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Efficacy of Propionic, Benzoic and Tartaric Acids in Preventing Biosynthesis of Aflatoxins in Poultry Feed

Ram Singh*, A.B. Mandal and Avishek Biswas

Division of Avian Nutrition and Feed Technology
Central Avian Research Institute, Izatnagar-243 122, India

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ABSTRACT

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A poultry feed was prepared using conventional feed ingredients which is free from aflatoxins. The moisture content of the feed was adjusted at 11, 13, 15 and 17%, respectively. The feeds with each level of moisture were then mixed with propionic, benzoic or tartaric acid each at various concentrations of 0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50%, respectively. Samples were taken in a 500 ml conical flask in duplicate, inoculated with fresh spores mould (*Aspergillus parasiticus* NRRL 2999) producing aflatoxins, incubated at room temperature for a period of one month and then analysed for the presence of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2). The results showed that at 11 % moisture level in feed, aflatoxin biosynthesis did not occur in any of the treatments. However, with the increase in moisture content in feed from 11 to 17%, there was increase in production of the aflatoxins. Complete inhibition of aflatoxins synthesis at 13% moisture level was achieved at 0.25% propionic or 0.30% benzoic or 0.40% tartaric acid concentration. The biosynthesis of any of the aflatoxins was completely inhibited at 0.50% propionic or benzoic acid in feed containing 15% moisture. However, propionic or benzoic or tartaric citric acid at 0.50% level in feed, failed to completely inhibit the synthesis of any of the four fractions of aflatoxins in feeds containing 17% moisture level, though with the increased concentrations of acids, the biosynthesis of total as well as individual fractions of aflatoxins decreased. It is thus concluded that the production of aflatoxin at 13% moisture level in poultry feed can be completely inhibited by adding propionic acid @ 0.25% or benzoic acid @ 0.30% or tartaric acid @ 0.40%. However, the level of organic acids varied with incresing level of moisture in the diet. Further, propionic acid was more efficaceous than benzoic or tartaric acid in inhibiting the synthesis of aflatoxins.

Keywords: Aflatoxin, *Aspergillus parasiticus,* Benzoic acid, Feed, Propionic acid, Tartaric acid.

INTRODUCTION

Presence of mycotoxins in feed is one of the major constraints in maintaining feed quality because the mycotoxins are widely present in feedstuffs around the world and may affect production even in very low concentration. These are low molecular weight

*Corresponding author: carirsingh@yahoo.co.in

toxic secondary metabolites produced by certain strains of filamentous fungi, which invade crops in the field, during harvesting or storage under favourable conditions. Although several hundred mycotoxins are known, the mycotoxins of most concern due to their toxicity and occurrence are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin and T-2 toxins (Iheshiulor *et al.*, 2011). *Aspergillus spp.* are primarily storage fungi that are found virtually everywhere in the world and produce aflatoxins (Coulombe *et al.*, 2005). Aflatoxins are fluorescent compounds, that are chemically classified as difurocoumarolactones and their biosynthesis by the producing fungi is via polyketide pathway (Smith and Moss, 1985). There are four major aflatoxins produced in feeds: Aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 can be classified as a highly toxic compound for most animal species, although it is extremely toxic for some highly susceptible species like ducklings and poults (Leeson *et al.*, 1995).

The toxicity of aflatoxins G_1, B_2 and G_2 is approximately 50, 20 and 10%, respectively, that of AFB_1 when tested against various animal species (Smith and Ross, 1991). The most widespread and the most studied group of mycotoxins, aflatoxins are of great concern in warm and humid climatic conditions like India (Singh *et al.*, 2010). The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which particular crop is grown, harvested or stored. Singh *et al.* (2010) conducted a survey in Uttar Pradesh and reported that ninety per cent of the maize samples were found to be positive for aflatoxin B_1 and the values ranged from 0.00 to 0.80 ppm with an average of 0.14 ppm of aflatoxin B_1. Aflatoxicosis in poultry causes listlessness, anorexia with lowered performance and increased mortality (Miazzo *et al.*, 2000), anemia (Oguz *et al.*, 2000), reduction of immune function (Oguz *et al.*, 2003), hepatotoxicosis and haemorrhage (Ortatatli and Oguz, 2001). Aflatoxins could be eliminated by growth inhibition of the fungus-producing strain. The aim of the present study was to assess the efficacy of propionic, benzoic and tartaric acids as mould growth inhibitor in poultry feed.

