The efficacy of various additives to reduce the toxicity of aflatoxin B_1 in broiler chicks

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Summary

Three-hundred and twenty 7-day-old Ross 308-strain broiler chickens were fed diets containing 0 or 125 ppb aflatoxin B_1 (AFB_1) from 7 to 28 days of age. Sodium bentonite (0.5%), yeast (*Saccharomyces cervisiae*) 0.2%, hydrated sodium calcium aluminosilicate (HSCAS) (0.5%), ammonia (0.5%), formycine (0.1%), and toxiban (0.1%) were added to the basal diet, as fed basis to determine the effects of these additives against aflatoxicosis. Diet free from aflatoxin, and diet containing aflatoxin (negative control group) were considered as comparison groups. Broiler chickens were divided into 32 groups of 10 with similar mean ± SD weight of 90 ± 0.64 g. Each experimental diet was replicated 4 times during 21 days. Body weight gain, daily weight gain, feed conversion ratio, daily and weekly feed intake, relative weight of organs (liver, intestines, heart, proventriculus and gizzard) and total serum protein were recorded. Relative weight of organs in chickens fed with diet containing AFB_1 alone were significantly greater ($P<0.01$) than that of those fed with other diets. Their body weight gain, daily weight gain, total serum protein concentration, however, were significantly lesser ($P<0.01$) compared with those fed with other diets. Experimental diets decreased the relative weight of organs in chickens fed with diets containing aflatoxin along with any of the experimental diets as compared with the negative control group. The feed conversion ratios were higher in chickens fed with diets containing aflatoxin. On the other hand, chickens receiving various additives in their diets showed an increase in body weight gains, serum total protein concentration and an improvement in the feed conversion ratio when compared with the negative control group ($P<0.01$). Generally, addition of the above compounds made an improvement against negative effects of AFB_1 in broiler chickens. Formycine was recognized to be the best additive in this respect.

Key words: Additives, Aflatoxin, Broiler chicks, Growth, Internal organs

Introduction

Aflatoxins (AFs), a class of mycotoxins produced by fungal species of genus *Aspergillu*s (*A. flavus* and *A. parasiticus*) are contaminants of feed ingredients routinely used for poultry rations (Wilson and Payne, 1994). AFs have been detected as contaminants of crops before and during harvesting and drying, in storage and after processing and manufacturing (Council for Agricultural Science and Technology, 1989). Among the different types of AFs produced, AF B_1 (AFB_1) is the most prevalent and potent and is often found in high concentrations in cereal grains and peanut meal (Gowda *et al*., 2004). Also, AFB_1 is considered to be one of the most protent hepatotoxins and a well-known hepatocarcinogen (Wilson and Payne, 1994). In many cases, AF contamination of feedstuffs may mean the difference between profit and loss to the poultry industry (Jones *et al*., 1982; Nichols, 1983; Hamilton, 1984) and negative effects on public health related to the human consumption of exposed animals and poultry (Ramos and Hernandes, 1997). AFs cause a variety of effects in poultry including poor growth and efficiency of feed conversion, increased mortality (Smith and Hamilton, 1970; Leeson *et al*., 1995) liver pathology, immunosuppression (Santin, 2000), and changes in relative organ weights (Edds and Bortell, 1983; Kubena *et*

al., 1990, 1993), kidney and spleen lesions (Glahn *et al*., 1991) and increased susceptibility to some environmental and infectious agents (Ibrahim *et al*., 2000; Oguz *et al*., 2003).

In addition to the preventive measures and decontamination technologies, in order to minimize the toxic effects of AF in contaminated feeds and feedstuffs, other approaches including physical, chemical and biological treatments have so far been employed (Goldblatt and Dollear, 1979; Anderson, 1983; Ibrahim *et al*., 2000; Miazzo *et al*., 2000; Oguz and Kurtoglu, 2000; Parlat *et al*., 2001; Rosa *et al*., 2001; Santin *et al*., 2003). Increased efforts are being undertaken in the areas of developing cost-effective procedures and products to effectively deal with the decontamination and remediation of feedstuffs contaminated with AFs.

The objective of this study was to evaluate the efficacy of various additives as dietary supplements for protection against toxicity of AFB_1 in broiler chickens.

