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The Effects of Sodium Humate and Aflatoxin B_1 on Body Weight of Broiler Chicks

Research Article

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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 B_1 (AFB_1) toxicity in the broiler chicken. Seventy-two (72) broiler hybrid Hybro birds were randomly divided into 4 groups: control group (G1) and 3 experimental groups (G2-G4) with addition of HuNa (G2); only AFB_1 (G3) and HuNa; and AFB_1 (G4). HuNa was added to a complete feed mixture daily for broiler chicks (1 g HuNa/100 g feed mixture) and AFB_1 at the concentration of 25 $\mu g.kg^{-1}$ of b.w. Parameters evaluated were body weight and serum levels of alkaline phosphatase (ALP). AFB_1 and HuNa were applied for broilers from 28 days of age. Statistically significant decrease activity of ALP ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 AFB_1 (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 ($P \leq 0.001$) in body weight was recorded for group G3 and G4 on 56^{th} 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 B_1, 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 poults 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 B_1, G_1, B_2, G_2, aflatoxin B_1 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 B_1, G_1, B_2, and G_2 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 AFB_1 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 AFB_1 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 an overall low performance, 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 bioactivated 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*. 2000). 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 (Sutabhaha *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 (Písaříková *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, trichothecene 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 AFB_1, 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 AFB_1 adsorption, -higher affinity to AFB_1, 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 AFB_1 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 AFB_1 on the activity of the selected enzyme ALP in broiler chicks. Suitable doses of the mineral preparation added to the feed mixture can results 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 were 218.18 ± 46.87g 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 $\mu g.kg^{-1}$ b.w. AFB_1 (G3); 1% HuNa feed mixture and 25 $\mu g.kg^{-1}$ b.w. AFB_1 (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%; Na_2O 15.3% of weight. Aflatoxin B_1 (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 $\mu g\ AFB_1.kg^{-1}$ 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.

Table 1 Nutrient content of the complete feed mixture for growing broilers HYD 02

Feed composition		Minerals		Vitamins	
Crude protein	235.0 g.kg^{-1}	Calcium	8.0 g.kg^{-1}	Vitamin A	10,000 IU.kg^{-1}
Metabolizable energy	12.9 MJ.kg^{-1}	Phosphorus	5.0 g.kg^{-1}	Vitamin D_3	2,000 IU.kg^{-1}
Methionine + cysteine	8.0 g.kg^{-1}	Sodium	3.0 g.kg^{-1}	Vitamin E	15.0 mg.kg^{-1}
Lysine	10.0 g.kg^{-1}	Copper	8.0 mg.kg^{-1}	Vitamin B_2	3.0 mg.kg^{-1}
Ash	70.0 g.kg^{-1}	Manganese	50.0 mg.kg^{-1}	Vitamin B_{12}	20.0 µg.kg^{-1}
Fibre	53.0 g.kg^{-1}	Zinc	50.0 mg.kg^{-1}	-	-

Table 2 Nutrient content of the final complete feed mixture for broilers HYD 03

Feed composition		Minerals		Vitamins	
Crude protein	210.0 g.kg^{-1}	Calcium	7.0 g.kg^{-1}	Vitamin A	8,000 IU.kg^{-1}
Metabolizable energy	13.3 MJ.kg^{-1}	Phosphorus	4.0 g.kg^{-1}	Vitamin D_3	12,00 IU.kg^{-1}
Methionine + cysteine	8.0 g.kg^{-1}	Sodium	2.0 g.kg^{-1}	Vitamin E	10.0 mg.kg^{-1}
Lysine	9.5 g.kg^{-1}	Copper	6.0 mg.kg^{-1}	Vitamin B_2	3.0 mg.kg^{-1}
Ash	66.0 g.kg^{-1}	Manganese	50.0 mg.kg^{-1}	Vitamin B_{12}	20.0 µg.kg^{-1}
Fibre	46.7 g.kg^{-1}	Zinc	50.0 mg.kg^{-1}	-	-

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 the 6%. Body weights of broilers were monitored on 20, 28, 35, 42, 49 and 56 days of age in individual broilers of the control and experimental groups. The birds were slaughtered by cervical dislocation and necropsied for gross lesions in liver.

Statistical analysis

The statistical evaluation of the results has been done by the program of microsoft Excel 7.0, using Student's t-test at ($P \leq 0.05$) and ($P \leq 0.001$) level of significance. For the calculation of means, values below the detection limits were set to zero.

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 AFB_1, 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).