MATERIALS AND METHODS

Prepararion of fungus inoculum

The lyophilized preparation of *Aspergillus parasiticus* NRRL 2999, obtained from U.S. Department of Agriculture, Peoria, Illinois, U.S.A was used in this study. This lyophilized preparation was revived on Potato Dextrose Agar (PDA) medium and maintained in mycotoxin laboratory of Central Avian Research Institute (CARI), Izatnagar. To get fresh spores of the fungus, the culture was sub-cultured on PDA medium slants and stored at 5°C.

A poultry feed (Broiler starter) was prepared using aflatoxin-free feed ingredients namely: yellow maize (54.625%); soybean meal (30%); sunflower cake (3%); rapeseed meal (5%); fish meal (4.5%) and standard premixes. The moisture content of the feed was adjusted at four levels of 11, 13, 15 and 17% respectively. Feeds having varied moisture levels were mixed with propionic, benzoic or tartaric acids each at 0, 0.05,

0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50% concentrations. One hundred gram samples from each of these preparations were taken in a 500 ml conical flask in duplicate. The flasks were inoculated with freshly harvested spores of *Aspergillus parasiticus* NRRL 2999, cotton-plugged and incubated at room temperature for a period of one month. The minimum and maximum temperature during the study period varied from 16.0 to 37.0°C. At the end of incubation period, the flasks were autoclaved at 15 lbs pressure for 15 min to destroy the live spores and dried in hot air oven at 80°C for the estimation of aflatoxins. The extraction and estimation of aflatoxins was undertaken as per Pons *et al*. (1966). Aqueous acetone was used for extraction of the toxin and measured using thin layer chromatography. Aflatoxin contents were quantified using UV-spectrophotometry.

RESULTS AND DISCUSSION

Aflatoxin contents (AFB_1, AFB_2, AFG_1 and AFG_2; and total) of poultry feed as influenced by various concentrations of propionic, benzoic and tartaric acids at variable moisture levels are presented in Table 1, 2 and 3, respectively. At 11% moisture level, aflatoxin biosynthesis did not occurred at any of the propionic, benzoic or tartaric acid concentrations including control suggesting that moisture level is the first limiting factor for the biosynthesis of aflatoxins in feed. This result is in agreement with Jones (2008), who reported that moisture is the single most important factor in determining how rapidly moulds will grow in feeds. However, Gosh *et al*. (1996) reported that aflatoxin biosynthesis occurred even at 10% moisture when groundnut cake was inocubated with pure culture of *Aspergillus flavus*. Lopez and Christensen (1976), on the other hand, reported that *Aspergillus flavus* did not invade maize samples even at 17.5% moisture, however, extensive growth appeared at 18.5% moisture level.

In the present study, at 13% moisture level, the biosynthesis of AFB_1 reduced from 0.05 ppm (control) to 0.03 ppm at 0.20% propionic acid (PA) concentration, whereas, the biosynthesis of AFB_2 reduced from 0.04 ppm (control) to 0.02 ppm at the same concentration of PA. The biosynthesis of AFB_1 and AFB_2 was completely inhibited at 0.25% or higher concentrations of PA. The biosynthesis of AFG_1 decreased from 0.02 ppm (control) to 0.01 ppm at 0.15% PA concentration, whereas, the AFG_2 content was 0.01 ppm at 0.05% PA level. Biosynthesis of total AF decreased from 0.12 ppm (control) to 0.05 ppm at 0.20% PA. Complete inhibition of aflatoxins synthesis at 13% moisture level was achieved at 0.25% PA concentration. In case of benzoic acid (BA) treatment, at 13% moisture level (Table 2), the biosynthesis of AFB_1 and AFB_2 reduced from 0.05 ppm (control) to 0.02 and 0.01 ppm, respectively at 0.25% BA concentration. The production of both B fractions was completely inhibited by 0.30% or higher concentrations of BA. The biosynthesis of AFG_1 decreased from 0.03 ppm (control) to 0.01 ppm at 0.25% BA, whereas, that of AFG_2 decreased from 0.02 ppm (control) to 0.01 ppm at 0.15% concentration. The complete inhibition of AFG_1 and AFG_2 was achieved by 0.30% and 0.20% levels of BA, respectively. Total AF production decreased from 0.15 ppm (control) to 0.04 ppm at 0.25% BA. Complete inhibition of all the aflatoxin fractions

