treatment	Antibody Titers of ND**	
		Experiment (I)	Experiment (II)
1	Control	9.33±0.33[a]	9.33±0.33[a]
2	AF	6.00±0.00[f]	6.00±0.0[bc]
3	AF+ AMC*	6.00±0.58[f]	3.67±0.33[e]
4	AF+ AMC**	6.33±0.33[e]	3.67±0.33[e]
5	OT	6.67±0.67[de]	6.67±0.67[b]
6	OT+ AMC*	7.33±0.33[c]	4.67±0.33[de]
7	OT+ AMC**	8.00±0.00[b]	5.00±0.0[cd]
8	AF+OT	6.33±0.33[e]	6.33±0.33[b]
9	AF+OT+ AMC*	6.67±0.33[de]	5.67±0.33[bcd]
10	AF+OT+ AMC**	7.00±0.58[cd]	6.33±0.33[b]

"a, b and c": There is significant difference (P< 0.05) between any two means, within the same column have the different superscript letter.
•• Antibody titers expressed as the log2of the reciprocal of the highest dilution in which Haemagglutination was observed macroscopically.
*AMCs in experiment (I): antimycotoxicosis compounds/ feed * Prod. A: combination of Eubacterium BBSH797 and Trichosporon mycotoxinivoran*, plant extract, algae extract. ** Prod. B: formed from nano-clay, seaweed extract, yeast cell wall, diatomaceous earth.
AMCs in experiment (II): antimycotoxicosis compounds/ drinking water * Prod. C: combination of bacterial cell wall extract, oligosaccharides, L-form bacteria extract, group of mycotoxin biotransforming enzymes, organic acid and salts, hepatic and renal tonic, vitamins. ** Prod. D: formed from isomalto-oligosaccharide, micronized-mannanoligosacharide, lactobacillus extract, and cholestyramine.

of chickens intoxicated with AF and/or OT groups in the both experiments were significantly (P<0.05) lowered when compared with the control group (Table 5). In the present study, the addition of product B in experiment (I) into the contaminated diets with AF and/or OT improve significantly (P<0.05) the antibody titers against ND vaccination in comparison with product A. On the other hand, the use of product A in experiment (I) and products C and D in experiment (II) did not have any beneficial effects against the negative effects of AF and/or OA on the humoral immune response.

Discussion

Mycotoxicoses, particularly aflatoxicosis and ochratoxicosis, are serious disease conditions with negative impact on poultry industry due to reduced performance in the exposed birds (Santin *et al.*, 2006; Vilar *et al.*, 2008; Resanovic *et al.*, 2009). In the present study, chicks intoxicated with AF and/or OT in the diet with and without treatment showed significantly (P<0.05) decrease in DWG and significantly (P<0.05) increased in FCR and mortality % as illustrated in Table (2). DFI and DWG

in the intoxicated groups with AF and/or OT (2, 5 and 8) significantly (P<0.05) decreased in comparison with control group. On the other hand, FCR in those groups showed significant (P<0.05) increase along the period 1-35d of both experiments when compared with control group. A negative effect on growth parameters of broiler chicken may be caused as a result of impairment of protein synthesis and metabolism by mycotoxins (Huff *et al.*, 1974). The reduction in growth performance due to intoxication with AF and/ or OT in the present study is consistent with the previous reports of Raju and Deveyowda (2000); Verma *et al.* (2004); Santin *et al.* (2006); Vilar *et al.* (2008). Interestingly, non-significant (P>0.05) difference between groups intoxicated with AF and / or OT was observed. These results came in accordance with those reported by Santin *et al.* (2006); Vilar *et al.* (2008) and in contrary to those reported by Raju and Devegowda (2000); Verma *et al.* (2004), probably due to the lower levels of mycotoxins used in the present study as compared to the previous studies. Moreover, a significant (P<0.05) increase in the mortality% of groups (2), (5) and (8), fed AF and/or OT contaminated diets in both experiments, indicated a direct relationship between mycotoxins in the diet and the mortality %. Similarly, Wang *et al.* (2006) reported highest mortalities in chickens feeding moldy corn.

