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Research Article

Effect of Commercial Toxin Binder, Native Probiotic Strains, Cell Wall Yeast and Aluminosilicate in Diets Contaminated with Aflatoxin, on the Expression of *GOT2, CYP450 1A5* Genes and Serum Concentrations of Liver Enzymes in Broiler Chickens

Mohsen BARATI [1] Mohammad CHAMANI [1] Seyed Naser MOUSAVI [2]
Seyed Abdollah HOSEINI [3] Maryam Taj Abadi EBRAHIMI [4]

[1] Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, IRAN
[2] Department of Animal Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Tehran, IRAN
[3] Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization, Karaj, IRAN
[4] Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, IRAN

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Abstract

This study was conducted to investigate the effect of cell wall yeast, aluminosilicate and some probiotic strains in diets contaminated with aflatoxin, on the expression of *GOT2, CYP450 1A5* genes and serum concentrations of liver enzymes including GGT, ALT, AST and LDH in broilers. 400 seven-days old chicks from strain Cobb 500 were used as a completely randomized design with 8 treatments, 5 replications. Treatments were included: 1- Negative control (NC), 2- NC + 1 mg/kg aflatoxin or positive control (PC), 3- PC + 1 kg/ton Toxeat® (a toxin binder), 4- PC + *Lactobacillus strains* (L), 5- PC + *Bacillus subtilis* JQ_{618} strain (B), 6- PC + *Saccharomyces cerevisiae*'s cell wall (Y), 7- PC + [PC+ BLY) B+ L+ Y(], 8- PC + Hydrated sodium calcium Aluminosilicate (HA). The serum concentrations of LDH, ALT, GGT and AST were increased in PC group at 42d ($P<0.05$). Tox®, L and BLY reduced serum levels of AST ($P<0.05$). Increased serum concentration of GGT was observed in PC treatment, decreased by HA, Y, B and L treatments ($P<0.05$). The results showed the upregulation of *GOT2, CYP450 1A5* in PC group. But Y, B, L and Tox® reduced the expression of *GOT2*. The groups receiving aflatoxin adsorbent compounds reduced the adverse effects of aflatoxin on increasing the expression of *CYP450 1A5*.

Keywords: *Aflatoxin, Broiler Chickens, Gene Expression, Liver Enzymes, Probiotic strains, Toxeat®*

Broiler Tavuklarda Aflatoksin ile Kontamine Diyette Ticari Toksin Bağlayıcı, Doğal Probiyotik Türleri, Maya Hücre Duvarı ve Aluminosilikatın *GOT2* ve *CYP450 1A5* Gen Ekspresyonları İle Karaciğer Enzimlerinin Serum Konsantrasyonları Üzerine Etkisi

Özet

Bu çalışma, broiler tavuklarda aflatoksin ile kontamine diyette maya hücre duvarı, aluminosilikat ve bazı probiyotik türlerinin *GOT2* ve *CYP450 1A5* genlerinin ekspresyonları ile GGT, ALT, AST ve LDH gibi karaciğer enzimlerinin serum konsantrasyonları üzerine etkisini araştırmak amacıyla gerçekleştirildi. 400 adet Cobb 500 yedi günlük civciv rastgele düzen içinde 8 uygulama ve 5 tekrar üzere kullanıldı. Uygulamalar şu şekilde gerçekleştirildi: 1- Negatif kontrol (NC), 2- NC + 1 mg/kg aflatoksin veya pozitif kontrol (PC), 3- PC + 1 kg/ton Toxeat® (toksin bağlayıcı), 4- PC + *Lactobacillus* türleri (L), 5- PC + *Bacillus subtilis* JQ618 türü (B), 6- PC + *Saccharomyces cerevisiae* hücre duvarı (Y), 7- PC + [PC+ BLY)B+ L+ Y(], 8- PC + Hidratlı sodyum kalsiyum Aluminosilikat (HA). LDH, ALT, GGT ve AST serum konsantrasyonları PC grubunda 42. günde arttı ($P<0.05$). Tox®, L ve BLY AST serum seviyesini düşürdü ($P<0.05$). PC uygulanan grupta artmış serum GGT konsantrasyonu gözlemlenirken HA, Y, B ve L uygulamaları bu seviyeyi düşürdü ($P<0.05$). Elde edilen sonuçlar, PC grubunda *GOT2* ve *CYP450 1A5* upregulasyonunu gösterdi. Ancak, Y, B, L ve Tox® GOT ekspresyonunu azalttı. Aflatoksin absorbe eden madde ilave edilen gruplarda *CYP450 1A5* ekspresyonu artarak aflatoksin tarafından oluşturulan olumsuz etkiler azaltılmıştır.

