Original Article

Protective Influence of Gamma Rays and Electron-Beam Irradiation with a Commercial Toxin Binder on Toxic Effects of Aflatoxin B_1 in Japanese Quails

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ABSTRACT

Background: This cross sectional study was conducted to evaluate the effect of γ-rays and electron-beam irradiation with a commercial toxin binder-milbond-TX on the performance, feed components, and meat quality of Japanese quails challenged with aflatoxin B_1.

Methods: Overall, 168 One-d-old chicks (Japanese quails) were allocated to eight treatments with three replicates based on a completely randomized design in a 2×4 factorial arrangement. Two levels of aflatoxin (Zero and 2 ppm) were considered as the essential factor. The secondary factor was involved in four levels (Control, 27 k Gy doses of γ-rays, electron-beam irradiation, and 0.3% commercial toxin binder-milbond-TX).

Results: *In vitro* condition showed that experiment diets do not have any effect on meat quality and feed components such as malondialdehyde, protein, fat, ash, and the dry matter. However, the highest and the lowest levels of feed intake and body weight gain were observed in the 7th treatment (2-ppm aflatoxin B_1 + electron-beam irradiation) and the 2nd treatment (2-ppm aflatoxin B_1 alone), respectively in 1-15 and 29-42 days ($P \leq 0.05$). In addition, the highest liver weight (1.73), spleen (0.57) and bursa (0.18) were seen in the second treatment (2 ppm aflatoxin B_1) alone ($P \leq 0.05$) at the age of 42 days.

Conclusion: γ-rays and electron-beam irradiation plus commercial toxin binder-milbond-TX can be used for aflatoxin B_1 absorption in poultry diets.

Keywords: Aflatoxin B_1, Irradiation, Performance, Toxin Binder.

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INTRODUCTION

Aflatoxins (AFs) are the most important members of the mycotoxin groups produced by toxigenic fungi, mainly *Aspergillus flavus* and *A. parasiticus* [1]. Aflatoxin B_1 (AFB1), B_2, G_1 and G_2 are the secondary metabolites of these fungi, with B_1 the most toxic and a known carcinogenic [2-4]. These fungi reproduce and grow in different conditions including humidity, temperatures, and pH [3]. AFB1 is activated to AFB1-8, 9-epoxide and forms adduct primarily at the N7 position of guanine. It is responsible for its mutagenic and carcinogenic effects [1, 4]. "In addition, lipid peroxidation and oxidative DNA damage are manifestations of AFB1-induced toxicity" [1]. AFB1 also causes poor performance, liver and meat lesions plus immunosuppression in animals [1, 5]. Significant increase in AFB1-induced lipid peroxidation in animal's meat, liver, and kidney has been mentioned earlier [6, 7].

Ameliorative effects of AFB1 toxicity in poultry diets were made by some absorbents such as hydrated sodium, calcium aluminosilicate (HSCA-millpond-TX) [8, 9] plus γ-rays [10] and electron-beam irradiation (EBI) [11]. Milbond-TX might also influence calcium and phosphorus utilization [8]. However, there is concern that some dietary milbond-TX might absorb small nutritional particles in the

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gastrointestinal tract. Electron beams can penetrate products with a 2 to 4-inch thickness, while gamma rays (GRs) can penetrate the entire product. Electron-beam irradiation has its advantages over GRs [11]. Additionally, it can contact the food product from both its top and bottom, leading to a uniform application. Thus, EBI is thought to have several advantages over GRs [11]. Using 10 kilogrey (kGy) γ- irradiation does not destroy the measurable effects on amino acids, while reduces microbial contamination and increases the endurance time of food nutrients [12]. Lack of appropriate equipment and facilities for irradiation in various parts is one of the drawbacks of this processing method and the main reason for the recent increase in its cost. Numerous reports have been published about the use of irradiated diet in animal nutrition such as the improvement in the digestibility of nutrients after irradiation in the dairy cow [13] and broilers [11].

The purpose of this study was to evaluate the effect of γ-rays and electron-beam irradiation with a commercial toxin binder (milbond-TX) on performance, meat quality and feed component in Japanese quail exposed to AFB1.

