The Effects of Sodium Humate and Aflatoxin B1 on Body Weight of Broiler Chicks

M. Skalická1* and B. Koréneková2

1 Institute of Nutrition, Dietetics and Feed Production, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic
2 Department of Hygiene and Food Industry Technology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

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*Correspondence E-mail: magdalena.skalicka@uvlf.sk
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ABSTRACT

The objective of this study was to investigate the protective effect of dietary natural sorbent-sodium humate (HuNa) on the prevention of aflatoxin B1 (AFB1) toxicity in the broiler chicken. Seventy-two (72) broiler hybrid Hydro birds were randomly divided into 4 groups: control group (G1) and 3 experimental groups (G2-G4) with addition of HuNa (G2); only AFB1 (G3) and HuNa; and AFB1 (G4). HuNa was added to a complete feed mixture daily for broiler chicks (1 g HuNa/100 g feed mixture) and AFB1 at the concentration of 25 μg.kg-1 of b.w. Parameters evaluated were body weight and serum levels of alkaline phosphatase (ALP). AFB1 and HuNa were applied for broilers from 28 days of age. Statistically significant decrease activity of ALP (P≤0.05) was recorded in group G3 of broilers from day 42 and 56 of age onwards in comparison to group G1. However, statistically increased activity of ALP (P≤0.05) was observed in group G4 in comparison to control group G1. The body weight of broilers was lower by about 180 g after exposure to AFB1 (G3) compared to control (G1). Also, the addition of HuNa increased the body weight of broilers by about 26.67 g at the end of the experiment in comparison to control group. Statistically significant increase (P≤0.001) in body weight was recorded for group G3 and G4 on 56th day of age, compared to 28 days. The results of our experiment showed that HuNa has a positive effect on the growth of broilers. And it could have been a suitable natural supplement for growing broilers against the adverse effects of aflatoxins.

KEY WORDS aflatoxin B1, alkaline phosphatase, body weight of broilers, broilers, sodium humate.

INTRODUCTION

Of all animals, the poultry is a type that is particularly sensitive to the toxic effects of aflatoxin from contaminated feed. However, among fowl there is wide variability in specific species sensitivity to these mycotoxins. Comparative toxicological studies in avian species have shown that ducklings and turkey poult are the most sensitive species to aflatoxins, quails show intermediate sensitivity, whereas chickens are the most resistant (Diaz et al. 2008). The aflatoxins are a group of secondary metabolites produced by certain strains of fungi, Aspergillus flavus and Aspergillus parasiticus species (Pittet, 1998; Denli and Okan, 2006). While there are a number of aflatoxins such as B1, G1, B2, G2, aflatoxin B1 is the most prevalent and highly hepatotoxic and carcinogenic in both human and animals (Williams et al. 2004; Denli et al. 2004; Sadeghi et al. 2013). The aflatoxins B1, G1, B2, and G2 are structurally similar secondary fungal metabolites that can be produced by Aspergillus flavus or Aspergillus parasiticus (Rauber et al. 2007). Avoidance of contaminated feed is rarely feasible and feeds that contain relatively low concentrations of AFB1 may still have deleterious effects on sensitive species such as poultry. Poultry are suggested to be the species...
most sensitive to its toxic effects (Doerr et al. 1983; Denli and Okan, 2006). In poultry even small amounts of AFB1 can cause a reduction in growth performance, hatchability and an increased susceptibility to bacterial and viral diseases and severe hepatotoxicosis (Kubena et al. 1998; Verma et al. 2003). Liver damage, decreased egg production and suppressed immunity have been noted in animals consuming relatively low dietary concentrations of aflatoxin. Liver is the target organ for aflatoxicosis because this is where most aflatoxins are biologically activated to the reactive 8, 9-epoxide form, which is capable of binding to proteins (Okan et al. 2004). These alterations produce changes in the biochemical parameters, mainly in enzyme levels and have been well documented in broiler chickens (Rosa et al. 2001), turkeys (Klein et al. 2002) quails (Oliveira et al. 2002) and ducks (Cheng et al. 2002). In serum, several clinical indicators are adversely affected by contaminated diet. They include serum urea, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (Yousef et al. 2003; Denli et al. 2004).

Risks associated with aflatoxin-contaminated food can be reduced by the use of specific processing and decontamination procedures (Park, 2002). Detoxification of aflatoxins by chemicals such as ammonium carbonate, ammonium bicarbonate, ammonium benzoate and humic acid has been reported (Sutabha et al. 1992; Denli and Okan, 2006).