Materials and Methods

Chickens and diets

Three-hundred and twenty 7-day-old, Ross 308-strain male chicks were provided from a commercial broiler producer. Individually weighed chicks were divided into 32 groups of 10 birds (four replicates per treatment) with similar mean ± SD body weight of 90 ± 0.64 g. The chicks were assigned to the following treatment groups: (1) Positive control diet, basal diet without additive; (2) basal diets + 0.1% formycine + 125 ppb AFB_1; (3) basal diet + 0.5% hydrated sodium calcium aluminosilicate (HSCAS) + 125 ppb AFB_1; (4) basal diet + 0.1% toxiban +125 ppb AFB_1; (5) basal diet + 0.2% *Saccharomyces cervisiae* + 125 ppb AFB_1; (6) basal diet + 0.5% sodium bentonite + 125 ppb AFB_1; (7) basal diet + 0.5% ammonia + 125 ppb AFB_1; and (8) basal diet + 125 ppb AFB_1 alone (negative control diet).

The chicks were housed in a temperature-controlled room with continuous lighting and were fed with a commercial ration (corn-soybean meal ration) to meet or exceed the critical nutrient requirements (NRC, 1994); they had access to feed and water ad libitum from 7–28 days of age. In addition, birds were inspected daily and any health related problems were recorded. The basal diet was supplemented with mineral and vitamins at levels recommended by NRC (1994), without added antibiotics, coccidiostats or growth promoters. Feed consumption and individual body weight (BW) were determined weekly and their feed conversion ratio was calculated. At 28 day of age, the study was terminated and one bird from each replicate was bled by wing vein for serum total protein analysis. Serum concentration of total protein was determined by biuret method according to the manufacturer's recommended procedure (Ziest Chem Diagnostics, Tehran, Iran) . After bleeding, birds were slaughtered and the liver, heart, proventriculus, gizzard and intestines were removed, cleaned and weighed.

Sodium bentonite, HSCAS, yeast (*Saccharomyces cervisiae*) and ammonia (analytical reagent grade) were provided from Merk, Chemical Co, Germany. Formycine (a mixture of formaldehyde, propionic acid, sodium bentonite and ammonia) and toxiban (a mixture of aluminosilicate and ammonium propionate), two commercial feed additives on the market, were purchased from IQF, Spain.

Aflatoxin production

AF produced isolate of *Aspergillus flavus* from pistachio nut was obtained from Rafsanjan Pistachio Research Center. The fungus was purified by single spore and cultured on potato dextrose agar (PDA) for four days at 25°C. Corn seeds were autoclaved three times in succeeding days. A 6-mm block of fungus from the edge of a growing colony was inoculated to 100 g sterilized seeds and incubated at 25°C for seven days. It was then autoclaved twice to kill the fungus. Contaminated seeds were dried in oven at 56°C for 48 hrs. The concentration of AFB_1 was measured by thin-layer chromatography (TLC-fluorometric densitometers, Camag-III, Germany). The dried corn seed containing 240 ppb AFB_1 was added to the basal diet to provide

the concentration of 125 ppb (μg/kg feed) of AFB_1.

Statistical analysis

The experiment was performed as a completely randomized design with four replicates of 10 chicks assigned to each of eight dietary treatments. Data were subjected to statistical analysis using the general linear models procedure of SAS software (SAS Institute, 1996). Variable means for treatments showing significant differences in the one-way ANOVA were compared using Duncan's multiple-range test. All statements of significance were based on the 0.01 level of probability.

Results

The effects of additives on feed conversion ratio, mean daily weight gain, body weight, daily and weekly feed consumption during experiment are presented in Table 1. Feed conversion ratio (kg of feed per kg of gain), mean daily weight gain and BW in the first, second and 3rd week of experiment were affected by treatments ($P<0.01$). There were 17, 12, 10, 10, 8 and 7.6% increase in daily feed consumption for treatments 5, 3, 7, 4, 6 and 2, respectively as compared with the negative control group. When compared with treatment 1, the chicks fed with the negative control diet, had lower daily feed consumption ($P<0.01$), with no statistically significant differences among other treatment groups. The weekly feed consumption was statistically significant among treatment for weeks 1 and 3 of the experiment ($P<0.01$). During the first week, the chicks, fed with treatment 1 had higher feed intake ($P<0.01$), when compared with the negative control. During the 3rd week, chicks fed with treatment 5 diet had higher feed consumption than those received treatments 2 and 7 ($P<0.01$). Treatment 1 had a better feed conversion ratio (1.56) than the negative control group (2.79) ($P<0.01$), with no differences among treatments 2, 3, 4 and treatment 1. There were 44, 33, 30, 30, 28, 27 and 22% improvement in feed conversion ratio for treatments 1, 2, 3, 4, 5, 6 and 7, respectively as compared with the negative control group. The mean daily weight gain (ADG) of treatment 1 was higher (30.8 g) than the negative control group (15.2 g) ($P<0.01$). There were no statistical differences among other treatments ($P>0.05$). Also ADG was significantly different between the negative control group and other treatments ($P<0.01$). ADG for treatments 2, 3, 4, 5, 6, 7 were increased by 60, 56, 52, 52, 42 and 36%, respectively, compared with the negative control group ($P<0.01$).

