Effect of Commercial Toxin Binder, Native Probiotic Strains, Cell Wall Yeast and Aluminosilicate in Diets Contaminated with Aflatoxin, on the Expression of \textit{GOT2}, \textit{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

Article Code: KVFD-2017-18022    Received: 14.05.2017    Accepted: 03.08.2017    Published Online: 05.08.2017

Citation of This Article

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 \textit{GOT2}, \textit{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 + \textit{Lactobacillus} strains (L), 5- PC + \textit{Bacillus subtilis} JQ618 strain (B), 6- PC + \textit{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 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 \textit{GOT2}, \textit{CYP450 1A5} in PC group. But Y, B, L and Tox® reduced the expression of \textit{GOT2}. The groups receiving aflatoxin adsorbent compounds reduced the adverse effects of aflatoxin on increasing the expression of \textit{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 \textit{GOT2} ve \textit{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 \textit{GOT2} ve \textit{CYP450 1A5} genlerinin ekspresyonları ile GGT, ALT ve LDH gibi karaciğer enzimlerinin serum konsantrasyonları üzerine etkisini araştırmak amacıyla gerçekleştirilmiştir. 400 adet Cobb 500 yedi günlük cıvçı tıraşte düzene 8 uygulama ve 5 tekrar üzere kullanıldı. Uygulamalar şu şekilde gerçekleştiriltiler: 1- Negatif kontrol (NC), 2- NC + 1 mg/kg aflatoksin veya pozitif kontrol (PC), 3- PC + 1 kg/ton Toxeat® (toksin bağlayıcı), 4- PC + \textit{Lactobacillus} türleri (L), 5- PC + \textit{Bacillus subtilis} JQ618 strain (B), 6- PC + \textit{Saccharomyces cerevisiae}’s cell wall (Y), 7- PC + [PC+ BLY ] B+ L+ Y[, 8- PC + Hidratlı sodyum kalsiyum Aluminosilikat (HA). \textit{GOT2} ve \textit{CYP450 1A5} genlerinin ekspresyonları serumdaki GGT, ALT ve AST koncentremleri arttı (P < 0.05). \textit{Tox®}, L ve BLY hem AST hem de ALT koncentrelerini düşürdü (P < 0.05). Aflatoksin absorbesi eden madde ilave edilen grupta \textit{CYP450 1A5} ekspresyonu arttı. Ancak, Y, B ve L ve \textit{Tox®} gen ekspresyonu azaldı.

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

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

Kafkas Univ Vet Fak Derg
23 (6): 953-960, 2017
DOI: 10.9775/kvfd.2017.18022

Kafkas Üniversitesi Veteriner Fakültesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article
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 B1 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 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 AFM1 [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 [8]. 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. [12] found the high ability of *Bacillus Subtilis* for reducing the effects or disable B1, M1, and G1 aflatoxins. Using 0.5 to 1 gram of glucomanann *Saccharomyces cerevisiae* per kg of aflatoxin-contaminated diet reduced the histological changes in the liver, kidney, spleen and bursa fabricius [13].

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 7×10⁶ 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 G2: 8 ppm, G1: 126 ppm, B1: 22 ppm, B2: 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 B1. 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* TDα, *Lactobacillus* TDα1, *Lactobacillus* TDβ, *Lactobacillus* TDβ10 and the amount of each of the bacteria was 1×10⁷ CFU/g

3) 1×10⁷ CFU/g of *Bacillus Subtilis* JQ618

4) *Saccharomyces cerevisiae*’s cell wall produced by Tak Gene Company (Tehran - Iran) as an organic component. The amount of mannann 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 TD14, TD4, TD15 strains, (L.)
- Group 5: PC + 1 kg/ton of feed Bacillus Subtilis JQ15 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 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. Then the amount of extracted RNA was measured by NanoDropTM 1000 spectrophotometer (Fisher Scientific, USA) [20]. To perform RT PCR, cDNA was obtained by using RevertAid First Strand cDNA Synthesis Kit (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 μL, 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 (%)**

