Original Article

In Vivo Assessment of Gamma Rays, Electron-beam Irradiation plus a Commercial Toxin Binder (Milbond-TX) As an Anti-Aflatoxin B_1 in a Chicken Model

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ABSTRACT

Background: *Aspergillus flavus* is the most important fungus for production of Aflatoxin B1 (AFB1). This study evaluated the ability of gamma rays (GRs) and electron-beam irradiation (EBI) to counteract the deleterious effects of aflatoxin B1 (AFB1) in a chicken model.

Methods: Overall, 168 one-day-old male Coturnix quails were assigned to eight treatments for 42 d in Tehran, Iran, in 2010 and 2011. Two dietary inclusion rates of AFB1 (0 and 2 ppm) and toxin binders, such as 0, 27 kGy doses of GRs, 27 kGy doses of EBI, and 0.3% of commercial toxin binder-milbond-TX, were tested in a 2×4 factorial manner. Serum biochemical parameters, immune response, and dietary treatments on factors associated with kidney and lipid profiles were determined on day 42.

Results: AFB1 significantly decreased the hematological parameters (Hematocrit in 21 and 42 d), immune response (White blood cell (WBC), heterophil to lymphocyte ratio (H/L) and sheep red blood cell (SRBC)), and blood chemical factors (glucose, albumin, total protein, and triglycerides) compared to the control diet (P<0.05). It also significantly increased the calcium, phosphorus, uric acid, and low-density lipoprotein (LDL) levels (P<0.05). The addition of toxin binders, such as GRs, EBI, and milbond-TX, in the contaminated diets significantly diminished the inhibitory effects of dietary AFB1 (P<0.05) on the hematological parameters, immune response, blood chemical factors, and factors associated with kidney and lipids profile with no differences compared to the control diet.

Conclusion: The addition of these toxin binders may reduce the adverse effects produced by the presence of AFB1 in Japanese quails' diets.

Keywords: Aflatoxin B_1, Electron-Beam Irradiation, Gamma Rays, Japanese Quail, Milbond-TX.

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INTRODUCTION

Aflatoxins (AFs), which are secondary metabolites of various *Aspergillus* species, such as flavus and parasiticus, commonly pollute an extensive variety of feedstuff [1, 2]. In addition, AFs are furanocoumarin compounds, and they consist of B_1, B_2, G_1, G_2, M_1, and M_2 [1]. These mycotoxins pollute a plethora of wide varieties of agricultural commodities, including oilseed meals, dried fruits, spices, and cereals [2, 3]. Aflatoxin B1 (AFB1) is most frequently experienced among the different kinds of AFs. It also is considered more poisonous than other AFs [1]. The liver is an important organ for the accumulation and metabolism of AFB1.

The metabolism of AFB1 after absorption has been studied thoroughly [3, 4]. In general, cytochrome P450 enzymes (CYP) (including CYP1A2, CYP3A4, and CYP2A6) in the liver and other tissues convert AFB1 to epoxides (AFB1-8, 9-exo-epoxide, and AFB1-8,9-endo-epoxide) and to AFM1, AFP1, AFQ1, and its reduced form, aflatoxicol [3, 5]. However, more data are required to comprehend completely the changes in the metabolism of poultry with species that are comparatively more sensitive to AFB1.

Gamma rays (GRs) and electron beams are two important sources of irradiation. In the gamma irradiation process, highly purified Co60 creates GRs to reach a constant and stable Ni60 state [6]. Electron-beam irradiation (EBI) utilizes

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accelerators that produce electron beams of energy directed toward the product by a magnet [7]. Electron beams can enter products with a 2- to 4-inch thickness, whereas GRs can enter into the entire product. Electron-beam irradiation has its advantages over GRs as follows: 1) It includes higher does rate capability, no nuclear waste, and switchable accelerators; 2) It can contact the food product from both the top and the bottom for a uniform application, therefore, more active elimination of bacteria. Therefore, EBI is considered to have a plethora of benefits over gamma irradiation [7].

Supplements of GRs and EBI have an anti-aflatoxin B1 effect in animals. In addition, in vivo reports on the effect of gamma rays (GRs) plus electron-beam irradiation (EBI) on blood parameters and immunity are very limited. Therefore, the objectives of this study were to investigate the effects of GRs and EBI on hematological parameters and immune response in Japanese quails.

MATERIAL AND METHODS

Irradiation and Dosimetry

This study was performed from 10 February 2010, to 10 May 2011 in the Animal Husbandry Unit of the Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran.

