Original Article

To Reduce the Effects of Experimental Aflatoxicosis in Broiler Chicks Using Specific Egg Yolk Immunoglobulin (IgY)

Ali Nakhaei[1], Nazar Afzali[2 *], Seyyed Javad Hosseini Vashan[3], Mohammad Amir Karimi Torshizi[4]

[1] Department of Animal Sciences, Faculty of Agriculture, University of Birjand, Birjand, Iran
[2] Department of Animal Sciences, Faculty of Agriculture, University of Birjand, Birjand, Iran
[3] Department of Animal Sciences, Faculty of Agriculture, University of Birjand, Birjand, Iran
[4] Department of Animal Sciences, Faculty of Agriculture, University of Tarbiyat modares, Tehran, Iran

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Abstract

Background: This experiment was conducted to evaluate the effects of the specific egg yolk immunoglobulin (IgY) on reducing the defects of experimental aflatoxicosis in broilers. **Materials and Methods:** In a completely randomized design, a total of 128 Ross 308 broiler chicks were used in 4 treatments, 4 replicates and 8 observations (chicks) for 42 days. Treatments were: 1) control; 2) diet containing 1 ppm aflatoxin B1; 3) diet contaminated with 1 ppm aflatoxin B1 + 0.75 % of egg yolk containing IgY; 4) diet contaminated with 1 ppm aflatoxin B1 + 1.5 % of egg yolk containing IgY. **Results:** The results showed that the use of aflatoxin containing diet significantly increased the feed conversion ratio, serum cholesterol concentration, serum gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST). Also, experimental aflatoxicosis resulted in reduced feed intake, weight gain, serum total protein and albumin concentrations ($p<0.05$); as well as the liver histopathologic lesions. IgY containing egg yolk (0.75% and 1.5%) added to the contaminated ration improved feed intake, weight gain and feed conversion ratio compared to treatment 2 ($p<0.05$). Compared to treatment 2, serum cholesterol concentration decreased while total protein concentration increased in treatments 3 and 4 ($p<0.05$). Liver tissue was approximately normal with mild effects on hepatocytes and mild cytoplasmic changes in chicks receiving treatments 3 and 4. **Conclusion:** It can be concluded that specific IgY is effective in reducing the defects of experimental aflatoxicosis as well as improving performance in broilers.

***Keywords**:* Aflatoxin B1, specific immunoglobulin, weight gain, feed conversion ratio.

***Corresponding Author:** Department of Animal Sciences, Faculty of Agriculture, University of Birjand, Birjand, Irannafzali@birjand.ac.ir

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Introduction

Aflatoxin B1 (AFB1) is one of the most hepatotoxic, genotoxic, and immunotoxic toxins to humans and animals. According to the International Agency for Research on cancer reports AFB1 is classified as the most important human carcinogen with no recognized safe dose (1). The toxic effect of AFB1 may be also attributed to its enhancement of the reactive oxygen species (ROS) level in living cells, which may attack lipids, proteins, and nucleic acids (2). Although many methods, including physical, chemical, and biological methods have been tried to degrade the AFB1, most of these processes have led to the formation of toxic residues or derivatives and

consequently reduced the food's nutritional values and organoleptic qualities (3).

Hens egg yolk immunoglobulin (IgY), is composed of two light chains and two heavy chains, like mammalian counterpart IgG (4). However, IgY can neither activate the mammalian complement system nor interact with human Fc receptors and mammalian rheumatoid factors. Additionally, chickens can produce a strong immune response against mammalian antigens owing to the phylogenetic distance between mammals and birds (5). Because of these advantages, IgY has been used for the treatment of various gastro enteric infectious diseases and against macromolecular protein toxins such as Shiga toxin, ricin toxin, botulinum toxins, and viper venom (6). IgY antibodies have many advantages over IgG antibodies such as strong avidity, scalable productivity, low assay background, and applicability to many immunoassays providing a strategy for improvement of assay performance and accuracy. Therefore, the purpose of this study was to evaluate the possibility of using specific IgY against aflatoxin B1 to reduce the effects of experimental aflatoxicosis on blood biochemistry and liver histopathology of broiler chicks.