MATERIAL AND METHODS

Irradiation and Feed Components

This study was performed in summer 2010 in the Animal Husbandry Unit of the Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran. Diets were formulated to meet the nutrition requirements of broilers (NRC, 1999). They were packed in 30 × 70 cm polyethylene bags (0.5 mm thick). Cobalt-60 with 27 kGy dose was employed for γ-irradiation (Gamma cell device, model PX-30, Nuclear Energy Organization, Beam Application Research Institute, Tehran, Iran). Moreover, electron-Beam accelerator (Rodotron) TT200 with 27 kGy dose was used for irradiation on the sample diets (Beam Application Research Institute, Yazd, Iran). Cellulose triacetate film was used to determine the homogeneity degree of the irradiation dose. The quantity of protein, fat, ash and dry matter (DM) in feed contaminated with AFB1 was evaluated using the AOAC method (Association of Official Analytical Chemist, 1990) [14].

Experimental Diets

Overall, 168 one-day-old Japanese quails were reared on the litter for 42 days and were evaluated using 2×4 factorial design; a completely randomized design with eight treatments, three replicates and seven chicks in each unit. The experimental treatments included T_1: control; T_2: 2 ppm of aflatoxin B_1 (AFB1); T_3: 27 kGy dose of γ-rays irradiation; T_4: 27 kGy dose of EBI; T_5: 0.3% of milbond-TX; T_6) 2 ppm of AFB1 + γ-rays; T_7: 2 ppm of AFB1 + EBI and T_8: 2 ppm of AFB1 + 0.3% milbond-TX. Feed and water were provided *ad libitum* throughout the experiment. The pure AFB1 vial and milbond-TX were provided by Zarin Gostar Sarina Company located in Kashmar, Khorasan Razavi Province, Iran. The feed composition analysis in basal diets is presented in Table 1.

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)

All animals were treated humanely and in compliance with the guidelines of the Poultry Research Center, Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran (12/40-4-R-A/BU). In addition, we used the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes.

Meat Quality and Performance

In this phase, the samples of femur muscle form each treatment were placed on plastic bags in -20 °C temperature at the end of the period (day 42). The meat anti-oxidant parameter such as malondialdehyde (MDA) was evaluated on first day and one month (day 30) after the end of the study. In addition, lipid oxidation was measured by thiobarbituric-acid test (TBA) plus colorimetric method and the spectrophotometer device (523 nm) in each samples [15]. In this method, the meat anti-oxidant parameters react with TBA and produce the purple color. The quantity of this qualitative parameter can be measured in tissues by the colorimetric and calibration methods. In addition, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were respectively measured at 1-15, 16-28 and 29-42 periods. The quails deprived from feed for 4 h and the residual feed was measured in each treat. Quail chicks were slaughtered at the end of each period (21 and 42 days) and their certain organs weight such as heart, liver, bursa and spleen was measured.

Statistical Analysis

Statistical analyses included analysis of variance (ANOVA); based on completely randomized design in 2×4 factorial arrangement was conducted in statistical analysis software (SAS Inst., 2001). Moreover, post hoc comparisons were made using Tukey-kramer multiple range test. Statistical significance was set at $P \leq 0.05$.

RESULTS

Gamma Rays, Electron-Beam Irradiation and Feed Components

The effect of γ-rays and electron-Beam irradiation on feed component in Japanese quail is presented in Table 2. As can be seen, γ-rays and EBI did not have any influence on feed components such as protein, fat, ash, and dry matter (DM). The protein levels in γ-ray treatment were less when compared with EBI treatment. However, the difference was not significant.

Table 2. Effect of γ-rays and electron-beam irradiation on feed component in Japanese quail.