Regardless of the contamination with mycotoxins, the adverse effects on performance were overcome and the mortality rates were significantly (P<0.05) decreased by the dietary supplementation of products (A or B) in experiment (I). These results confirm previous studies by Shi *et al.* (2009) who found that the dietary supplementation with montmorillonite nanocomposite recover the adverse effect of AF on performance in broiler chickens. Hanif *et al.* (2008) found that the administration of OT with a toxin deactivator containing the yeast *Trichosporon mycotoxinivoran* attenuated the harmful effects of OT on the performance in broiler chickens. In the present study, addition of product A to single contaminated diets with AF or OT showed significant (P<0.05) decrease in FCR and mortality% than product B while product B in contaminated diet with both AF and OT showed significant (P<0.05) improvement of FCR than product A (Table 2). These variations may be attributed to the different components of each product and their mode of action to eliminate the negative effect of mycotoxins on performance. AMC product A influence in feed as mycotoxin modifier may be due to its components, yeast (*Trichosporon mycotoxinivorans*) and bacteria (*Eubacterium BBSH*[797]), that biodegrade mycotoxins into less toxic metabolites and act in the intestinal tract of animals prior to the absorption of mycotoxins while product B containing nanoclay, diatomaceous earth, seaweeds extracts and yeast cell walls that are classified as a wide mycotoxin binder binding the mycotoxins in the gastrointestinal tract (GIT) of the animal and form toxin binder complex which eliminated via the feces (EFSA, 2009; Devreese *et al.*,2013).

In experiment (II) (Table 2), the addition of product C or D in drinking water reported significantly (P<0.05) decrease in the mortality%. It could be attributed to their excellent contents, which play an important role as a chelating agent in the sequestration of the mycotoxin through the GIT, preventing them from absorption, and so help in getting rid of these toxins outside the body. Although non-significant (P>0.05) difference in FCR of these groups when compared with the intoxicated groups. On contrary, Shareef and Omar (2012) reported that the Synertox® (contains the same components of product C) in the drinking water able to counteract negative effect of AF on performance of broiler chickens. Also, Pizzolitto *et al.* (2013) recorded a protective effect of the yeast (Saccharomyces cerevisiae) in the drinking water against aflatoxicosis in broiler chickens. This is probably due to the short administration period of AMCs used in the current study as compared

to the previous studies.

Based on the local market prices of feed ingredients and selling price of live broiler chickens, FC, TC, TR and NR are calculated and summarized in Table (3). In experiment (I), the addition of product A to the contaminated diets with AF or OT in group (3) and (4) gave significant (P<0.05) improvement to TR and NR than product B, while in the chicks intoxicated with both AF and OT, product B was more efficient than product A. Moreover, non-significant (P>0.05) difference was reported in treatment of the chicks intoxicated with AF and/or OT by product C or D. These results may be attributed to decrease WG and increase mortality % as a result for the intoxication with AF and/or OT and also depend on the inactivation and detoxification properties of the used products (Marţi *et al.*, 2011).

Significant (P<0.05) decrease in EE and REE in all intoxicated groups with AF and/or OT was found when compared with control group (Table 4 and Figs. 1- 2). The addition of product A to the single contaminated diets with AF or OT in-group (3) and (4) gave significant (P<0.05) increase in the EE than product B. Although supplementation of product B to diet contaminated with AF and OT in group (10) was more efficient than product A. Interestingly, no significant (P>0.05) difference in EE and REE was found when using the product C or D in the drinking water for treatment of the intoxicated chicks with AF and/or OT. These results were in agreement with the report of Abdelaziz *et al.* (2015) who showed significant reduction of EE and REE with the use of Mycofix plus® or other natural feed additives when compared to those fed control diet for broiler chicks. Similarly, using hydrates sodium calcium aluminosilicate, rice hulls or Mycofix plus® were particularly reduced EE and REE of broiler chicks when compared with those fed on the control diet (El Faham *et al.*, 2015). On the other hand, these results disagree with those of Marţi *et al.* (2011) who reported the dietary inclusion of Mycofix MTV® in broiler chicken feed imposed supplementary expenses and also generated better incomes and revenues, compared to control group.