Anahtar sözcükler: *Aflatoksin, Broiler Tavuk, Gen Ekspresyonu, Karaciğer Enzimleri, Probiotik türleri, Toxeat®*

İletişim (Correspondence)
+98 912 3221336
m.chamani@srbiau.ac.ir

INTRODUCTION

Mycotoxins are secondary metabolites of fungi which the possibility of their presence in foods can be provided by the conditions of production, transportation and incorrect storage. Aflatoxins are a group of mycotoxins that are produced by certain fungal species, especially *Aspergillus flavus* and *Aspergillus parasiticus* [1]. Aflatoxin B_1 has the most biological activity. Aflatoxin and Aflatoxicosis due to the consumption of contaminated diets in poultry are accompanied with symptoms such as decreased performance, liver damages and immunosuppression [2]. The investigations revealed that Cytochrome P450 enzyme[1] produced by *CYP1A5* and CYP3A37 genes is specifically responsible for the conversion of aflatoxin to the other metabolites at in *in vitro* and *in vivo* conditions [3,4]. CYP450 1A5 enzyme has high affinity for binding and metabolizing the metabolites of aflatoxin as well as the detoxification of AFM_1 [5]. Aflatoxin metabolites can be attached to the DNA and RNA and changed the level of gene expression [6]. Exposure to aflatoxin in poultry causes changes in liver enzymes gene expression levels including Xenobiotic neutralizers, cell cycle regulators, oxidative stress, DNA damages recovery, amino acid metabolizers, cell proliferation, immunity and fatty acids metabolism [7]. Aflatoxin contaminated diet leads to disturbance of the natural process of enzyme gene expression, one of these enzymes is AST that *GOT2* gene is responsible for its production. AST (*GOT2* gene expression product) is responsible for catalyzing the reversible transfer of α- amine between aspartate and glutamate [8]. The upregulation of *GOT2* affected by aflatoxin consumption causes to increase serum levels of AST, which this increment causes damages to the liver, kidneys and heart [9,10]. Researchers have been pointed out increasing the concentration of AST, ALT, LDH in the presence of aflatoxin in diets for broilers [10].

Since prevention from aflatoxin contamination is often impossible, so different methods of detoxification of mycotoxins is highly considered [6]. Among the various methods of detoxification, the impact of aluminosilicate compounds efficiency in reducing the effects of aflatoxin in *in vitro* and *in vivo* conditions has been proved [11]. Due to the limitations of aluminosilicate consumption, using biological compounds is on the agenda of nutritionists because of their numerous advantages. It has been proved that using diets based on the probiotic compounds especially *Lactobacilli* in poultry diets, have the ability to reduce aflatoxin effects on the gene expression of liver enzymes such as genes for amino acids and fat metabolizing enzymes [12]. Gao *et al*.[13] found the high ability of *Bacillus Subtilis* for reducing the effects or disable B_1, M_1 and G_1 aflatoxins. Using 0.5 to 1 gram of glucomannan *Saccharomyces cerevisiae* per kg of aflatoxin-contaminated diet reduced the histological changes in the liver, kidney, spleen and bursa fabricius [14].

[1] *Chicken Cytochrome P450 1A5*

In this study the expression of *GOT2* (Gallus gallus Aspartate transaminase), *CYP450 1A5* genes and serum concentrations of liver enzymes including Gammaglutamyl transpeptidase (GGT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH), also the possible effects of various organic, mineral and biological additives were investigated under the influence of aflatoxin contaminated diets in broiler chickens because of great economic losses of aflatoxins to the poultry industry.

MATERIAL and METHODS

Aflatoxin Production

The required aflatoxin was produced by contaminating rices with *Aspergillus flavus* (PTCC 5004) (Prepared from the microbial treasures of Scientific and Industrial Research Organization of Iran). For this purpose, 1 mL of *Aspergillus flavus* suspension, containing 7×10^6 fungal spores were added to rice and were cultivated for 7 days at 28°C temperature. After fungal growth and toxin production, rice was dried by using oven at 70°C and finally its powder was obtained. Qualitative and quantitative aflatoxin content in rice powder was measured by HPLC (Waters Alliance e2695 equipped with 2475 fluorescence detector, USA) [15,16]. The content of aflatoxin in rice samples were G_2: 8 ppm, G_1: 126 ppm, B_2: 22 ppm, B_1: 289 ppm and the total concentration of aflatoxins was 445 ppm. In order to prepare the experimental diets, rice powder with a certain composition and level of aflatoxin was added and mixed to the basal diet up to a concentration of 1 mg/kg of aflatoxin B_1. According to the extent permitted of aflatoxin in poultry diets (0.02 mg/kg of feed) so contamination of the basal diet was 50 times of the extent permitted [17]. The basal diet had no aflatoxin.