Methods

Ethical approval. The research was carried out at the University of Birjand (the poultry research farm) in July 2017.

Toxin production. In the in vitro stage, Aflatoxin B1 was produced by culture of Aspergillus parasiticus (NRRL 2999) on potato dextrose agar. Then the AFB1 production on rice was done according to the method described by Shotwell et al. (7). The concentration of Aflatoxin B1 was measured (75.4 ppm) with HPLC (Testa Quality Control Laboratory, Mashhad, Iran).

Bird immunization and IgY production. Immunization of laying hens and production of IgY was performed using the conjugated aflatoxin B1-bovine serum albumin (Sigma, Germany). The injection was carried out in three stages at 14 days intervals. Injections were associated with 0.2 ml of complete Freund's adjuvant in the first and incomplete Freund's adjuvant in subsequent injections. In the first and second injections, 200 micrograms and in the third injection, 100 micrograms of the conjugate per bird was used. 14 days after the first injection, daily eggs were obtained and kept refrigerated (8).

Experimental model. In the in vivo stage one-day-old Ross's vaccinated broilers were obtained from a local commercial hatchery. One hundred twenty-eight chicks were randomly distributed to 4 dietary groups with 4 replicates of 8 chicks in each. Water and feed were available ad libitum. The experimental treatments were: 1) control; 2) diet containing 1 ppm aflatoxin B1 (contaminated control); 3) diet contaminated with 1 ppm aflatoxin B1 + 0.75 % of egg yolk containing IgY; 4) diet contaminated with 1 ppm aflatoxin B1 + 1.5 % of egg yolk containing IgY. The average body weight gain and feed consumption were calculated during the experimental period.

Blood chemical and histopathology. At the end of the trial, when broilers were 42 days old, 8 birds per treatment were randomly selected for blood collection from the wing vein for biochemical analysis. Blood was centrifuged at 2500 × g for 15 minutes and the serum separated and preserved at -20°C until biochemical analyses. Serum concentrations of total protein (TP), albumin (ALB), aspartate aminotransferase (AST), cholesterol (CHOL), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using an automatic analyzer (Gesan Chem 200, Italy) with commercial test kits (Pars Azmoon, Iran). After blood collection, the 8 previously selected birds were euthanized for pathological examination. Liver tissue samples were collected in 10% neutral buffered formalin. Upon fixation, samples were dehydrated in graded alcohol series, cleared in xylene, and embedded in paraffin wax. Ten-µm sections were cut and stained with hematoxylin and eosin (H&E; Thermo Shandon, 15275, USA) (9).

Statistical analysis. Data were subjected to statistical analysis using the general linear model procedure of SAS software (10). Treatment means showing significant differences in the one-way analysis of variance were compared using Tukey's general linear model test. All the statements of significance were based on the 0.05 probability level.

Table1. The effect of different treatment on feed intake, body weight gain and feed conversion ratio in different experimental periods

	Periods (days)	Treatments*				P.Value	±SEM
		1	2	3	4		
Feed Intake (gr/period)	1-10	168.50	163.50	157.90	160.30	0.0812	2.53
	11-24	1127.47a	977.78b	1113.44a	1186.69a	0.0015	28.26
	25-42	2165.47a	1727.81b	2105.94a	2236.09a	0.0001	47.15
	1-42	3461.69a	2874.34b	3388.13a	3591.53a	0.0001	50.48
Body Weight Gain (gr/period)	1-10	116.09ab	113.09b	121.87ab	126.56a	0.0309	2.94
	11-24	722.50a	650.44b	758.59a	762.19a	0.0010	15.72
	25-42	1178.25a	795.52b	1257.19a	1271.22a	0.0001	30.07
	1-42	2013.84a	1562.05b	2137.66a	2159.97a	0.0001	36.50
Feed Conversion Ratio (gr/gr)	1-10	1.49a	1.45ab	1.38a	1.33b	0.0282	0.03
	11-24	1.56	1.50	1.46	1.55	0.3602	0.04
	25-42	1.83b	2.17a	1.67b	1.76b	0.0001	0.04
	1-42	1.71b	1.84a	1.58c	1.64bc	0.0001	0.02