However, prevention is unquestionably the best method for controlling mycotoxin contamination. One of the ways to eliminate the toxic effects on animal health is to use various sorbents (zeolites, kaolins, humic compounds) in agriculture and veterinary medicine (Desheng et al. 2005; Sehu et al. 2007). The idea of using humates as feed additives in animal nutrition is new. Humic acids stabilize the intestinal microflora, ensuring an improved nutrient utilization and feed efficiency (Pisafiková et al. 2010). Sodium humate (HuNa) is a fraction of humic substances, which can be extracted from soil by various reagents and which is insoluble in dilute acid. Studies show humic substances are effective in removing various mycotoxins, such as aflatoxin, fumonisin, trichotheceene and zearalenone (Madden et al. 1999). The efficiency of these alternative absorbers depends on the chemical structure of the toxin (Huwig et al. 2001). A number of methods, including physical, chemical and biological techniques have been used to protect animals from the toxic effects of AFB1, but most of these methods are costly, time-consuming, and only partially effective (Sheng-Qun Ye et al. 2009). Many authors demonstrate the use of natural sorbents (zeolites, HuNa) to reduce the content of heavy metals and toxins from soil, sediment, sewage (Ghosh et al. 1997; Spark et al. 1997), as well as the reduction of heavy metals and toxins from the animal body (Skalická et al. 2002a; Skalická et al. 2002b). However, the efficiency of HuNa for aflatoxins is not clearly demonstrated in poultry.

Sheng-Qun Ye et al. (2009) found that sodium humate has several advantages on AFB1 adsorption, higher affinity to AFB1, not adsorb other nutrients, the complex was very stable in different pH phosphate buffer. However, like other assay in vitro, this study did not replace the adsorption conditions of the gastrointestinal tract animals and in vivo studies are needed to assess the efficiency of adsorption AFB1 under practical conditions. Thus, the research reported was conducted to determine the efficiency of HuNa for protection against the toxicity of aflatoxin in broiler chicks. The aim of this study was to evaluate the effects of HuNa and AFB1 on the activity of the selected enzyme ALP in broiler chicks. Suitable doses of the mineral preparation added to the feed mixture can result in the improvement weight gain of feed.

**MATERIALS AND METHODS**

The experiments were performed in accordance with the ethical requirements for animal handling approved by the University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic. Its Ethic Commission approved these experiments and attached a number to these experiments (No. 8/2003).

The experiment was carried out on hybrid Hybro broilers of both sexes, from 20-56 days of age. Average body weight of broilers was 218.18 ± 46.87 g at 20 days of age.

The broilers (n=72) were randomly divided into 4 groups with 18 chickens per group: control group with regular diet including no additional supplement (G1); and 3 experimental groups with 1% HuNa feed mixture (1 g HuNa/100 g feed mixture) (G2); 25 µg.kg⁻¹ b.w. AFB1 (G3); 1% HuNa feed mixture and 25 µg.kg⁻¹ b.w. AFB1 (G4). The sodium humate (HuNa-salt of natrium acid) used was sourced from the territory of most (Czech Republic). The composition of HuNa that was used in our experiment is as follow: water 16.7%; humic acid 63.2%; ash 20.1%; Na2O 15.3% of weight. Aflatoxin B1 (Sigma Chemical Co., USA), was dissolved in a small volume of ethanol solution (2.5 mg/mL ethanol). One hundred microliters (100 µL) from ethanol solution were diluted in 100 mL physiological solution. The final dose (25 µg AFB1, kg⁻¹ of body weight) was applied to the drinking water for broiler.

Broilers were fed with a commercial diets: diet for growing broilers (from 20-42 of age) (Table 1) and final feed mixture for broilers (from 42-56 of age) (Table 2). The complete feed mixture used for this experiment were tested for any possible residual aflatoxin, following the official method of sampling and analysis of feed.
The composition of the feed was in accordance with Decree of the Ministry of Agriculture of the Slovak Republic (Decree of the Ministry of Agriculture of the Slovak Republic No. 440, 2016).

The experimental conditions complied with the requirements for ethical standards of welfare of animal treatment. The broiler chicks were reared on deep litter and the microclimatic conditions complied with the requirements for fattening of broilers. Broilers were reared under a conventional temperature regimen at 21°C. The relative humidity was maintained between 60-70%. Feed and water were provided ad libitum.

The activity of ALP was determined on days 20, 28, 42 and 56 by collecting the blood from Vena cutanea ulnaris. The serum samples were stored at -20°C until analyses.