Our results revealed that EBI values were lower in the intoxicated chicks with AF and/or OT. In experiment (I), chickens supplemented with product A recorded significant (P<0.05) increase in EBI values with single contaminated diets. On the other hand the addition of product B in diet contaminated with both AF and OT revealed significant (P<0.05) increase in EBI than product A. Moreover, treatment by product C and D showed significant (P<0.05) improvement in EBI for chickens fed contaminated diet with OT when compared with group 5 (untreated contaminated with OT) (Table 4). It is generally accepted that EBI positively influenced by the growth performance parameters (BW, DWG, FCR and recorded mortality %). This explanation was supported by Diaz (2001) who found that FCR and WG have been improved in chicken broilers which received certain mycotoxin detoxifying additives even in the absence of mycotoxin from feed, the used product acted similarly to a growth promoter. Usage of the dietary inclusion of mycotoxin detoxifying agents (Mycofix MTV® 1 or 3 %) in broilers generated 50% cut of mortality rate, also better EBI results compared to control group as reported by Marţi *et al.* (2011). Another suggestion reported by Agboola *et al.* (2015), was the use of toxin binder as an alternative to antibiotics can improve performance of broilers especially in the first 3 weeks of life as its potential to control growth of enteropathogenic bacteria, improve intestinal morphology and improve absorption of nutrients through reduction or prevention of mycotoxin absorption across the GIT.

Regarding to the results of humoral immune response of chickens to ND vaccination using HI test, the antibody titers of chickens intoxicated with AF and/or OT groups in the both experiments were significantly (P<0.05) lowered when com-

pared with the control group (Table 5). Similarly, Verma *et al.* (2004); Kalorey *et al.* (2005); Sakhare *et al.* (2007) observed suppression of the mean antibody titers in chickens given AF and/or OT. Also, El Nabarawy *et al.* (2016) reported that exposure of broilers to 23 ppb OT and 16 ppb AF resulted in significance decrease in humoral immune response against ND vaccination applied by different routes. Moreover, humoral immune response in the broiler chickens significantly (P<0.05) decreased by experimental aflatoxicosis (Ibrahim *et al.*, 2000; Manafi *et al.*, 2012) or ochratoxicosis (Santin *et al.*, 2006; Elaroussi *et al.*, 2006; Awaad *et al.*, 2011; Jayaramu *et al.*, 2012; Hanif and Muhammad, 2015). These findings may be due to the fact that mycotoxins have been implicated as potent inhibitors of the avian immune response by interfering with DNA and protein synthesis (Oguz *et al.*, 2000). Mycotoxins impose oxidative stress, stimulate apoptosis and involved in gene expression regulation. These changes were responsible for immunosuppressive action of mycotoxins and their interference with vaccination programs (Surai and Mezes, 2005).

In the present study, the addition of product B in experiment (I) into the contaminated diets with AF and/or OT improve significantly (P<0.05) the antibody titers against ND vaccination in comparison with product A. Similarly, addition of dietary mycotoxin binders, as sodium bentonite and clinoptilolite, were effective in ameliorating the suppressive effect of AF on the humoral immunity (Ibrahim *et al.*, 2000; Oguz *et al.*, 2003; Pasha *et al.*, 2007). On the other hand, the use of product A in experiment (I) and products C and D in experiment (II) did not have any beneficial effects against the negative effects of AF and/or OT on the humoral immune response. These findings were in disagreement with Diaz *et al.* (2005) and Hanif and Muhammad (2015) who showed that Mycofix plus® was capable of counteracting the deleterious effects of AF and OT on humoral immune response of birds against ND. Also, Shareef and Omar (2012) reported the ability of Synertox® to restore the reduction of antibody titer caused as a negative effect of AF to those of the control group in broiler chickens. The efficacy of product B over other products partially ameliorate the negative effects of mycotoxins on humoral immune response could be attributed to its content from yeast cell wall extract that had a potent immunomodulatory effect, evoked immune response and enhanced vaccination effectiveness (Awaad *et al.*, 2011).

Conclusion

This study has shown that the feed supplementation of AMCs, improve (P<0.05) significantly the adverse effects of AF and/or OT in broilers. But there were no significant difference with the treatment of AF and/ or OT intoxicated chicks with AMCs in drinking water except significant decrease in mortality %.

Acknowledgement

References

Abdelaziz, M.A.M. El-Faham, A.I., Nematallah, G.M., 2015. Using natural feed additives as alternative antimycotoxins in broiler diets. Egyptian Poultry Science 35, 291-310.

Agboola, A.F., Omidiwura, B.R.O., Odu, O., Odupitan, F.T., Iyayi, E.A., 2015. Effect of probiotic and toxin binder on performance, intestinal microbiota and gut morphology in broiler chickens. Journal of Animal Science Advances 5, 1369-1379.