Treatment	CP(%)	EE(%)	Ash(%)	DM(%)
Control	22.76	4.820	7.530	91.16
GI	20.83	4.740	7.060	90.06
EBI	21.33	4.780	7.170	89.60
SEM±	0.320	0.006	0.041	0.380
P-value	0.64	0.41	0.83	0.41

Dry matter (DM); γ-irradiation (GI); Electron-Beam irradiation (EBI)

Meat Quality and Performance

The meat quality factors of experimental diets are summarized in Table 3. There was no significant effect on the meat antioxidant levels such as the MDA factor when the quails received different diets. However, the highest (3.8) and lowest (2.3) MDA factor were observed in the second treatment (2 ppm AFB1 alone) and the fourth treatment (EBI alone), respectively, at 30 days after the meat was frozen ($P \geq 0.05$). The Japanese quail's performance is presented in three different periods in Tables 4 to 6. Compared to the control diets, the interaction effects of FI and BWG were significant in the 2nd treatment (2 ppm AFB1 alone) at the starter (1-15 days), finisher (29-42 days) and total periods ($P \leq 0.05$) (Table 4 and 5). In addition, the highest and lowest levels of the FI and BWG in the starter (1-15 day) and finisher (29-42 day) periods occurred in the 7th treatment (2 ppm AFB1 + EBI) and the 2nd treatment (2 ppm AFB1 alone), respectively ($P \leq 0.05$). In contrast, the interaction effects of FCR were not significant in different diets (Table 6), but the main effects of the second treatment (2 ppm of AFB1 alone) in the finisher (29-42 day) and total period was significant with 0.05 probability level.

Table 3. Effect of different dietary treatments on meat quality factor; Malondialdehyde (MDA (gr MDA/kg)) in Japanese quail at 0 and 30 days after storage in freezer (-20 °C).

Treatment		0 days	30 days
1) Control		1.5	2.8
2) 2 ppm AFB1		1.6	3.8
3) GI		1.2	2.7
4) EBI		1.1	2.3
5) Toxin binder (Milbond)		1.3	2.8
6) 2 ppm AFB1 + GI		1.4	2.6
7) 2 ppm AFB1+ EBI		1.2	2.9
8) 2 ppm AFB1+ milbond		1.2	3.1
SEM±		0.18	0.08
P-value	Aflatoxin	0.23	0.71
	Additives	0.45	0.25
	Interaction	0.33	0.19

γ-irradiation (GI); Electron-Beam irradiation (EBI)

Table 4. Effect of different dietary treatments on feed intake (FI) in Japanese quail.

Treatment		1-15 days	16-28 days	29-42 days	Total Period
1) Control		101.01 a	196.16	294.0 a	591.5 c
2) 2 ppm AFB1		97.01 b	195.43	288.3 b	580.7 d
3) GI		102.5 a	197.33	295.6 a	595.5 a
4) EBI		102.3 a	197.01	295.3 a	594.6 a
5) Toxin binder (Milbond)		102.0 a	196.01	294.6 a	592.6 c
6) 2 ppm AFB1 + GI		102.6 a	196.66	296.8 a	596.5 ab
7) 2 ppm AFB1+ EBI		113.1 a	204.01	314.6 a	609.3 a
8) 2 ppm AFB1+ milbond		102.0 a	197.01	295.0 a	594.1 bc
SEM±		4.39	14.72	4.32	22.10
P-value	Aflatoxin	0.012	0.018	0.0231	0.0111
	Additives	0.026	0.931	0.0018	0.0061
	Interaction	0.014	0.981	0.0141	0.0012

(a,b,c) Main effect means within a column lacking a common superscript differ significantly ($P<0.05$).
γ-irradiation (GI); Electron-Beam irradiation (EBI)

Table5. Effect of different dietary treatments on body weight gain (BWG) in Japanese quail.

Treatment		1-15 days	16-28 days	29-42 days	Total Period
1) Control		91.8 bc	180.2	282.7 a	432.2 b
2) 2 ppm AFB1		89.0 c	180.1	275.0 c	351.5 c
3) GI		94.2 ab	183.3	281.1 ab	474.8 a
4) EBI		93.3 ab	182.9	280.6 ab	451.7 b
5) Toxin binder (Milbond)		91.4 bc	181.1	279.6 ab	445.1 b
6) 2 ppm AFB1 + GI		95.0 a	181.8	282.6 ab	411.1 bc
7) 2 ppm AFB1+ EBI		99.6 a	189.3	300.8 ab	438.2 b
8) 2 ppm AFB1+ milbond		91.8 bc	180.6	278.7 b	400.6 bc
SEM±		2.26	14.58	4.24	12.10
P-value	Aflatoxin	0.821	0.53	0.741	0.041
	Additives	0.0002	0.68	0.002	0.011
	Interaction	0.016	0.97	0.026	0.032