The concentrations of alkaline phosphatase (ALP) in blood serum were determined with enzymatic methods on a spectrophotometer using Bio-La-Tests (PLIVA–Lachema Brno Ltd., Czech Republic), at a wavelength of 420 nm. Reproducibility of the method was better than 6%.

Results and Discussion

The most prevalent symptom of aflatoxicosis in poultry and livestock is reduced growth rate and thus a poor performance. Data presented in Tables 3 and 4 show the effect of dietary treatments on weight and activities of ALP, respectively.

In all experimental groups prior to application of AFB1, the mean levels of ALP were found to be 13.73 μkat.l⁻¹ on 20 day of age of the broilers, with maximum level at 16.2 μkat.l⁻¹. The activity of ALP in control group during the experiment was 4.17 μkat.l⁻¹ on 28th day of age and decreased to 2.14 μkat.l⁻¹ on 56th day (Table 4).

The obtained results show that the activity of ALP decreased on day 28, as well as on day 56 of age of the broilers. These changes in the activity of ALP were observed in control group (4.17; 2.49 and 2.14 μkat.l⁻¹), in experimental groups G2 (2.30; 2.1 and 1.71 μkat.l⁻¹), G3 (1.55; 1.63 and 1.42 μkat.l⁻¹) and G4 (3.12; 2.17 and 2.37 μkat.l⁻¹) in comparison to values (13.73 μkat.l⁻¹) on 20 day of age of the broilers.

On the other hand, the activity of ALP in group G4 (3.12; 2.17 and 2.37 μkat.l⁻¹) was decreased only on day 42 and slightly increased on day 56 of age of the broilers in comparison to values of control group. The metabolic activity and neurohumoral regulation of the organism has an impact on levels of ALP, which vary depending on the age (Blahovec and Šlesárová, 1991).

A decrease in the activity of ALP was in chickens as early as the age of 1-7 weeks. The values of activity of ALP are higher in all young and growing animals. The main source of ALP in the serum of the young is osteoenzyme. Alkaline phosphatase is released into the blood during injury and during such normal activities as bone growth and pregnancy. High blood levels of alkaline phosphatase may indicate disease in bone or liver (Jindal et al. 1993). Alkaline phosphatase activity is a very useful serum biochemical indicator of liver disease (Fernandez and Kidney, 2007) and is known to be indicative of induced hepatobiliary disease (Kaplan, 1987).

The statistical evaluation of the results has been done by the program of microsoft Excel 7.0, using Student's t-test at (P≤0.05) and (P≤0.001) level of significance. For the calculation of means, values below the detection limits were set to zero.
Garaleviciene et al. (2001) observed that the activity of ALP was increased after feeding moulded diets contained low concentration of ochratoxin A. Increase in the level of ALP due to the dietary aflatoxin B1 has also been reported by Sadeghi et al. (2013). Broilers exposed to AFB1 in group G3 had lower ALP levels after day 42 and 56 of age in comparison with the control group G1. These results are comparable to those described by Edrington et al. (1997) and Miall et al. (2005), who observed the effects of aflatoxin B1 on decrease ALP serum enzyme activity. The reductions in concentrations of ALP observed in this study are probably of impaired protein synthesis and have been reported previ-

### Table 3: Weight of broilers

<table>
<thead>
<tr>
<th>Groups</th>
<th>28 day</th>
<th>35 day</th>
<th>42 day</th>
<th>49 day</th>
<th>56 day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Min</strong> G1</td>
<td>350.00</td>
<td>500.00</td>
<td>800.00</td>
<td>1600.00</td>
<td>1750.00</td>
</tr>
<tr>
<td>Max</td>
<td>660.00</td>
<td>1060.00</td>
<td>1350.00</td>
<td>1750.00</td>
<td>2010.00</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>563.00</td>
<td>843.33</td>
<td>1194.17</td>
<td>1680.00</td>
<td>1910.00</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>81.28</td>
<td>181.87</td>
<td>150.30</td>
<td>67.82</td>
<td>108.63</td>
</tr>
</tbody>
</table>

**Min:** minimal levels; **Max:** maximal levels; **Mean:** mean body weight; **SD:** standard deviation.

G1: control group; G2: natural sorbent-sodium humate (HuNa); G3: aflatoxin B1 (AFB1) and G4: natural sorbent-sodium humate (HuNa) and aflatoxin B1 (AFB1).