[a,b] Within the same row, means with different letters are significantly different.
*1) control; 2) diet containing 1 ppm aflatoxin B1; 3) diet contaminated with 1 ppm aflatoxin B1 + 0.75 % of egg yolk containing IgY; 4) diet contaminated with 1 ppm aflatoxin B1 + 1.5 % of egg yolk containing IgY.

Table2. Effect of different treatments on serum lipid, protein and liver enzymes (mg/dl) in broiler chicks at 42 days

Treatments*	TP	GGT	ALB	AST	LDL	HDL	TG	CHOL
1	2.60a	23.50	1.55a	301.75ab	35.00	74.50	91.75	127.50ab
2	2.15ab	23.50	1.35ab	279.50b	43.00	79.25	96.75	154.75a
3	1.96b	25.00	1.30b	344.33ab	34.50	76.00	85.33	126.33ab
4	2.40ab	26.33	1.46ab	360.67a	30.33	75.66	89.50	119.00b
P.Value	0.0145	0.3943	0.0305	0.0184	0.0179	0.6866	0.4473	0.0216
SEM±	0.12	1.31	0.05	16.83	3.41	2.79	12.32	7.24

[a, b] The mean of the letters in different columns is statistically significant.
*1) control; 2) diet containing 1 ppm aflatoxin B1; 3) diet contaminated with 1 ppm aflatoxin B1 + 0.75 % of egg yolk containing IgY; 4) diet contaminated with 1 ppm aflatoxin B1 + 1.5 % of egg yolk containing IgY.

Results

Performance. The effect of different treatments on feed intake, body weight gain and feed conversion ratio in the starter (1 to 10 days), grower (11 to 24 days), finisher (25-42 days) and entire period (1 to 42 days) is shown in Table 1. Contamination of the feed with 1 mg kg-1 of AFB1 reduced the feed intake of broiler chicks in grower, finisher, and the entire period of the experiment, which was significantly different from the control treatment ($p<0.05$). Adding egg yolks containing specific IgY to the contaminated control improved the feed consumption of broilers compared to the treatment 2 during the starter, finisher, and the entire period of the experiment ($p<0.05$). The addition of 1.5% egg yolk containing specific IgY to the contaminated diet (treatment 4) resulted in the highest feed intake which had a significant difference compared to the contaminated control group ($p<0.05$). Feeding chicks by contaminated feeds (1 mg kg-1 of AFB1) showed a significant weight loss in all experimental periods compared to the control group. IgY in the egg yolk added to the contaminated control group caused an increase in the weight of the broilers compared to the contaminated control treatment in all experimental periods ($p<0.05$). The highest weight gain in broiler chicks belonged to the treatment received 1.5% of egg yolk containing specific IgY, which is significantly higher than the contaminated control group in the starter, grower, finisher, and the entire of the experiment ($p<0.05$). Contamination of chicken feed with 1 mg kg-1 of AFB1 increased feed conversion ratio compared to the control group ($p<0.05$). Using IgY effectively reduced the effects of aflatoxin. Egg yolk containing immunized IgY against AFB1 at the levels of 0.75 and 1.5% reduced the feed conversion ratio compared with the contaminated control