(a,b,c) Main effect means within a column lacking a common superscript differ significantly ($P<0.05$)
γ-irradiation (GI); Electron-Beam irradiation (EBI)

Table 6. Effect of different dietary treatments on feed conversion ratio (FCR) in Japanese quail.

Treatment		1-15 days	16-28 days	29-42 days	Total Period
1) Control		1.26	2.21	2.98	2.12
2) 2 ppm AFB1		1.25	2.14	3.03	2.11
3) GI		1.24	2.21	3.02	2.13
4) EBI		1.25	2.20	3.03	2.11
5) Toxin binder (Milbond)		1.28	2.18	2.99	2.11
6) 2 ppm AFB1 + GI		1.22	2.30	2.94	2.11
7) 2 ppm AFB1+ EBI		1.24	2.26	2.95	2.12
8) 2 ppm AFB1+ milbond		1.27	2.21	3.00	2.13
±SEM		0.0016	0.011	0.02	0.00046
P-value	Aflatoxin	0.48	0.49	0.012	0.028
	Additives	0.33	0.61	0.990	0.520
	Interaction	0.98	0.57	0.810	0.670

γ-irradiation (GI); Electron-Beam irradiation (EBI)

Relative Weight of Internal Organs

The weight of internal organs on the 21st and 42nd days is listed in Tables 7 and 8. The interaction effect showed that the weight of the organs, such as spleen, bursa, proventriculus, gizzard and heart, were not significant at the age of 21 days, with the exception of the liver (*P*≤0.05) (See Table 7). The highest (2.88) and lowest (2.42) liver weights were seen in the second (2 ppm of AFB1 alone) and fourth (EBI alone) treatment (*P*≤0.05), respectively. On the contrary, the weight of internal organs such as spleen, bursa and liver was significant at the age of 42 days (*P*≤0.05). The main effect of AFB1 alone on internal organs such as spleen, bursa, proventriculus, gizzard and liver was significant (*P*≤0.05), (Table 8). Furthermore, the heaviest weights of spleen (0.57), bursa (0.18) and liver (1.73) were attributed to the second treatment (2 ppm of AFB1 alone), (*P*≤0.05). However, the weight of internal organs in the third (γ-irradiation alone), fourth (EBI alone) and fifth treatment (milbond-TX) did not show any differences.

Table 7. Effect of different dietary treatments on relative weight of internal organs in Japanese quail at 21 days of age.

Treatment		Spleen	Bursa	Proventriculus	Gizzard	Heart	Liver
1) Control		0.093	0.12	0.40	2.29	0.87	2.46 b
2) 2 ppm AFB1		0.040	0.097	0.36	2.24	0.81	2.88 a
3) GI		0.061	0.11	0.45	2.21	0.88	2.46 b
4) EBI		0.082	0.11	0.43	2.25	0.87	2.42 b
5) Toxin binder (Milbond)		0.038	0.14	0.44	2.29	0.94	2.44 b
6) 2 ppm AFB1 + GI		0.064	0.11	0.36	2.23	0.87	2.58 b
7) 2 ppm AFB1+ EBI		0.074	0.10	0.37	2.23	0.86	2.53 b
8) 2 ppm AFB1+ milbond		0.085	0.11	0.39	2.21	0.89	2.54 b
±SEM		0.0011	0.0012	0.004	0.018	0.008	0.083
P-value	Aflatoxin	0.83	0.22	0.04	0.60	0.36	0.210
	Additives	0.83	0.72	0.80	0.96	0.55	0.120
	Interaction	0.13	0.72	0.94	0.93	0.96	0.041

(a,b) Main effect means within a column lacking a common superscript differ significantly (*P*<0.05).
γ-irradiation (GI); Electron-Beam irradiation (EBI)

Table 8. Effect of different dietary treatments on relative weight of internal organs in Japanese quail at 42 days of age.