During the 2nd, 3rd and 4th weeks of age, birds in the negative control had significantly lower BW gain ($P<0.01$). During the 2nd week of age, (8 to 14 d) treatments 7, 2, 4, 3, 5 and 6 increased the BW gain by 49, 43, 42, 30, 29 and 23%, respectively, compared to the negative control group. As a result, treatment 7 (0.5% ammonia) had the highest BW gain (49%) among others.

The weight gain for treatments 3, 2, 4, 5, 6 and 7 was increased by 34, 31, 31, 30, 29 and 20%, respectively, during days 15 to 21 (the 3rd week of age), compared with the negative control group.

In contrast to the 2nd week, treatment 7 had the lowest increase in weight gain during days 22 to 28 (4th week of age). During days 22 to 28, there was no significant difference ($P>0.05$) among treatments 2, 3, 4, 5, 6 and 7 in weight gain; here were 47, 43, 41, 41, 33 and 28% increase in weight gain for treatments 2, 3, 4, 5, 6 and 7, respectively, as compared to the negative control group. The effects of the experimental diets on relative organ weights (g per 100 g of BW) and serum total protein are presented in Table 2.

Relative weights of liver, heart, proventriculus, gizzard and intestines were affected by treatments ($P<0.01$) and were increased by dietary AFB_1. Treatments 3, 4, 6 and 7 did not provide total protection as evidenced by relative liver weight to that were intermediate among those of treatments 1, 2, 5 and 8. Relative liver weight was increased by 177% in the negative control group compared with treatment 1 ($P<0.01$); treatments 4, 7, 6, 3, 5 and 2 decreased relative liver weight by 35, 40, 43, 47, 53 and 64%, respectively, compared with the

Table 1: Effects of various additives on feed consumption, feed conversion ratio, daily weight gain and body weight in broiler chickens

Treatments*	Feed consumption (g/d)	Weekly feed intake (g/bird)			Feed conversion ratio (kg/kg)**	Daily weight gain (g)	BW (g)		
		Day 14	Day 21	Day 28			Day 14	Day 21	Day 28
1	47.7^{a}	193.00^{a}	243.75	565.00^{a}	1.56^{c}	30.8^{a}	253.6^{a}	611.4^{a}	736.0^{a}
2	44.8^{ab}	185.00^{a}	240.00	526.25^{cd}	1.89^{bc}	24.3^{b}	210.8^{b}	373.0^{b}	600.1^{b}
3	46.8^{a}	187.43^{a}	233.75	540.00^{bc}	1.94^{bc}	23.6^{b}	191.8^{b}	381.0^{b}	584.8^{b}
4	45.9^{a}	176.25^{ab}	228.75	542.50^{bc}	1.96^{bc}	23.1^{b}	210.4^{b}	370.6^{b}	574.4^{b}
5	48.6^{a}	178.75^{ab}	230.00	558.75^{ab}	2.01^{b}	23.1^{b}	190.5^{b}	368.8^{b}	574.4^{b}
6	45.0^{ab}	162.50^{bc}	217.50	542.50^{bc}	2.05^{b}	21.5^{b}	181.1^{bc}	364.9^{b}	540.3^{b}
7	45.9^{a}	177.00^{ab}	230.00	535.00^{cd}	2.18^{b}	20.6^{b}	220.3^{ab}	341.6^{bc}	521.8^{b}
8	41.7^{b}	150.00^{c}	208.75	516.25^{d}	2.79^{a}	15.2^{c}	147.6^{c}	283.8^{c}	407.6^{c}
SE	0.45	2.75	3.19	3.10	0.03	0.79	9.82	14.90	20.83