The diet samples were divided into four equal portions and packed in polyethylene bags in preparation for irradiation. Cobalt-60 with 27 kGy doses [8] was used for γ-irradiation (Gamma cell device, model PX-30, Nuclear Energy Organization, Beam Application Research Institute, Tehran, Iran) (Fig. 1). Moreover, the samples were exposed to a 10-megavolt (MeV) electron beam by using a Rhodotron accelerator model TT-200 (IBA Co., Belgium) installed at the Yazd Irradiation Center, Radiation Applications Research School, Atomic Energy Organization, Iran(Fig. 2). All irradiations were performed at room temperature. Cellulose triacetate film was used to determine the degree of homogeneity of the irradiation dose.

Figure 1. Gamma cell device, model PX-30, Nuclear Energy Organization, Beam Application Research Institute, Tehran, Iran.

Figure 2. Rhodotron accelerator model TT-200 (IBA Co., Belgium) installed at the Yazd Irradiation Center, Radiation Applications Research School, Atomic Energy Organization, Iran.

Ethical Approval

All animals received humane care in compliance with the guidelines of the Poultry Science Department at Tarbiat Modares University, Tehran, Iran.

Birds and Diets

One-day-old male Coturnix quails (168 in total) (body weight, 9 ± 0.5 gr) were allotted randomly into eight treatment groups, with three replicates in a 2 × 4 factorial experiment. The birds of each group were reared on the litter for 42 d. Feed and water were given ad libitum. The experimental treatments included: 1) control; 2) 2 ppm of aflatoxin B1 (AFB1); 3) 27 kGy dose of γ-rays irradiation; 4) 27 kGy dose of EBI; 5) 0.3% of milbond-TX; 6) 2 ppm of AFB1 + γ-rays; 7) 2 ppm of AFB1 + EBI and 8) 2 ppm of AFB1 + 0.3% milbond-TX. They received a commercial diet formulated to meet or exceed the nutritional requirements of Japanese quails, as recommended by the National Research Council [9] (Table 1). The pure AFB1 vial (Sigma Alorich-Art No. 6636) and milbond-TX (MIL-A-48611) were provided by Zarin Gostar Sarina Company located in Khorasan Razavi Province, Kashmar, Iran. In addition, all animals received humane care in compliance with the guidelines of Poultry Science Department at Tarbiat Modares University, Tehran, Iran.

Table 1. Analysis of feed composition in Japanese quail.

ME (Kcal Per kg)	3000
CP (Percent)	23.0
EE (Percent)	6.00
Methionine (Percent)	0.64
Lysine (Percent)	1.40
Methionine (Percent)+Cysteine	1.00
Calcium (Percent)	1.00
Available phosphorus (Percent)	0.45
Sodium (Percent)	0.20

Metabolizable energy (ME); Crude protein (CP); Ether extract (EE)

Serum Biochemistry

Two birds from each treatment group were selected randomly. At the end of the trial, blood was gathered from the brachial vein. The clear serum was gathered in pasteurized disposable plastic tubes and stored at -20 ºC. The samples were sent for analysis to evaluate total protein, albumin, calcium, phosphorus, glucose, uric acid, creatinine, triglyceride, LDL-C, and HDL-C by means of as a clinical chemistry auto analyzer (Tokyo Boeki, TMS, 1024, Japan) with commercial test kits (Spinreact, Spain). The red blood cell (RBC) and white blood cell (WBC) counts also were identified by a haemocytometer method using Natt-Herrick solution; haematocrit values were measured by the microhaematocrit method at d 21 and 42. Moreover, 100 leukocytes per sample were calculated by heterophil to lymphocyte separation as an optical microscope. The heterophil-to-lymphocyte ratio (H/L) was calculated and documented. Sheep's red blood cell (SRBC) values were evaluated by the hemagglutination inhibition test (HI).

Statistical Analysis

The data were subjected to ANOVA as a completely randomized design in the factorial procedure [10]. Tukey's test was used for multiple comparisons when a significant interaction was detected. All statements of significance were based on probability (P<0.05).

RESULTS

The data presented in Table 2, 3 and 4 show the effects of dietary treatments on blood chemical factors, factors associated with kidney and lipids profile, hematocrit and immune response in Japanese quails. Feeding AFB1 alone caused significant decreases in serum total protein (2.47 g/dl), albumin (2.33 g/dl), glucose (190.38 mg/dl), and total triglyceride (129.09 mmol/l), while a significant increase was found in calcium (9.58 mg/dl), phosphorus (6.47 mg/dl), uric acid (7.14 mg/dl), and LDL-C (202.86 mmol/l), (P<0.05; Tables 2 and 3). Dietary treatment had no significant effect on creatinine and HDL-C compared to the control diet (Table 3). AFB1 also caused significant changes in hematocrit and immune response in Japanese quails (P<0.05; Table 4). Compared to the control, WBC (5.31 10^6 mm^3), haematocrit at day 21 (36.1%) and day 42 (32.1%), H/L (0.51%), and SRBCs (4.12 log_2) values were decreased (P<0.05), but RBC counts (6.27 10^6 mm^3) did not differ significantly by AFB1 (Table 4).