Adsorbent Materials

Adsorbent compounds investigated in this study were including:

1) Toxeat®, a commercial toxin binder based on biological compounds, produced by Tak Gene Company (Tehran - Iran) contains *Lactobacilli*, *Bacilli* and Iranian native cell wall yeast based on Aluminosilicate (as a career)

2) *Lactobacilli* strains including *Lactobacillus* TD_4, *Lactobacillus* TD_{15}, *Lactobacillus* TD_3, *Lactobacillus* TD_{10} and the amount of each of the bacteria was 1×10^7 CFU/g

3) 1×10^7 CFU/g of *Bacillus Subtilis* JQ_{618}

4) *Saccharomyces cerevisiae*'s cell wall produced by Tak Gene Company (Tehran - Iran) as an organic component

The amount of mannan and glucan in the used cell wall yeast were analyzed by Tak Gene Zist Company and were respectively 430.26 mg/kg and 569.73 mg/kg.

5) Hydrated sodium calcium Aluminosilicate as a mineral component (HA)

Isolated strains of bacteria which are commercial products of Tak Gene Zist Company (Tehran - Iran) were selected among a collection of over 200 indigenous microorganisms of Iran based on their high ability of detoxification.

Experimental Treatments

In this study a total of 400 seven-day old chicks from a broiler breeder strain (Cobb 500) were used. The chicks were randomly divided into 8 treatments, 5 replications and 10 chicks in each replication (in equal proportions of male and female) and were fed with experimental diet from 7 to 42 days of age. The experimental treatments were as follows:

- Negative control group: Basal diet, (NC.)
- Positive control group: Basal diet + 1 mg/kg of feed aflatoxin, (PC.)
- Group 3: PC + 1 kg/ton of feed Toxeat®, (a commercial toxin binder) (Tox®)
- Group 4: PC + *Lactobacillus* TD_3, TD_4, TD_{10}, TD_{15} strains, (L.)
-Group 5: PC + 1 kg/ton of feed *Bacillus Subtilis* JQ_{618} strain, (B.)
- Group 6: PC + 1 kg/ton of feed *Saccharomyces cerevisiae*'s cell wall, (Y.)
- Group 7: PC + 1 kg/ton of feed the content of treatments L, B and Y, (BLY.)
- Group 8: PC + 15 kg/ton of feed Hydrated sodium calcium Aluminosilicate, (HA.)

The used feedstuffs were sent to the Tak gene laboratory for analyzing compounds by using NIR method. Diets were prepared for starter (7-14 days of age), grower (15-28 days of age) and finisher (29-42 days of age) periods. The amounts of feedstuffs and nutrient composition of the experimental diets are shown in *Table 1*. The chicks were vaccinated against infectious bronchitis, Newcastle and Gumboro but no medical program has run during the entire experimental period. In all process of the experiment the temperature and lighting control systems have been set based on the broiler husbandry instruction manuals (Cobb 500). During the experimental period, the environmental conditions were the same for all groups and given *ad libitum* access to the water and feed. The bird care and used procedures were approved by standard committee of Karaj Animal Science Research (approval date: 19/02/2016; No: 10036).

Evaluation of Liver Enzymes

At the end of the experiment (42 days of age) 3 mL of blood was taken from each chick, that way 2 randomly chicks from each pen (replicate) and 10 chicks of each treatment were selected to measure the serum levels of liver ezymes. The serum was separated for measuring the levels of GGT, ALT, AST and LDH. Analysis of serum samples was carried out with ELISA technique by using Elx 800 ELISA Reader, BioTek and commercial kits for poultry (ALT ELISA Cat. No.: MB S266858, AST ELISA Cat. No.: MB S740867, GGT ELISA Cat. No.: MB S934604, LDH ELISA Cat. No.: MB S736903) produced by MyBiosource American company [10].

Evaluation of CYP450 1A5, GOT2 Gene Expression

At the end of experiment 3 chicks from 3 replications of each treatment were selected and the birds were anesthetized by carbon dioxide gas and slaughtered through the cervical vertebra movement. Their liver samples were taken immediately after slaughter and transferred to the laboratory in vicinity of ice. A total amount of 30 mg of liver tissue were measured and all cellular RNA content of liver samples was extracted by using the instructions of GeneJetTM RNA Purification Thermo kit (Fisher Scientific, USA, Cat no. K0731) instantly [18,19]. Then the amount of extracted RNA was measured by NanoDropTM 1000 spectrophotometer (Thermo Fisher Scientific, USA) [20]. To perform RT PCR, cDNA was obtained by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA, Cat no. K1621), according to the manufacturer instructions [18]. To carry out the quantitative RT-PCR, the volume of reaction was set on 25 uL, in which cDNA concentration and the final concentration of SYBR green were respectively set on 6.25 and 0.25 ng/µL [21]. In this study, β-actin was used as a housekeeping control that under these conditions