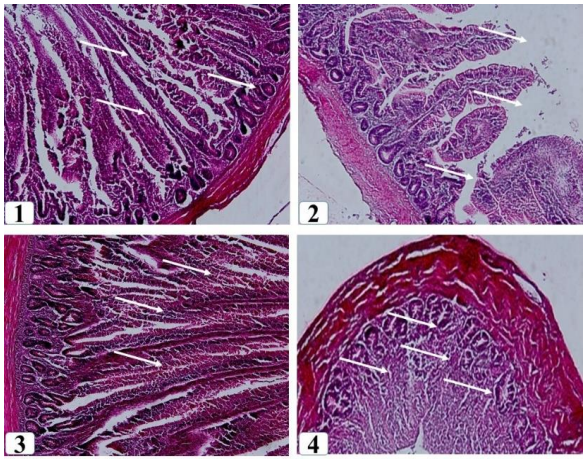

Figure 1. Liver histopathology in different treatments. 1) control; 2) diet containing 1 ppm aflatoxin B1; 3) diet contaminated with 1 ppm aflatoxin B1 + 0.75 % of egg yolk containing IgY; 4) diet contaminated with 1 ppm aflatoxin B1 + 1.5 % of egg yolk containing IgY.(Arrows show widespread destruction, cytoplasmic cavities and decayed fat).

treatment during the finisher and the entire periods of the experiment ($p<0.05$).

Blood biochemical. The effect of experimental treatments on proteins, lipids and serum enzymes of broiler chicks is presented in Table 2. The results indicate that the highest level of blood cholesterol observed in broilers fed 1 mg kg-1 of AFB1(contaminated control) chickens. Egg yolk anti-AFB1 immunoglobulin reduced serum cholesterol content in broilers compared with the contaminated control group. The lowest serum cholesterol belonged to the treatment with 1.5% of immunized egg yolk, which was significantly lower than that of contaminated control ($p<0.05$). Triglycerides concentration in different treatments did not show a significant difference. A numerical increase observed in serum triglyceride concentration of chickens fed 1 mg kg-1 of AFB1. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations were not significantly different between treatments. Serum concentration of aspartate aminotransferase (AST) enzyme increased by 1 mg kg-1 of AFB1, which is significantly different from control treatment ($p<0.05$). Serum total protein and albumin concentration were different among experimental treatments. The lowest total protein and albumin concentrations observed in chickens fed aflatoxin, showing a significant difference ($p<0.05$) compared to control treatment. Specific IgY in egg yolks added to the contaminated control diet at levels of 0.75 and 1.5%, led to a numerical increase in total protein and albumin content of broiler chicks in comparison with the contaminated control group. There was no significant difference in serum concentration of gamma glutamine transferase enzyme (GGT) between treatments.

Histopathology. Pathological photomorphography of the liver tissue in broilers receiving various experimental diets is shown in Fig-1. At the end of the experimental period (day 42), liver tissue of the broilers fed aflatoxin-infected ration showed the penetration of mononuclear cells in portal areas, widespread destruction, cytoplasmic cavities and decayed fat in hepatocytes. Liver tissue was approximately normal with mild effects on hepatocytes and mild cytoplasmic changes in chicks receiving treatments 3 and 4. Supplementation of diets contaminated with one mg kg-1 aflatoxin by the specific immunoglobulin (0.75% and 1.5%), showed the high ability of the IgY to absorb Aflatoxin B1 and reduce its effects on the liver of the broilers.

Discussion

Feed intake and weight gain (Table 1) were significantly different between treatments ($P<0.01$), and the values were lower in treatment negative control, as expected, with declines of 50 and 35%, respectively, when compared with positive control. These reductions were due to the high absorption of AFB1 by the gastrointestinal tract. The metabolism of this compound in the liver generates toxic metabolites that cause liver injury and inhibition of protein synthesis, culminating in anorexia, as also reported by Yunus et al. (11). Studies using only AFB1 has demonstrated the impact of this mycotoxins on animal performance. In this study, the highest reductions can be associated with AFB1, as evidenced by Miazzo and his colleagues, who used levels of 2.5 mg kg-1 of AFB1 and reported an 11% reduction in weight gain of broilers from 21 to 42 days (12).