The addition of γ-rays and electron-beam irradiation with 27 kGy doses plus a commercial toxin binder (milbond-TX) to an AF-containing diet significantly improved the toxic effects of AFB1 on the blood chemical factors, lipids profile, hematocrit, and immune response (P<0.05). However, the dietary addition of GI, EBI, and milbond-TX to the AF-treated did not produce any significant changes in the creatinine, HDL-C, and RBC levels compared with the other treatments (Tables 3 and 4). The GI and EBI at 27 kGy were effective in the destruction of AFB1 in the contaminated feed.

Table 2. Effect of different dietary treatments on hematological parameters (%) and immune response (Log_2) in Japanese quail.

Treatment		RBC (%-day 42)	WBC (%-day 42)	Hematocrit at day 21 (%)	Hematocrit at day 42 (%)	H/L (Log_2-day 42)	SRBC (Log_2-day 42)
1) Control		6.33	5.48 a	45.66 ab	37.66 b	0.65 a	5.51 a
2) 2 ppm AFB1		6.27	5.31 c	36.1 c	32.1 c	0.51 b	4.12 b
3) GI (kGy)		6.38	5.39 b	43.66 abc	40.66 ab	0.62 a	6.14 a
4) EBI (kGy)		6.35	5.42 ab	47.66 a	42.66 a	0.62 a	5.51 a
5) Milbond (%)		6.35	5.41 ab	46.33 ab	41.1 ab	0.66 a	5.11 a
6) 2 ppm AFB1 + GI		6.26	5.38 bc	39.66 abc	39.33 ab	0.63 a	4.51 a
7) 2 ppm AFB1+ EBI		6.31	5.45 ab	41.1 abc	41.33 ab	6.21 a	5.14 a
8) 2 ppm AFB1+ milbond		6.34	5.41 ab	38.33 bc	38.33 ab	6.11 a	4.54 a
±SEM		0.015	0.017	2.25	2.87	0.004	0.05
P-value	Aflatoxin	0.027	0.001	0.001	0.001	0.005	0.003
	Additives	0.943	0.003	0.011	0.019	0.044	0.021
	Interaction	0.873	0.004	0.034	0.031	0.003	0.013

(a,b,c) Main effect means within a column lacking a common superscript differ significantly (P<0.05).
Red blood cell (RBC); White blood cell (WBC); Heterophil to lymphocyte ratio (H/L); Sheep red blood cell (SRBC); 27 kGy doses of γ-irradiation (GI); 27 kGy doses of electron-Beam irradiation (EBI)

Table 3. Effect of different dietary treatments on blood chemical factors at the end of period (day 42).

Treatment		**Total protein (gr/dl)**	**Albumin (gr/dl)**	**Calcium (mg/dl)**	**Phosphorus** (mg/dl)	Glucose (mg/dl)
1) Control		5.87 ab	2.91 a	7.93 b	5.07 c	220.82 a
2) 2 ppm AFB1		2.47 d	2.33 b	9.58 a	6.47 a	190.38 b
3) GI (kGy)		4.91 bc	3.03 a	7.26 bc	5.81 abc	230.19 a
4) EBI (kGy)		6.78 a	3.23 a	6.45 c	5.71 bc	235.35 a
5) Milbond (%)		4.66 bc	3.02 a	7.46 bc	5.25 c	226.71 a
6) 2 ppm AFB1 + GI		5.43 b	3.23 a	7.97 b	6.23 ab	228.83 a
7) 2 ppm AFB1+ EBI		5.25 bc	3.03 a	7.29 bc	5.29 c	220.91 a
8) 2 ppm AFB1+ milbond		5.23 bc	3.28 a	8.35 b	5.51 c	232.48 a
±SEM		0.26	0.11	0.44	0.14	11.46
P-value	Aflatoxin	0.003	0.002	0.004	0.001	0.003
	Additives	0.042	0.021	0.034	0.045	0.002
	Interaction	0.031	0.022	0.011	0.007	0.033

(a,b,c) Main effect means within a column lacking a common superscript differ significantly (P<0.05).
27 kGy doses of γ-irradiation (GI); 27 kGy doses of electron-Beam irradiation (EBI)

Table 4. Effect of different dietary treatments on factors associated with kidney and lipids profile at the end of period (day 42).