Table 1. *Composition and analysis of basal diets (%)*

Ingredients %	Starter (1-14 days)	Grower (15-28 days)	Finisher (29-42 days)
Corn	55	46.08	45
Soybean meal	39	29	32.6
Soybean oil	1	1.05	3.8
Wheat	-	20	15
Oyster shell-flour	1.3	1.17	1
NaCl	0.2	0.2	0.1
Premix[1]	3.5	2.5	2.5
Analysis			
AME (kcal/kg)	2995	2987	3121
Crude protein (%)	22.58	19.25	20.23
Digestible lysine (%)	1.156	0.923	0.994
Calcium (%)	1.068	0.87	0.812
Total Available phosphorus (%)	0.546	0.42	0.424
Digestible methionine (%)	0.528	0.423	0.434
Digestible methionine + cysteine (%)	0.834	0.698	0.717
Na (%)	0.212	0.187	0.145
Cl (%)	0.248	0.225	0.163

[1] Permix Vitamin and Mineral analysis: *Vitamin A: 1000 IU; vitamin D_3:3500 IU; vitamin E: 40 IU; vitamin K_3: 2 mg; vitamin B_1: 2 mg; vitamin B_2: 5 mg; vitamin B_3: 35 mg; vitamin B_5: 13 mg; vitamin B_6: 1.5 mg; vitamin B_{12}: 0.01 mg; vitamin B_9: 1.6 mg; Biotin: 1.5 mg; I: 1.25 mg; Cu: 16 mg; Zn: 100 mg; Se: 0.3 mg; Mn: 120 mg; Fe: 40 mg; Choline chloride: 350 mg; Betaine:150 mg; ME (kcal/kg) 2837; CP: 12.5%; TSAA: 6.3%; Dig Lys: 1.8%; Dig Thr: 0.85%; Ca: 21.88%; Na: 2.45%; AP: 11.5%*

no changes will occure in its expression levels [22] and PCR was designed for 80 cycles so that 15 sec at 94°C for Denaturation, 30 sec at 60°C for Annealing and 30 sec at 72°C for Elongation were intended. In the final stage, the results of fluorescence were collected and investigated by SYBR Green combined with the expanding DNA. In this research primer sequences of *GOT2* and *CYP1A5* genes were respectively designed according to the previous reports [23,24] and β-actin gene was designed based on both mentioned reports *Table 2*. Each sample was performed in 3 replications. The amount of ΔCT was obtained by subtracting the cycle threshold of sample from the amount of CT for β-actin gene. The group with the highest amount of ΔCT means it has the lowest gene expression [21]. The amount of ΔCT was calculated by Livak and Schmittgen method [25].

Statistical Analysis

The results of the experiment were analyzed as a completely randomized design with 8 treatments and 5 replications per treatment. Data were analysed by using the GLM procedures SAS version 9.2 [26] and differences between the treatments were compared by Duncan's multiple range test and the value of significance level was 0.05.

of aflatoxin on ALT serum levels with less efficiency than the treatment fed the cell wall yeast (P<0.05). Also Hydrated sodium calcium Aluminosilicate could inhibit the adverse effects of aflatoxin on increasing ALT serum levels but this effect was more limited in comparison with other investigated compounds (P<0.05). Studying the changes in serum levels of GGT showed the serum level increment of this enzyme in the positive control group (P<0.05). L., B., Y. and HA. groups were controled the incremental effects of aflatoxin on serum levels of GGT (P<0.05) whereas Tox® and BYL. groups had a lower effect on inhibiting adverse effects of aflatoxin in comparison with other groups (P<0.05).

Evaluation the Expression of GOT2 and CYP450 1A5

The results of the Tox®, L., B., Y., BYL. and HA. effects on the expression of *GOT2* and *CYP450 1A5* are presented in *Fig 1-4*. As it can be observed in *Fig. 1*, the greatest increase was for CYP450 1A5 gene expression in PC. group (P<0.05) and the other treatments showed the lowest rate of increase in the expression of this gene compared to endogenous control (P<0.05) and there were no significant differences between other treatments

Table 2. *Sequences of the investigated genes and Housekeeping gene*

Target	Accession No (GenBank)	Sequence (5'-3')	Product Length	Source
GOT2	M12105	S: ATCCTCATCCGTCCCATGTA A: GTCAGTGATGTGCTGCCAGT	201 bp	Rosebrough *et al.*[23]
CYP1A5	XM015278761	S: TCACCATCCCGCACAGCA A:AAGTCATCACCTTCTCCGCATC	201bp	Zhang *et al.*[24]
-actinβ	L08165	S: TGCGTGACATCAAGGAGAAG A: TGCCAGGGTACATTGTGGTA	300bp	Li *et al.*[22]; Rosebrough *et al.*[23]; Zhang *et al.*[24]