Dietary exposure to AFB1 and other aflatoxins leads to lower weight gain and absolute body weights in both chickens and turkeys (13). Reduced feed intake and decreased efficiency of nutrient usage both contribute to this impaired growth during aflatoxicosis (13). Feed efficiency was significantly suppressed ($P< 0.05$) in 1000 µg kg-1 AF fed group during all the three periods. Poor feed conversion noted with the

AFB1 seems to have mediated decreased nutrient utilization. Yegani et al. (14) with broiler breeders showed that chickens are sensitive to the feeding of mycotoxins with respect to feed efficiency.

AFB1 adducts with biomolecules cause damage to hepatocytes that impairs metabolic functions of the liver during AFB1 exposure. This is exemplified by AFB1-reduced total serum protein levels, as the liver is responsible for production of most circulating proteins (13). Aflatoxicosis negatively affects albumin, globulin, cholesterol, and triglyceride levels in serum (15). Protein content likely declines because AFB1-DNA adducts inhibit transcription or translation and AFB1-lysine adducts result in protein degradation or excretion. Reduced synthesis of enzymes in the liver would have systemic effects on poultry metabolism (16). Quist et al. (17) showed that total protein levels are significantly reduced in turkeys poisoned with Previous studies performed with high levels of AF (2.5-5 mg/kg diet) showed significant decreases in serum total protein and albumin. The results also revealed that treatment with IGY prevented changes in AST activity, supporting the idea that these IGY may provide protection against toxic effects of AFB1. The addition of IGY to the AFB1 diet prevented the decrease in these AF-sensitive serum proteins in the present study.

Triglyceride levels, in turn, were not influenced by mycotoxins. These data agree with Maciel et al. (18), who reported no differences in plasma triglyceride content between the positive and negative control containing 5 mg kg-1 of aflatoxins.

The present study clearly indicates significant increase in cholesterol concentration in the aflatoxin-treated. Exact mechanism for a significant rise in cholesterol content in liver is not clearly understood. This might be due to fatty infiltration and degeneration of hepatocytes during aflatoxicosis as toxin is fat soluble. Once brought to the liver through hepatic portal system, fat present in the liver cells might dissolve toxin and retain it. Verma et al. (19) have reported significant rise in cholesterol in aflatoxinfed rabbits. Rastogi et al. (20) also reported increased cholesterol in rat liver.

AFB1 is known to be hepatotoxic and causes genetic damage (21). In the present study, significant increases in the levels of AST and ALT were observed upon treatment with AFB1 during 42 days. This increase in the values of serum AST and ALT might be due to hepatotoxic effects of AFB1 which is in agreement with the findings of Fani Makki et al. (22).

Critical to protein synthesis, enzymatic metabolism and detoxification processes, the liver is the primary site of AFB1 activation and therefore toxicity (23). Aflatoxicosis in poultry is characterized by an enlarged, pale, and friable liver although relative liver weight can initially decrease, longer exposure to dietary AFB1 raises the relative weight of the liver and causes pale or yellowed pigmentation (13). Both acute and chronic AFB1 consumption by poultry cause other hepatic lesions. Common histopathological signs of AFB1-induced liver damage include focal necrotic hepatocytes or hemorrhages (24). Acute damage initiates inflammatory responses and leads to leukocyte infiltration and proliferation in the liver (25). In poultry, chronic AFB1 consumption is mutagenic and leads to remodeling of liver tissues. Hyperplasia of bile duct epithelial cells or oval cells develops first, followed by periportal fibrosis and nodular tissue regeneration (26).

Conclusion

It can be concluded that specific IgY is effective in reducing the defects of experimental aflatoxicosis as well as improving performance in broilers.

Conflicts of Interest

All authors have no conflicts of interest to express.

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