Treatment		Spleen	Bursa	Proventriculus	Gizzard	Heart	Liver
1) Control		0.12 b	0.084 bc	0.35	1.85	0.84	1.45 b
2) 2 ppm AFB1		0.57 a	0.18 a	0.17	1.61	0.83	1.73 a
3) GI		0.095 ab	0.15 ab	0.34	1.42	0.83	1.46 b
4) EBI		0.054 b	0.03 abc	0.37	1.64	0.84	1.44 b
5) Toxin binder (Milbond)		0.07 b	0.10 abc	0.27	1.60	0.82	1.45 b
6) 2 ppm AFB1 + GI		0.088 b	0.068 c	0.31	1.62	0.86	1.55 b
7) 2 ppm AFB1+ EBI		0.052 b	0.15 a	0.34	1.47	0.84	1.54 b
8) 2 ppm AFB1+ milbond		0.056 b	0.14 ab	0.38	1.60	0.82	1.52 b
±SEM		0.0061	0.0012	0.007	0.054	0.0023	0.032
P-value	Aflatoxin	0.047	0.011	0.031	0.032	0.93	0.11
	Additives	0.038	0.012	0.33	0.44	0.67	0.025
	Interaction	0.0014	0.015	0.095	0.37	0.96	0.031

(a,b,c) Main effect means within a column lacking a common superscript differ significantly (*P*<0.05).
γ-irradiation (GI); Electron-Beam irradiation (EBI)

DISCUSSION

The mechanism of action of GRs plus EBI on performance, feed components and meat quality in Japanese quails is not clearly understood. Currently, it seems that irradiation can be referred to AFB1 absorbent on meat quality in poultries. AFs decrease the quantity of total soluble carbohydrates. Non-starch polysaccharides (NSP) digestion increases the adhesion of gastro intestinal tract and finally reduces the digestion and adsorption of nutrients [1, 4]. So that, the absorption of some anti-oxidant vitamins such as C and E will decrease but the level of tissues oxidation will increase unnaturally. Milbond-TX decreases the MDA oxidation plus lipids peroxidation, also its can lead to more effective elimination of bacteria by reduction of free radicals [16].

In this study, 27 kGy doses of γ-rays and EBI did not significantly influence on meat quality and feed components in diet. This result is similar with previous studies [8, 13, 17]. In contrast, other authors reported that γ-rays improves the performance parameters and increases the nutrient digestibility by reduction of the phytic acid, tripsin and kimotripsin [18]. Accordingly, γ-rays in low doses can decrease the toxic effects of AFB1 and improve the digestibility of nutrients [17]. Even though electron-beams are less penetrating than the γ-rays, they are also thought to have an effect on the quality of the product [11]. Both sources are active in declining bacteria in food products like poultry. However, this evaluation showed that electron-beam irradiation is more effective than the γ-rays on meat quality.

In this study, 27 kGy doses of γ-rays plus EBI and 0.3% milbond-TX affected on FI, BWG and certain organ weight such as bursa, liver and spleen in Japanese quails. Hepatic injuries and increase the liver weight is a classic sign of AFB1 consumption [1, 5]. The lipoproteins increase the liver lipids and is the main reason for increase the liver weight. Accordingly, each factor that stimulates the immune system can affect on the weight of bursa and spleen [19, 20]. There is much evidence about the effects of AFB1 on increasing the relative weight of certain organs such as liver, kidney, heart, preventriculus, gizzard, spleen and pancreas in broilers [5]. Furthermore, the adverse effects of AFs on performance depend to the AF concentration in diet [2, 5]. The toxic effects of AFB1 on the liver and the immune system decrease the feed intake in broilers [1]. The aluminosilicates can control the anion- on meat quality balance, increases the activity and stimulation of intestinal enzymes and increase the feed digestion [21, 22].

CONCLUSION

Dietary supplementation with γ-rays, electron-beam irradiation and Milbond-TX are able to decrease the AFB1-toxicity in Japanese quails.

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