***S:** Sense, **A:** Anti-sense*

RESULTS

According to the the results, increasing the concentration of LDH and AST enzymes was observed in PC. group (P<0.05), but no differences were observed for serum concentrations of these two enzymes in other treatments (P>0.05) *(Table 3)*. However the serum level differences of these two enzymes was significant between PC. and other groups (P<0.05). So organic, mineral and biological compounds could well prevent the negative effects of aflatoxin on serum levels of LDH and AST. This result was obtained from the comparison of adsorbent receiving along with aflatoxin groups and NC. group that the results of this groups had no significant differences with NC. group (P>0.05). The results showed that serum levels of ALT were increased in PC. group significantly (P<0.05). Aflatoxin inhibitor compounds were able to control the increment of serum enzymes levels in all groups in comparison with positive control group. Such that there were no significant differences between NC. and Y. groups (P>0.05). Tox®, L., Y., BYL. and B. groups were inhibited the negative effects

Table 3. *Effect of Tox®, L., B., Y., BYL. and HA. on serum concentrations of liver enzymes in broiler chickens (Cobb 500) fed by diets contaminated with aflatoxin at 42 days of age*

Treatment	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)
NC	419.38±0.13^{b}	43.8±1.26^{b}	14.8± 0.78^{d}	7.42±0.41^{c}
PC	420.67±0.41^{a}	65.1±6.64^{a}	20.2±1.04^{a}	9.02±0.54^{a}
Tox®	419.78±0.15^{b}	47.8± 1.5^{b}	16.8±0.41^{c}	8.46±0.36^{ab}
L.	419.70±0.06^{b}	47.0±0.64^{b}	16.3±0.79^{c}	8.14±0.29^{b}
B.	419.72±0.08^{b}	47.2±0.83^{b}	16.2±0.25^{c}	8.02±0.51^{b}
Y.	419.47±0.07^{b}	44.7±0.71^{b}	15.7±0.35^{cd}	8.07±0.43^{b}
BLY	419.61±0.13^{b}	46.1±1.29^{b}	16.2±0.41^{c}	8.42±0.52^{ab}
HA	419.61±0.72^{b}	47.7±5.06^{b}	18.1±1.65^{b}	8.17±0.39^{b}
SEM	0.08	1.12	0.3	0.1
P-value	0.0001	0.0001	0.0001	0.0005

***GGT:** γ-glutamyltransferase, **ALT:** Alanine amino-transferase, **AST:** aspartae amino-transferase, **LDH:** Lactate dehydrogenase **NC:** Negative control; **PC:** positive control*

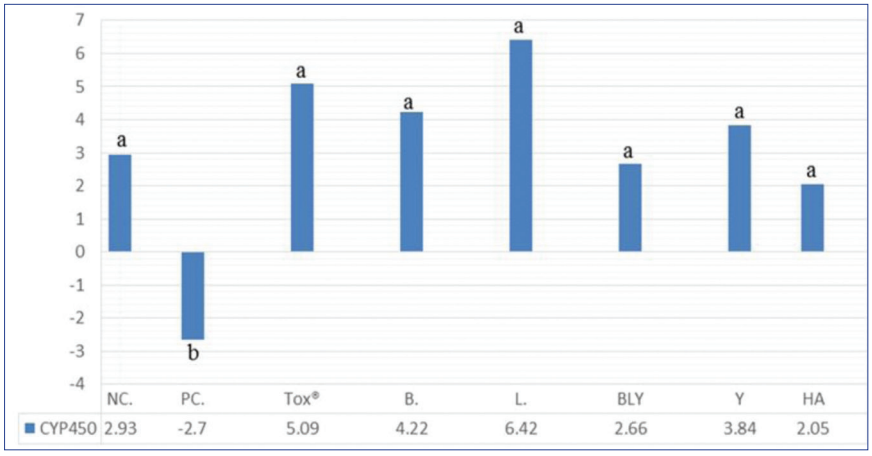
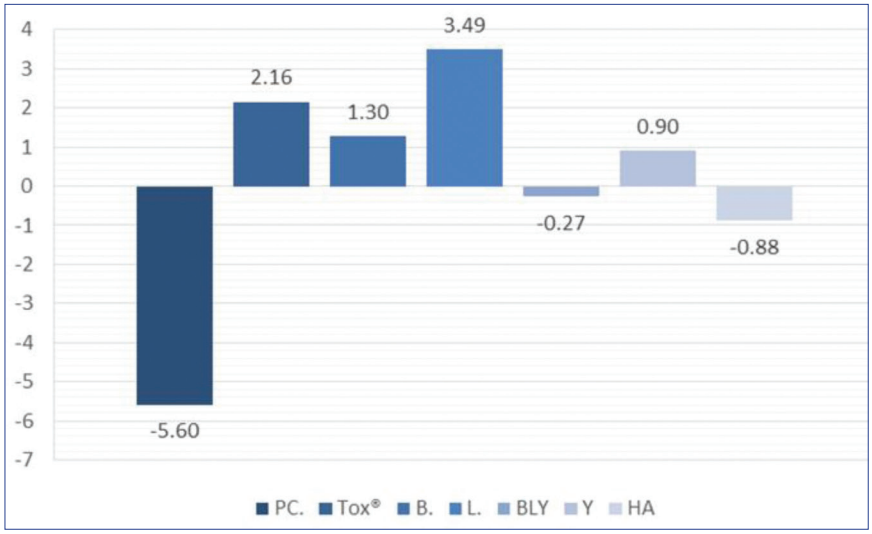