Andretta, I., Kipper, M., Lehnen, C.R., Hauschild, L., Vale, M.M., Lovatt, P.A., 2011. Meta-analytical study of productive and nutritional interactions of mycotoxins in broilers. Poultry Science 90, 1934–1940.

Awaad, M.H.H., Atta, A.M., Abd El-Ghany, W.A., Elmenawey, M., Ahmed K., Hassan, A.A., Nada, A.A., Abdelaleem, G.A., 2011. Effect of a specific combination of mannan-oligosaccharides and β-glucans extracted from yeast cell wall on the health status and growth performance of ochratoxicated broiler chickens. Journal of American Science 7, 82-89.

Council for Agricultural Science and Technology (CAST), 2003 Mycotoxins: risks in plants, animal and human systems. Task force report No. 139, Ames, Iowa, USA, p. 99.

Devreese, M., Backer, P. De, Croubels, S., 2013. Different methods to counteract mycotoxin production and its impact on animal health. Vlaams Diergeneeskundig Tijdschrift 82, 181-190.

Diaz, G., 2001. Mycofix plus 3.0 trial with broilers fed with DAS contaminated feed, Universidad Nacional de Colombia, Bogota, Biomin trials, www.biomin.net.

Diaz, G.J., Cortes, A., Roldan, L., 2005. Evaluation the efficacy of four additives against the adverse effects of T-2 toxin in growing broiler chicken. J. Appl. Poult. Res. 14, 226-231.

Duncan, D.B., 1955. New multiple range and multiple F test. Biometrics 11, 1-42.

European feed safety agency (EFSA), 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. http://www.efsa.europa.eu/en/supporting/pub/22e.htm

El Faham, A.I., Ali, N.G.M., El Sanhoury, M.H.S., 2015. Comparison of hydrated sodium calcium aluminosilicate, yeast cell wall and rice hulles on counteracting aflatoxin in broiler chicken diets. Egyptian Poultry Science 35, 673- 689.

El Nabarawy A.M, Madian, K., Aly, E.A.H, Madbouly, Y., 2016. The effect of mycotoxins on immune response of broilers to the live ND vaccines applied by different routes. Middle East Journal of Applied Sciences 6, 51-58.

Elaroussi M.A., Mohamed, F.R., El Barkouky, E.M., Atta, A.M., Abdou A.M., Hatab, M.H., 2006. Experimental ochratoxicosis in broiler chickens. Avian Pathology 35, 263-269.

Food and Agriculture Organization (FAO) 1983. Post-harvest losses in quality of food grains. Food and Nutrition Paper No. 29, 103.

Hanif, N.Q., Muhammad, G., 2015. Immunotoxicity of ochratoxin A and role of *Trichosporon mycotoxinivorans* on the humoral response to infectious viral disease vaccines in broilers. Pakistan Journal of Zoology 47, 1683-1689.

Hanif, N.Q., Muhammad, G., Siddique, M., Khanum, A., Ahmed, T., Gadahai, J.A., Kaukab, G., 2008. Clinico-pathomorphological, serum biochemical and histological studies in broilers fed ochratoxin A and a toxin deactivator (Mycofix Plus). British Poultry Science 49, 632-642.

Hoerr, F.J., 2013. Mycotoxicoses chapter 31 In: Swayne, D. E. editor. Diseases of poultry. 13th ed., Blackwell Publishing Ltd., pp. 1271–1287.

Huff, W.E., Wyatt, R.D., Tucker, J.L., Hamilton, P.B., 1974. Ochratoxicosis in broiler chicken. Poultry Science 53, 1585-1591.

Ibrahim, I.K., Shareef, A.M., Joubory, K.M.T., 2000. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Research in Veterinary Science 69, 119-122.

Jayaramu, G.M., Satyanarayana M.L., Ravikumar, P, Narayanaswamy, H.D., 2012. Evaluation of the immune status of broiler chicken during ochratoxin, citrinin and their combined toxicity. International Journal of Advanced Biological Research 2, 756-759.

Kalorey, D.R., Kurkure, N.V., Ramgaonkar, J.S., Sakhare, P.S., Shubhangi, W., Nigot, N.K., 2005. Effect of Polyherbal Feed Supplement "Growell" during Induced Aflatoxicosis, Ochratoxicosis and Combined Mycotoxicoses in Broilers. Asian Australian Journal of Animal Science 14, 677-679.