### Table 4: The alkaline phosphatase (ALP) activity in blood serum of broilers

<table>
<thead>
<tr>
<th>Groups</th>
<th>28 day</th>
<th>42 day</th>
<th>56 day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Min</strong> G1</td>
<td>3.00</td>
<td>1.59</td>
<td>1.64</td>
</tr>
<tr>
<td>Max</td>
<td>5.65</td>
<td>4.42</td>
<td>2.83</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>4.17</td>
<td>2.49</td>
<td>2.14</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.08</td>
<td>1.90</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**Min:** minimal levels; **Max:** maximal levels; **Mean:** mean ALP activity; **SD:** standard deviation.

G1: control group; G2: natural sorbent-sodium humate (HuNa); G3: aflatoxin B1 (AFB1) and G4: natural sorbent-sodium humate (HuNa) and aflatoxin B1 (AFB1).

* (P≤0.05).
The liver showed signs of damage. Livers of broilers fed control group. mental broiler chicks (G3 and G4) in comparison with the there is observed slight decrease in weight of the experi-

ments and stimulate the metabolism. This effect is due to the fact that humic substances are capable of binding and re-

moving of heavy metals and xenobiotics from the body. It appears that ALP is one of the mediators of aflatoxicosis in broiler chickens. This problem, however, needs further de-

tailed studies. In the second part of the study body weight of experimental chicks was evaluated after supplementation of sodium humate and addition of aflatoxin B1. Aflatoxins causes a reduction in body weight (Giambrone et al. 1985) severe economies losses and health problems in the poultry industry (Miazzo et al. 2005). Average body weight of broilers were 218.18 ± 46.87 g at 20 days. In group G1, at the end of the experiment, the body weight increased of 1 691.82 g (Table.3).

For example, the quartz-type sorbents do not impart equal protection against aflatoxicosis (Kubena et al. 1998). After adding various concentrations of hydrated sodium calcium aluminosilicate (HSCAS) to the feed of Japanese quails a marked decrease in harmful effects of aflatoxin B1 was observed during digestion (Sehu et al. 2007). The maximum AFB1 adsorption capacity of a sodium bentonite in the diet from southern Argentina was estimated (Rosa et al. 2001). These results suggest that effects of AF treatment were ameliorated when sodium bentonite was used in the broiler chick diets. Data presented in Table 3 show that there is observed slight decrease in weight of the experimental broiler chicks (G3 and G4) in comparison with the control group.

Also the reduction in body weight of the experimental broiler chicks (G3) exposed to aflatoxin B1 were observed in comparison with the groups with HuNa (G2). The mean body weight in group G4 was 152.5 g higher in comparison with the group G3 on day 56 of the age. During the experiment the mean body weight of broiler chicks in group G2 was 26.67 g higher in comparison to the control chickens. Statistically significant increases (P≤0.001) in body weight was observed in the groups G3 and G4 on 56th day of age, compared to 28 days.

Similar decrease in body weight of broilers following the consumption of aflatoxin B1 was recorded by Tessari et al. (2005). Rauber et al. (2007) demonstrated the toxicity of aflatoxins of several doses (20, 50, 100, 200, 500 and 1000 ppb) added to the feed of turkey poult. Also showed, tur-

keys from treatments that received up to 200 ppb aflatoxins had lower weight compared with the other treatments. The presence of aflatoxins 50 ppb still did not affect body weight of turkeys.

The data collected from examination of the body weight on day (49 and 56 of age) in the G4 (sodium humate and aflatoxin B1) in comparison with G3 (aflatoxin B1) was higher, respectively. At the end of the experiment on day 56 of age, broilers in groups G2, which has been fed only HuNa had better weight gain compared with other groups. Effects of oxihumate in prevention of aflatoxicosis in chicks were suggested by Jansen van Rensburg et al. (2006).

The adsorptive mechanism of aflatoxin B1 on montmoril-

lonite was determined by Desheng et al. (2005). The montmorillonite, when added to the diet of broiler chicken at 0.5%, significantly diminished the adverse effects of feeding.

This research provided information on the effects of humic substances on metabolic function and blood chemistry in broilers during experimental aflatoxicosis. The result of the research indicated that the changes in activity of ALP in our experiment are likely to be associated with a HuNa. It is known that humic substances catalyse the enzymatic re-

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ers fed with commercial diets. The result indicated that re-

duction in the activity of ALP in the experiment can be as-

sociated with the addition AFB and increased activity occurred on addition HuNa and AFB1. Probably ALP is also an indicator of aflatoxicosis in broilers. This problem, however, needs further detailed studies.

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