Treatment		**Uric acid (mg/dl)**	**Creatinine (mg/dl)**	**Triglyceride (mmol/l)**	**LDL (mmol/l)**	HDL **(mmol/l)**
1) Control		5.55 b	0.028	178.78 a	191.16 abc	106.11
2) 2 ppm AFB1		7.14 a	0.031	129.09 b	202.86 a	89.19
3) GI (kGy)		5.55 b	0.031	173.09 a	188.45 bc	95.19
4) EBI (kGy)		5.63 b	0.031	172.34 a	181.1 c	94.18
5) Milbond (%)		5.29 b	0.035	177.4 a	196.46 ab	96.94
6) 2 ppm AFB1 + GI		5.54 b	0.034	175.28 a	191.41 a	95.19
7) 2 ppm AFB1+ EBI		5.53 b	0.030	174.77 a	198.98 abc	94.17
8) 2 ppm AFB1+ milbond		5.32 b	0.032	177.19 a	193.33 abc	96.61
±SEM		0.11	0.0024	32.53	45.43	10.15
P-value	Aflatoxin	0.003	0.022	0.002	0.004	0.037
	Additives	0.072	0.511	0.066	0.044	0.072
	Interaction	0.015	0.721	0.027	0.011	0.093

(a,b,c) Main effect means within a column lacking a common superscript differ significantly (P<0.05).
27 kGy doses of γ-irradiation (GI); 27 kGy doses of electron-Beam irradiation (EBI); Low-density lipoprotein (LDL); High-density lipoprotein (HDL)

DISCUSSION

Chronic and sub-clinical aflatoxicosis cases were identified and recognized through specifying variations in serum biochemical and haematological parameters before important symptoms become apparent [11]. These parameters are delicate signs of toxic impacts of AF on blood samples. In addition, these toxic impacts occurred in the present study. Because of the hepatotoxic impacts of AF characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism, the researcher observed the decreased serum total protein, albumin, glucose, and triglyceride values and increased calcium, phosphorus, and uric acid, along with LDL levels in this present study (P<0.05; Tables 2 and 3) [12, 13]. The increased serum calcium, phosphorus, and uric acid values (P<0.05) were related to the osteoporosis and nephrotoxic impacts of AF, which was consistent with other research [2, 12].

The liver is considered the target organ for AF, because it is the organ where most AFs are bioactivated to the reactive 8, 9-epoxide form, known to bind DNA and proteins, damaging the liver structures and increasing the liver's weight [3]. Antibody production was suppressed against both T-dependent, such as WBC and SRBCs antigens, and that this suppression was observed during the secondary response and thus suggests a defect in the clonal expansion of antigen-specific heterophil and lymphocyte after antigen re-exposure [14]. In addition, AF exposure has been shown to reduce resistance to various bacterial, viral, and protozoan diseases in poultry [15].

Significant gross and histopathological lesions also were reported in the haemopoietic and immune system organs in different aspects of aflatoxicosis [16]. In this paper, important decreases were observed in SRBCs, WBC, heterophil to lymphocyte ratio, and haematocrit counts (P<0.05; Table 4). Moreover, these findings are consistent with other reports, which illustrated the supportive impacts of AF on haematopoiesis and immune responses [14, 16]. Our findings sustained statement of above studies, and the decrease in SRBCs and WBC counts proposed that the toxin elicited inflammatory response of the chicks [14].

In addition, radiation is used commonly several medical, agricultural, and industrial situations. Gamma rays and electron-beam irradiation were shown to significantly reduce the adverse effects of AFB1 in Japanese quails [17]. In addition, they increase membrane cholesterol levels, cause the oxidation of membrane protein, thiol groups, and lipid peroxidation, and impair of the membrane permeability barrier [2]. Our results demonstrated that acute 27-kGy dose of gamma ray and electron-Beam radiation (EBI) exposure could improve blood chemical, lipid profile, and immune response against dietary AFB1 in Japanese quails. We demonstrated that irradiation with 27 kGy did not affect RBC and SRBCs levels. "Other studies have also demonstrated significant rise in plasma Hb, potassium and LDH" [7, 17]. About interaction effects of AFB1 and gamma rays plus electron-Beam irradiation on blood chemical, lipid profile and immune response in poultry, we could not find any article to compare our results with previous researchers.

CONCLUSION

We believe further research is needed to determine the intraction effects of AF, GRs, and EBI on blood biochemical factors in poultry. Addition of GRs, EBI, and Milbond-TX in diets can reduce the toxic effects of AFB1 in Japanese quails.

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