Manafi, M., Umakantha, B., Mohan, K., Swamy, H.D., 2012. Synergistic effects of two commonly contaminating mycotoxins (aflatoxin and T-2 toxin) on biochemical parameters and immune status of broiler chickens. World Application Science Journal 17, 364–367.

Marţi, R., Vacaru-Opriş, I., Radu-Rusu, R.M., 2011. Productive performances and economic efficiency of chicken broilers receiving mycotoxin contaminated feed and dietary detoxificated additive. Lucrări Ştiinţifice 56, 390-393.

National Research Council, 1994. Nutrition Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Oguz H, Hadimli H.H., Kurtoglu, V., Erganis, O., 2003. Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Revue de Medicine Veterinaire 154, 483–486.

Oguz, H., Kececi, T., Birdane, Y.O., Onder, F., Kurtoglu, V., 2000. Effect of clinoptilolite on serum biochemical and hematological characters of broiler chickens during experimental aflatoxicosis. Research in Veterinary Science 69, 89–93.

OIE, 2012. Terrestrial Manual. Newcastle Disease. Chapter 2. 3.14.

Pasha, T.N., Mahmood, A., Khattak, F.M., Jabbar M.A, Khan. A.D, 2007. The Effect of feed supplementation with different sodium bentonite treatments on broiler performance. Turkish Journal of Veterinary and Animal Sciences 32, 245- 248.

Pizzolitto, R.P., Armando, M.R., Salvano, M.A., Dalcero, A.M., Rosa, C.A., 2013. Evaluation of Saccharomyces cerevisiae as an antiaflatoxicogenic agent in broiler feedstuffs. Poultry Science 92, 1655–1663.

Raju, M.V.L.N., Devegowda, G., 2000. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). British Poultry Science 41, 640-650.

Resanovic, R.M., Nešic, K.D., Nesic, V.D., Palic, T.D., Jacevic, V.M., 2009. Mycotoxins. In: Poultry Production. Proc. Nat. Sci, Matica Srpska Novi. Sad. 116, 7-14.

Sakhare, P.S., Harne, S.D., Kalorey, D.R., Warke, S.R., Bhandarkar, A.G., Kurkure N.V., 2007. Effect of Toxiroak® polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Veterinarski 77, 129-146.

Santin, E., Paulillo, A.C., Nakagui, L.S.O., Alessi, A.C., Maiorka, A., 2006. Evaluation of yeast cell wall on the performance of broilers fed diets with or without mycotoxins. Brazilian Journal of Poultry Science 8, 221–225.

Shareef, A.M., Omar, E.K.S., 2012. Effect of Synertox® on broiler health and performance during aflatoxicosis. Iraqi Journal of Veterinary Sciences 26, 27-34.

Shehab, A.E.M., 2008. Some studies on broiler chicken nutrition. Thesis, Master of Veterinary Medicine, Benha University, Egypt.

Shi, Y., Xu, Z., Sun, Y., Wang, C. Feng, J., 2009. Effects of two different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. Turkish Journal of Veterinary and Animal Science 33, 15–20.

Shotwell, O.L., Hessltine, C.W., Staublefield, R.D., Soreson, W.G., 1966. Production of aflatoxins on rice. Appl. Microbial. 14, 425-442.

Surai, P.F., Mezes, M., 2005. Mycotoxins and immunity. Theoretical consideration and practical applications. Praxis Veterinaria 53, 71-88.

Trenk, H.L., Butz, M.E., Chu, F.S., 1971. Production of ochratoxins in different cereal products by *Aspergillus ochraceus*. Applied Microbiology 6, 1032-1035.

Verma, J., Johri, T.S., Swain, B.K., Ameena, S., 2004. Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. British Poultry Science 45, 512-518.

Vilar, E.A., Stamford, T.L.M., Bastos, S.T.G., Filho, E.V., Souza, E.L., 2008. Total aflatoxin and ochratoxin A in the liver, kidneys and plasma of experimentally contaminated chickens. Spanish Journal of Agricultural Research 6, 373-377.

Wang, R.J., Fui, S.X., Miao, C.H., Feng, D.Y., 2006. Effects of different mycotoxin adsorbents on performance, meat characteristics and blood profiles of avian broilers fed mold-contaminated corn. Asian Australian Journal of Animal Science 19, 72-79.

Zain, M.E., 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15, 129-144.