Table 1. Aflatoxin contents (ppm) of poultry feed as influenced by various concentrations of propionic acid

Moisture†		Propionic acid (PA) concentration (%)										
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
13%	AFB1	0.05	0.05	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
	AFB2	0.04	0.04	0.03	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	AFG1	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG2	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.12	0.12	0.09	0.06	0.05	0.00	0.00	0.00	0.00	0.00	0.00
15%	AFB1	0.45	0.43	0.43	0.40	0.38	0.38	0.39	0.30	0.20	0.09	0.00
	AFB2	0.32	0.33	0.32	0.29	0.28	0.25	0.22	0.20	0.12	0.05	0.00
	AFG1	0.22	0.21	0.20	0.19	0.16	0.13	0.11	0.05	0.00	0.00	0.00
	AFG2	0.20	0.20	0.18	0.15	0.14	0.11	0.10	0.02	0.00	0.00	0.00
	Total	1.19	1.17	1.13	1.03	0.96	0.87	0.82	0.57	0.32	0.14	0.00
17%	AFB1	0.60	0.61	0.55	0.55	0.52	0.52	0.50	0.49	0.45	0.40	0.35
	AFB2	0.41	0.40	0.36	0.37	0.38	0.38	0.35	0.33	0.30	0.25	0.22
	AFG1	0.28	0.22	0.21	0.20	0.19	0.19	0.18	0.18	0.15	0.09	0.07
	AFG2	0.24	0.20	0.19	0.18	0.18	0.15	0.13	0.12	0.11	0.07	0.06
	Total	1.53	1.43	1.31	1.30	1.27	1.24	1.16	1.12	1.01	0.81	0.70

†At 11% moisture level, aflatoxin biosynthesis did not occurred at any of the propionic acid concentrations including control.

production at 13% moisture level was achieved at 0.30% BA concentration. With regard to tartaric acid (TA) treatment, at 13% moisture level (Table 3), the biosynthesis of AFB_1 and AFB_2 reduced from 0.07 and 0.06 ppm (control) to 0.01 ppm at 0.35% TA concentration. The production of both B fractions was completely inhibited by 0.40% or higher levels of TA. The biosynthesis of AFG_1 and AFG_2 decreased from 0.03 ppm (control) to 0.01 ppm at 0.30% TA concentration. The complete inhibition of AFG_1 and AFG_2 was achieved by 0.35% level of TA. Total AF production decreased from 0.19 ppm (control) to 0.02 ppm at 0.35% TA. Complete inhibition of all the aflatoxin fractions production at 13% moisture level was achieved at 0.40% TA concentration. Singh and Mandal (2014) also reported that the production of aflatoxin at 13% moisture level can be completely inhibited by adding fumaric acid @ 0.20% or citric acid @ 0.45%. In present study, the contents of total aflatoxins as well as individual fractions decreased with the increasing levels of acids. Growth of *Aspergillus flavus* and aflatoxin production in maize containing 13% moisture was also reported by Sauer and Burrough (1974).

The production of AFB_1 reduced from 0.45 (control) to 0.09 ppm and that of AFB_2, 0.32 (control) to 0.05 ppm at 0.45% PA. Whereas, AFG_1 and AFG_2 biosynthesis reduced from 0.22 (control) to 0.05 ppm and 0.20 (control) to 0.02 ppm, respectively at 0.35% PA concentration and 15% moisture level (Table 1). Complete inhibition of

AFG_1 and AFG_2 biosynthesis were recorded at 0.40% PA. In case of total AF, the biosynthesis of total AFs reduced from 1.19 ppm (control) to 0.14 ppm at 0.45% PA. Complete inhibition of all the aflatoxin fractions production at 15% moisture level was achieved at 0.50% PA concentration.