$^{a-c}$: means presented in a column with different superscripts differ significantly (P<0.01). *(1) Positive control diet, basal diet without additives; (2) basal diet + 0.1% formycine + 125 ppb AFB_1; (3) basal diet + 0.5% HSCAS + 125 ppb AFB_1; (4) basal diet + 0.1% toxiban + 125 ppb AFB_1; (5) basal diet + 0.2% *Saccharomyces cervisiae* + 125 ppb AFB_1; (6) basal diet + 0.5% sodium bentonite + 125 ppb AFB_1; (7) basal diet + 0.5% ammonia + 125 ppb AFB_1 and (8) basal diet + 125 ppb AFB_1 alone (negative control). **Kilograms of feed per kilogram of gain. SE = Standard error

Table 2: Effects of various additives on relative organ weights and serum total protein in broiler chickens

Treatment*	Liver	Heart	Proventriculus	Gizzard	Intestines	Serum total protein (g/dL)
	g/100 g BW					
1	1.87^{d}	0.52^{bc}	0.62^{b}	2.07^{c}	3.89^{cd}	4.23^{a}
2	1.87^{d}	0.46^{bc}	0.46^{b}	2.16^{c}	3.53^{d}	3.95^{a}
3	2.79^{bc}	0.56^{bc}	0.68^{ab}	3.29^{b}	4.91^{abc}	3.23^{bc}
4	3.37^{b}	0.62^{b}	0.57^{b}	3.19^{b}	5.53^{ab}	3.10^{bc}
5	2.44^{cd}	0.49^{bc}	0.58^{b}	2.85^{bc}	3.89^{cd}	2.93^{c}
6	2.95^{bc}	0.37^{c}	0.55^{b}	3.05^{b}	4.06^{cd}	3.18^{bc}
7	3.09^{bc}	0.66^{b}	0.62^{b}	2.76^{bc}	4.57^{bcd}	3.50^{b}
8	5.19^{a}	1.10^{a}	0.92^{a}	4.67^{a}	5.88^{a}	2.50^{d}
SE	0.19	0.05	0.07	0.19	0.25	0.10

$^{a-c}$: means presented in a column with different superscripts differ significantly (P<0.01). *(1) Positive control diet, basal diet without additives; (2) basal diet + 0.1% formycine + 125 ppb AFB_1; (3) basal diet + 0.5% HSCAS + 125 ppb AFB_1; (4) basal diet + 0.1% toxiban + 125 ppb AFB_1; (5) basal diet + 0.2% *Saccharomyces cervisiae* + 125 ppb AFB_1; (6) basal diet + 0.5% sodium bentonite + 125 ppb AFB_1; (7) basal diet + 0.5% ammonia + 125 ppb AFB_1 and (8) basal diet + 125 ppb AFB_1 alone (negative control). SE = standard error

negative control group. Similar relative liver weights were found for treatments 1 and 2 (1.87). Relative heart weights decreased (P<0.01) by 40, 44, 49, 55, 58, 66 and 112% by treatments 7, 4, 3, 5, 2, 6 and 1, respectively, as compared with the negative control group. There were no significant differences among treatments 1, 2, 3, 4, 5 and 7, except between the negative control group and the rest of the treatments in relative heart weight. Treatment 2 had the lowest relative proventriculus weight (0.46) with no significant differences (P>0.05) among treatments except a difference between the negative control group and other treatments; only treatment 3 was not different from the negative control group (P<0.01). As compared with the negative control group, treatments 1, 3, 7, 5, 4, 6 and 2 decreased the relative proventriculus weight by 49, 26, 33, 37, 38, 40 and 50%, respectively. There were no significant differences among treatments 1, 2, 5 and 7 for the relative gizzard weights (P>0.05). Treatments 7, 5, 6, 3, 2 and 1 decreased the relative gizzard weight by 30, 32, 35, 39, 41, 54 and 125%, respectively, as compared with the negative control group. Treatment 2 had the lowest relative intestine weight (3.53) among treatments with significant differences (P<0.01) between treatments 1, 2, 5, 6, 7 and the negative control group. Treatments 4, 3, 7, 6, 5 and 2 decreased the relative intestine weight by 6, 16, 22, 31, 30,

34 and 40%, respectively, as compared with the negative control group.