Fig 1. Effect of Tox®, L., B., Y., BYL. and HA. on *CYP450 1A5* liver enzyme gene expression in broiler chickens (Cobb 500) fed by diets treated with aflatoxin, figure has been normalized by β-actin gene (Δct), P value = 0.0152 - SEM = 0.73

Fig 2. Effect of Tox®, L., B., Y., BYL. and HA. on *CYP450 1A5* liver enzyme gene expression in broiler chickens (Cobb 500) fed by diets treated with aflatoxin, compared to control group without aflatoxin (ΔΔct)



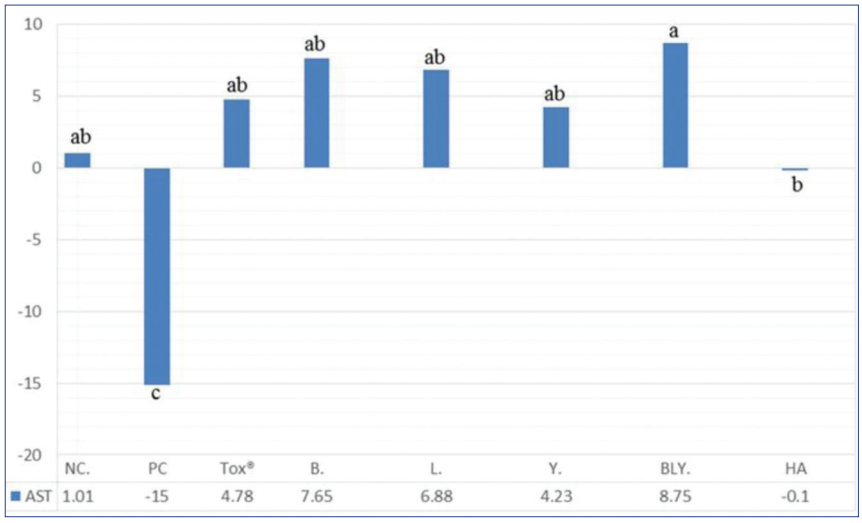


Fig 3. Effect of Tox®, L., B., Y., BYL. and HA. on *GOT2* liver enzyme gene expression in broiler chickens (Cobb 500) fed by diets treated with aflatoxin, figure has been normalized by β-actin gene (Δct), P value = 0.0008 - SEM = 1.7

received various kinds of additives compared with NC. treatment (P>0.05).

No significant treatment differences were observed between groups that received various types of additives compared with NC. group (P>0.05) but in comparison with PC. the differences were highly significant (P<0.05). Numerical comparisons of the groups received aflatoxin inhibitor indicated that L. and Tox® treatments in *CYP450 1A5* gene expression compared to endogenous control had the minimum changes. In comparing the results of *CYP450 1A5* gene expression with NC. group (ΔΔct), the highest in-