Table 3 Weight of broilers

Groups		Weight of broilers (g)				
		28 day	35 day	42 day	49 day	56 day
G1	Min	350.00	500.00	800.00	1600.00	1750.00
	Max	660.00	1060.00	1350.00	1750.00	2010.00
	Mean	563.00	843.33	1194.17	1680.00	1910.00
	SD	81.28	181.87	150.30	67.82	108.63
G2	Min	310.00	310.00	700.00	1000.00	1700.00
	Max	650.00	470.00	1250.00	1750.00	2150.00
	Mean	565.80	767.50	1116.67	1548.33	1936.67
	SD	89.20	93.17	93.17	206.73	147.60
G3	Min	410.00	460.00	920.00	700.00	1000.00
	Max	670.00	950.00	1250.00	2000.00	2150.00
	Mean	529.00	786.00	1101.00	1450.00	1730.00***
	SD	82.25	88.97	98.82	501.25	357.16
G4	Min	350.00	510.00	550.00	1350.00	1500.00
	Max	650.00	900.00	1500.00	1750.00	2350.00
	Mean	506.36	732.00	1150.00	1572.00	1882.50***
	SD	103.08	109.53	305.16	160.22	374.02

Min: minimal levels; Max: maximal levels; Mean: mean body weight and SD: standard deviation.
G1: control group; G2: natural sorbent-sodium humate (HuNa); G3: aflatoxin B_1 (AFB_1) and G4: natural sorbent-sodium humate (HuNa) and aflatoxin B_1 (AFB_1).
*** ($P \leq 0.001$).

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

Groups		Activities of ALP ($\mu kat.l^{-1}$)		
		28 day	42 day	56 day
G1	Min	3.00	1.59	1.64
	Max	5.65	4.42	2.83
	Mean	4.17	2.49	2.14
	SD	0.08	1.90	0.49
G2	Min	1.32	1.16	1.80
	Max	2.59	3.35	2.27
	Mean	2.30	2.10	1.71
	SD	0.63	0.80	0.41
G3	Min	0.65	1.49	0.85
	Max	2.55	1.85	1.73
	Mean	1.55	1.63	1.42
	SD	0.55	0.12	0.33
G4	Min	2.66	1.28	1.50
	Max	3.47	2.96	3.12
	Mean	3.12	2.17*	2.37*
	SD	0.34	0.41	0.74

Min: minimal levels; Max: maximal levels; Mean: mean ALP activities and SD: standard deviation.
G1: control group; G2: natural sorbent-sodium humate (HuNa); G3: aflatoxin B_1 (AFB_1) and G4: natural sorbent-sodium humate (HuNa) and aflatoxin B_1 (AFB_1).
* ($P \leq 0.05$).

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 B_1 has also been reported by Sadeghi *et al*. (2013).

Broilers exposed to AFB_1 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 Miazzo *et al*. (2005), who observed the effects of aflatoxin B_1 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 previously (Kubena *et al*. 1998; Abo-Norag *et al*. 1995; Denli *et al*. 2004). Also, the decreases in activity of ALP associated with accepting aflatoxin in chickens were recorded by Raina *et al*. (1991) and Phillips *et al*. (1994).

The significant increase ($P \leq 0.05$) of activity ALP (2.17 and 2.37 $\mu kat.l^{-1}$) were recorded in experimental group G4 fed on feed containing sodium humate and aflatoxin B_1 in comparison with group G3 fed only aflatoxin B_1 (1.63 and 1.42 $\mu kat.l^{-1}$) on days 42 and 56 of age. The increase in ALP activity of broilers, in group G4 (HuNa and AFB_1), could be due to HuNa addition into diet and has a high mycotoxin adsorption capacity. Humates have the ability to block colonization of pathogens in the gastrointestinal tract

(Jansen van Rensburg *et al*. 2006). The protective effect of humate appears to involve sequestration of aflatoxins in the gastrointestinal tract and reduction in bioavailability of aflatoxin (Ghahri *et al*. 2010). At the end of the experiment and after pathological and anatomical dissections, we found that the liver showed signs of damage. Livers of broilers fed diets containing AFB_1 (group G3) and combination of AFB_1 and HuNa (G4) were friable, enlarged, yellowish and had rounded borders. In this study we observed moderate hepatic steatosis. Livers from groups G1 and G2 were not included because they had normal appearances. Liver is considered to be the organ that is most affected with toxic effect of AFB_1 and the changes of activity ALP testify hepatotoxicity (Beers *et al*. 1992; Espada *et al*. 1992; Gawai *et al*. 1992; Tessari *et al*. 2006). ALP too belongs among the enzymes used in the diagnosis of hepatopathies. The ALP also should be considered as indicator of metabolic changes due to the toxic noxa, such as aflatoxin.

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 reactions and stimulate the metabolism. This effect is due to the fact that humic substances are capable of binding and removing 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 detailed studies. In the second part of the study body weight of experimental chicks was evaluated after supplementation of sodium humate and addition of aflatoxin B_1. Aflatoxins causes a reduction in body weight (Giambrone *et al*. 1985) severe economics 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 B_1 was observed during digestion (Sehu *et al*. 2007). The maximum AFB_1 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 B_1 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 ($P \leq 0.001$) 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 B_1 was recorded by Tessari *et al*. (2006). 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 poults. Also showed, turkeys from treatments that received up to 200 pbb 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 B_1) in comparison with G3 (aflatoxin B_1) 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 B_1 on montmorillonite 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.

CONCLUSION

This research provided information on the effects of humic substances on metabolic function and blood chemistry in broilers during experimental aflatoxicosis. Application of HuNa caused increase of body weight compared with broilers fed with commercial diets. The result indicated that reduction in the activity of ALP in the experiment can be associated with the addition AFB and increased activity occurred on addition HuNa and AFB_1. Probably ALP is also an indicator of aflatoxicosis in broilers. This problem, however, needs further detailed studies.

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