<table>
<thead>
<tr>
<th>Ingredients %</th>
<th>Starter (1-14 days)</th>
<th>Grower (15-28 days)</th>
<th>Finisher (29-42 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AME (kcal/kg)</td>
<td>2995</td>
<td>2987</td>
<td>3121</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>22.58</td>
<td>19.25</td>
<td>20.23</td>
</tr>
<tr>
<td>Digestible lysine (%)</td>
<td>1.156</td>
<td>0.923</td>
<td>0.994</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.068</td>
<td>0.87</td>
<td>0.812</td>
</tr>
<tr>
<td>Total Available phosphorus (%)</td>
<td>0.546</td>
<td>0.42</td>
<td>0.424</td>
</tr>
<tr>
<td>Digestible methionine (%)</td>
<td>0.528</td>
<td>0.423</td>
<td>0.434</td>
</tr>
<tr>
<td>Digestible methionine + cysteine (%)</td>
<td>0.834</td>
<td>0.698</td>
<td>0.717</td>
</tr>
<tr>
<td>Na (%)</td>
<td>0.212</td>
<td>0.187</td>
<td>0.145</td>
</tr>
<tr>
<td>Cl (%)</td>
<td>0.248</td>
<td>0.225</td>
<td>0.163</td>
</tr>
</tbody>
</table>

1. Premix Vitamin and Mineral analysis: Vitamin A: 10000 IU; vitamin D3:3500 IU; vitamin E: 40 IU; vitamin K3: 2 mg; vitamin B1: 2 mg; vitamin B2: 5 mg; vitamin B3: 35 mg; vitamin B6: 13 mg; vitamin B7: 1.5 mg; vitamin B9: 0.01 mg; vitamin B12: 1.6 mg; Biotin: 1.5 mg; folic acid: 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%
Effect of Commercial Toxin ...

RESULTS

According to 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 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 Table 2. 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: ATCCTCATCCGATCCCATGTA; A: TCCTCATCCGATCCCATGTA | 201 bp | Rosebrough et al. [23] |
| CYP1A5 | XM01527861 | S: TCACCATCCGGACAGCA; A: AAGTCATCACCTTCTCCGCATC | 201 bp | Zhang et al. [24] |
| β-actin | L08165 | S: TGGGTAGATCAAGGAGAAG; A: TGCCAGGTTACATTTGTGGA | 300 bp | Li et al. [25]; Rosebrough et al. [26]; Zhang et al. [27] |

SourceProduct LengthSequence (5’-3’)Accession No (GenBank)Target

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LDH (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>419.38±0.13 b</td>
<td>43.8±1.26b</td>
<td>14.8±0.78b</td>
<td>7.42±0.41b</td>
</tr>
<tr>
<td>PC</td>
<td>420.67±0.41 a</td>
<td>65.1±6.64a</td>
<td>20.2±1.04a</td>
<td>9.02±0.54a</td>
</tr>
<tr>
<td>Tox®</td>
<td>419.78±0.15 b</td>
<td>47.8±1.5b</td>
<td>16.8±0.41c</td>
<td>8.46±0.36a</td>
</tr>
<tr>
<td>L.</td>
<td>419.70±0.06 b</td>
<td>47.0±0.64b</td>
<td>16.3±0.79b</td>
<td>8.14±0.29b</td>
</tr>
<tr>
<td>B.</td>
<td>419.72±0.08 b</td>
<td>47.2±0.83b</td>
<td>16.2±0.25b</td>
<td>8.02±0.51b</td>
</tr>
<tr>
<td>Y.</td>
<td>419.47±0.07 b</td>
<td>44.7±0.71b</td>
<td>15.7±0.35b</td>
<td>8.07±0.43b</td>
</tr>
<tr>
<td>BLY</td>
<td>419.61±0.13 b</td>
<td>46.1±1.29b</td>
<td>16.2±0.41c</td>
<td>8.42±0.52b</td>
</tr>
<tr>
<td>HA</td>
<td>419.61±0.72 b</td>
<td>47.5±0.56b</td>
<td>18.1±1.65b</td>
<td>8.17±0.39b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.08</td>
<td>1.12</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

GGT: γ-glutamyltransferase, ALT: Alanine amino-transferase, AST: aspartate amino-transferase, LDH: Lactate dehydrogenase, NC: Negative control; PC: positive control;
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 (∆∆ct), the highest in-
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 [36]. 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 [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 increase 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 up-regulation 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 [14]. 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 Toxate® 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 synergic 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.

REFERENCES


2 Reproduction and increment of cells
Effect of Commercial Toxin ...

10.1159/000218748