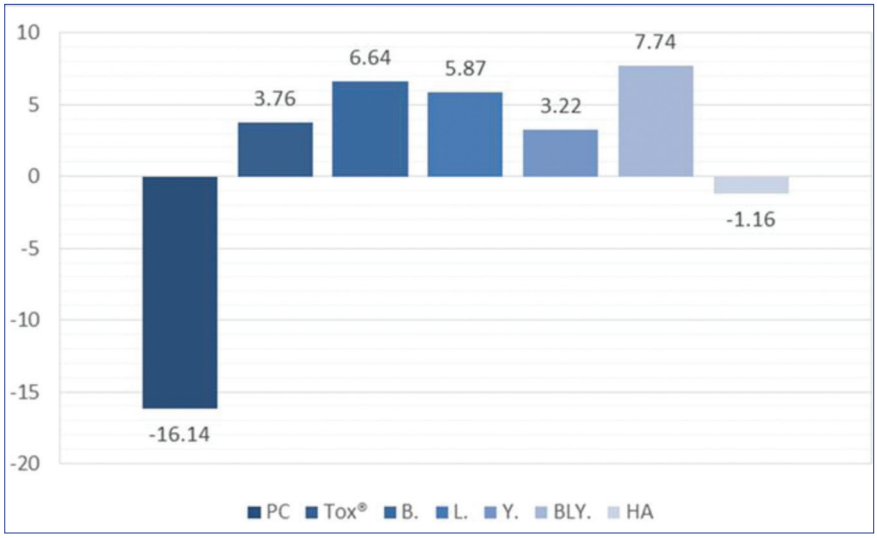


Fig 4. Effect of Tox®, L., B., Y., BYL. and HA. on *GOT2* liver enzyme gene expression in broiler chickens (Cobb 500) fed by diets treated aflatoxin, compared to control group without aflatoxin (ΔΔct)

crease of gene expression was in PC. Group and then was observed in HA. group *(Fig. 2)*. Also the results showed that the groups receiving an aflatoxin adsorbent in comparison with NC. group were able to control the effects of aflatoxin, but in comparison (ΔΔct) between all groups, L. and Tox® groups could more control the upregulation of *CYP450 1A5* gene expression.

The results of *GOT2* gene (Δct) are shown in *Fig. 3*. The PC. treatment significantly upregulated the expression of *GOT2* in comparison with endogenous control ($P<0.05$) and there were no significant differences between the other treatments ($P>0.05$), however BLY. group had the lowest gene expression and in terms of *GOT2* gene (Δct) B., L., Y., Tox® and NC. groups did not show any significant differences with each other ($p>0.05$). HA. treatment could inhibit the adverse effects of aflatoxin on upregulation of *GOT2* gene expression compared to endogenous control, but it had less ability in comparison with the other groups contain an inhibitor factor. The comparison of (ΔΔct) for *GOT2* gene showed that B., Y., L., BYL. and Tox® groups could control the adverse effects of aflatoxin on the expression of this gene, however among all groups BLY. had more effectiveness efficiency. The highest increase for *GOT2* gene expression (ΔΔct) was in PC. group. The results of (ΔΔct) are given in *Fig. 4*.

DISCUSSION

Aflatoxin has been considered as a threat to poultry nutrition from almost 50 years ago till now. This contamination makes extensive lesions in poultry and heavy economic losses to this industry by weakening the immune system and performance and also it is a threat to human health as a consumer of contaminated protein products [27]. Aflatoxins are the reason of a wide range of metabolic damages, including, liver lesions, changes in genes expression especially liver enzymes and genes involved in the metabolism of this toxin [28]. Measuring the amount of serum concentrations of liver enzymes is a good way to assess liver damages [29]. Some researchers showed that consuming aflatoxin will increase the concentration of liver enzymes especially AST, ALT and LDH [10]. In this study, an increment in serum levels of liver enzymes (ALT, AST, LDH) was observed in PC. group. Shi *et al.*[30] reported the increasment of ALT, AST and GGT enzymes due to feeding a diet contaminated with aflatoxin to broiler chickens. Researches have been proved that increased serum levels of GGT and AST, is used as an indicator for investigating liver and kidney toxicity [31]. According to what was mentioned, increased serum levels of these two enzymes (GGT, AST) in PC. group can be attributed to the liver and kidney damages in broilers fed with aflatoxin. According to the fact that many of the metabolic activities (fat and protein metabolism) and immunity (production of cytokines, chemokines, maturation of immune cells) are related to liver, therefore damage to this tissue leads to a disturbance in the immunity system function and metabolic pathways of fat and protein. All adsorbents used in this study caused to control the adverse effects of aflatoxin on increasing serum concentration of AST and GGT, but L., B., Y. and HA. groups were jointly showed better results for both mentioned enzymes. The findings of this study were in agreement with the results of Aravind *et al.*[32]. In another study, 14% increase for AST and 17% increase for ALT serum levels were observed in chickens fed aflatoxin contaminated diets [33]. One of the symptoms for hyperplasmy is a significant increase in serum levels of ALT and GGT [34]. Kasmani *et al.*[10] reported that using *Bacillus* will control aflatoxin effects on increasing liver enzyme concentrations (AST, ALT, LDH). Also in another research [32], using cell wall yeast could control the aflatoxin effects on increasing liver enzyme levels (ALT, AST, GGT) that these results are consistent with our findings for B. and Y. groups. Investigating the results of changes in liver enzymes gene expression indicated that increase in gene expression related to interleukins, liver enzymes and especially enzymes involved in the metabolism of aflatoxin occurs