Serum total protein was significantly ($P<0.01$) higher in treatments 1 (4.23) and 2 (3.95) and lower in the negative control group (2.50). Treatments 1, 2, 7, 3, 4, 5 and 6 increased serum total proteins by 69, 58, 40, 29, 24, 17 and 13%, respectively, compared with the negative control group. Only two very weak chickens in the negative control group died during day 14–21 in the present experiment.

Discussion

AFs are important to the poultry industry because of their toxicity and frequency of occurrence in food stuffs. The toxicity with AFs in poultry has been well documented (Huff *et al*., 1988). The lower food intake, daily weight gain and higher food conversion ratio observed in chicks fed with AFB_1 alone (the negative control group) as compared with other treatments ($P<0.01$) were consistent with previous reports on the performance depressing effects of AF (Edds and Bortell, 1983; Kubena *et al*., 1990). AF-contaminated food decreases the activities of several enzymes important to the digestion of carbohydrates, proteins, lipids, and nucleic acid in broiler chicks (Campbell *et al*., 1983). AF has been found to induce nutritional deficiency, resulting in depressed BW gain, hepatomegaly and proventriculus (Huff *et al*., 1986; Kubena *et al*., 1990). Boden and Jensen (1985) stated that the nutritional deficiency induced by AF could have disrupted the activity of the digestive enzymes and the absorption of essential nutrients. Additives were able to ameliorate the toxic effects of AFB_1 on performance of the chicks, except the intermediate effect of treatment 2 on food consumption.

Higher daily food intake (17%), daily weight gain (60%) and better feed conversion ratio (33%) were observed with treatments 5 and 2, respectively, as compared with the negativecontrol group which showed a better performance with treatment 2. During the first, 2nd and 3rd week of the experiment, the birds in treatment groups 7, 3 and 2 had an increase in BW gain by 49, 34 and 47%, respectively, compared with the negative control group. Treatment 2 (formycine) had better effects on feed conversion ratio, mean daily weight gain during the experiment and BW at 4th week of age.

The liver is considered to be the primary target organ of AF, and in poultry, the relative weight of liver is increased by lower levels of AF more than that of other organs (Smith and Hamilton, 1970; Huff *et al*., 1986). AF is a hepatotoxin causing an excessive build-up of hepatic lipids with hepatomegaly, proliferation of biliary ducts (Adav and Godinwar, 1997) and hepatocellular carcinoma (Hamilton, 1978). In the present study, we found the protective effects of additives with respect to the liver damage as indicated by relative liver weights, with the lowest liver weight (63%) for treatment 2 compared to the negative control group. Kubena *et al*. (1990) reported an increase in liver and kidney weights in broiler chicks when fed with AF.

Among dietary treatments, treatments 6, 5, 3 and 2 were more effective (66, 51, 54 and 39%, respectively) in decreasing the relative weights of heart, proventriculus, gizzard and intestine, respectively, compared with the negative control group. Mycotoxins are known to irritate the proventriculus and gizzard of the gastrointestinal tract, thus causing an increase in the relative weights of these organs (Huff and Doerr, 1981). In other word, treatment 2 (formycine) was as effective as treatment 1 (the positive control) in reducing the toxic effects of AFB_1 on relative organ weights.

The levels of serum total protein and albumin are sensitive indicators of aflatoxicosis. The serum total protein decreased (41%) among chicks consuming with the negative control feed (AFB_1 alone) when compared with the treatment 1 (the positive control; without AFB_1), which is in agreement with previous reports (Tung *et al*., 1970, 1975; Huff *et al*., 1986; Kubena *et al*., 1990). Decrease in concentration of whole plasma proteins and albumin have been proposed, as indicators of the alternation in protein synthesis observed in aflatoxicosis (Kubena *et al*., 1993; Abo-Norage *et al*., 1995). Treatment 2 (formycine) was the most effective additive for reducing the toxic effects of AFB_1 on

serum total protein. In the present study, the protective effects of additives, except for additives 2 (formycine) and 4 (toxiban) on performance of the chicks were in agreement with previous reports (Kubena *et al*., 1990; Ibrahim *et al*., 2000; Miazzo *et al*., 2000; Baptista *et al*., 2002; Satin *et al*., 2003; Thiesen, 2003; Gowda *et al*., 2004).

The results indicated that when used in conjunction with other good management practices, formycine may be used as another mean for the development of an integrated approach for the preventive management of AFB_1-contaminated food stuffs in broiler chicks.

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