under the influence of aflatoxin [28]. It seems that changes in gene expression levels in the liver occurs by using aflatoxin contaminated diets therefore acute and chronic aflatoxicosis occur due to the liver proliferation[2] [12]. Studies on the impact of diets contaminated with aflatoxin on gene expression in liver enzymes showed that the presence of toxin in the diet causes to upregulation of *CYP450 1A* gene. This cytochrome exists in chickens and turkeys and is consists of *CYP450 1A4* and *CYP450 1A5* subfamilies. Reports indicated that *CYP450 1A5* expression increases more faced with aflatoxin [35]. Yarru *et al*.[7] expressed that the expression of *CYP450* has increased by effect of aflatoxin and this causes to oxidative stress and in continue liver damage and death occur in poultry. The results of current research (PC. group) in the field of *CYP4501A5* gene expression are completely corresponded with those of previous studies mentioned. Groups L. and Tox® additives as the aflatoxin adsorbents, caused to control the upregulation of *CYP450 1A5* gene. Increasing *CYP450 1A5* expression is important because it causes to increase oxidative stress and consequently death occurs in poultry [7]. So it appears that control the expression of *CYP450 1A5* and adjusting its expression near to its level in negative control treatment under the influence of studied compounds in the diets has been protected chickens against oxidative stress caused by aflatoxin.

AFBO production was affected by *CYP450* and high affinity of this compound for binding to DNA and RNA that causes to damage to DNA and create carcinogens [36]. Control the gene expression of *CYP450 1A5* can help to reduce damages to DNA, which was observed in all treatments and of course with the higher capacity and efficiency in L. and Tox® groups. According to obtained data, chickens fed with *Lactobacillus* and Toxeat® commercial combination showed higher ability to inhibit adverse effects of aflatoxin and possible damages to DNA. More study were on serum level of liver enzyme (AST) and fewer reviews have been conducted on gene expression of this enzyme, but since investigating effects of Xenobiotics such as aflatoxin on liver enzymes, through the study of gene expression in liver enzymes or their serum concentrations or catalytic activity is possible [24], so the result of serum level of this enzyme was compared with other studies. In several experiments elevation in serum level AST was reported in broiler chickens fed diets containing aflatoxin [10]. Increasing *GOT2* gene expression (the producer of aspartate aminotransferase) was observed in PC. group of the present study in comparison with endogenous control gene and NC.

Kasmani *et al*.[10] reported that the addition of *Bacillus* to the diets contaminated by aflatoxins reduced serum level AST. Also the researchers expressed a reduction in serum level of AST by adding a commercial toxin binder containing *Saccharomyces cerevisiae* cell wall to the diets contaminated with aflatoxin in comparison with the group without additives [37]. Monson *et al*.[38] stated that probiotics in diets contaminated with aflatoxin reduces gene expression of liver enzymes and proteins. The result of *GOT2* gene expression in PC. group was consistent with the findings of Sridhar *et al*.[39] which showed the increasing of AST serum levels by consuming aflatoxin contaminated diets. Also L., B., Y. and BLY. groups in comparison with β-actin control gene (Δct), could well inhibit aflatoxin effects on *GOT2* upregulation and even there were no significant differences with NC. group. But between all groups receiving a factor as toxin adsorbent, BLY. treatment revealed more ability to inhibit aflatoxin effects on *GOT2* gene expression. The results of ΔΔct demonstrated that L., B. and BLY. groups could control *GOT2* gene expression with a better efficiency. What mentioned is in agreement with the findings of Kasmani *et al*.[10] and Yildirim *et al*.[37], in the field of AST serum levels.

According to the results of *GOT2* and *CYP450 1A5* expression and compare (Δct) and (ΔΔct) of these genes and also serum levels of liver enzymes (ALT, AST, LDH, GGT), it can be concluded that using probiotics and prebiotics in diets contaminated with aflatoxin caused to control the adverse effects of this toxin on increasing the gene expression of serum levels of liver enzymes. Compare the results of L., B., Y. groups with BLY. and Tox® groups indicated that applying several biological factors together, due to the synergistic effects of these compounds together for control negative effects of aflatoxin were efficient. Review the results of HA. group with B., L., Y. and BLY. groups determined that mineral factors have a less ability than probiotics and prebiotics to control the effects of aflatoxin on poultry, however by observing the results of Toxeat® which is a commercial biologic product based on aluminosilicate, it can be concluded that the use of HA. alongside the biological factors can help the absorption of aflatoxin in the presence of biological factors. Control the effects of aflatoxin and its absorption by Tox® and BLY. groups reduce damages to the liver and this leads to performance improvement and reduced mortality.

2 Reproduction and increment of cells

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