These results were in agreement with those of Gowda *et al*. (2004) who reported that propionic acid at 0.05–0.5% completely inhibited aflatoxin production. In case of BA treatment, the AFB_1 and AFB_2 production decreased from 0.50 (control) to 0.10 ppm and 0.36 (control) to 0.06 ppm, respectively at 0.45% BA treatment of 15% moisture level (Table 2). The biosynthesis of AFB_1 and AFB_2 was completely inhibited at 0.50% concentration of BA. The biosynthesis of AFG_1 and AFG_2 was completely inhibited at 0.45 and 0.40% concentrations of BA, respectively. The total AFs production in control group was 1.34 ppm which reduced to 0.16 ppm at 0.45% BA. Complete inhibition of all the aflatoxin fractions production at 15% moisture level was achieved at 0.50% BA concentration. Gowda *et al*. (2004) also reported that benzoic acid at 0.2% concentration completely inhibited aflatoxin production. With regard to TA treatment at 15% moisture level (Table 3), the AFB_1 and AFB_2 production decreased from 0.50 (control) to 0.11 ppm and 0.42 (control) to 0.09 ppm, respectively at 0.50% TA level, indicating that 0.50% TA concentration could not completely inhibit the synthesis of B aflatoxins. However, the synthesis of AFG_2 was completely inhibited at this (0.50% TA) concentration.

Table 2. Aflatoxin contents (ppm) of poultry feed as influenced by various concentrations of benzoic acid

Moisture[†]		Benzoic acid (BA) concentration (%)										
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
13%	AFB1	0.05	0.05	0.04	0.04	0.03	0.02	0.00	0.00	0.00	0.00	0.00
	AFB2	0.05	0.04	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00
	AFG1	0.03	0.02	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00
	AFG2	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.15	0.13	0.10	0.09	0.05	0.04	0.00	0.00	0.00	0.00	0.00
15%	AFB1	0.50	0.48	0.45	0.44	0.41	0.40	0.35	0.29	0.19	0.10	0.00
	AFB2	0.36	0.34	0.30	0.26	0.28	0.26	0.20	0.19	0.15	0.06	0.00
	AFG1	0.26	0.25	0.24	0.22	0.20	0.17	0.15	0.08	0.02	0.00	0.00
	AFG2	0.22	0.21	0.20	0.18	0.16	0.14	0.12	0.03	0.00	0.00	0.00
	Total	1.34	1.28	1.19	1.10	1.05	0.97	0.82	0.59	0.36	0.16	0.00
17%	AFB1	0.62	0.62	0.57	0.56	0.54	0.54	0.51	0.50	0.48	0.45	0.34
	AFB2	0.40	0.39	0.38	0.39	0.36	0.37	0.35	0.35	0.33	0.28	0.20
	AFG1	0.31	0.24	0.23	0.22	0.21	0.20	0.19	0.17	0.15	0.10	0.08
	AFG2	0.25	0.21	0.21	0.19	0.18	0.19	0.15	0.14	0.12	0.06	0.05
	Total	1.58	1.46	1.39	1.36	1.29	1.30	1.20	1.16	1.08	0.89	0.67

[†]At 11% moisture level, aflatoxin biosynthesis did not occurred at any of the benzoic acid concentrations including control.

Table 3. Aflatoxin contents (ppm) of poultry feed as influenced by various concentrations of tartaric acid

Moisture†		Tartaric acid (TA) concentration (%)										
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
13%	AFB1	0.07	0.05	0.06	0.06	0.03	0.03	0.03	0.01	0.00	0.00	0.00
	AFB2	0.06	0.05	0.05	0.04	0.04	0.03	0.02	0.01	0.00	0.00	0.00
	AFG1	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.00	0.00	0.00	0.00
	AFG2	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00
	Total	0.19	0.16	0.16	0.15	0.11	0.10	0.07	0.02	0.00	0.00	0.00
15%	AFB1	0.50	0.49	0.50	0.48	0.46	0.46	0.44	0.40	0.32	0.20	0.11
	AFB2	0.42	0.38	0.36	0.35	0.32	0.31	0.30	0.28	0.20	0.12	0.09
	AFG1	0.30	0.30	0.28	0.25	0.24	0.21	0.20	0.19	0.10	0.05	0.04
	AFG2	0.31	0.25	0.22	0.24	0.22	0.20	0.19	0.12	0.06	0.01	0.00
	Total	1.53	1.42	1.36	1.32	1.24	1.18	1.13	0.99	0.68	0.38	0.24
17%	AFB1	0.72	0.70	0.70	0.68	0.68	0.68	0.65	0.60	0.56	0.51	0.49
	AFB2	0.50	0.50	0.49	0.49	0.48	0.45	0.42	0.40	0.35	0.21	0.18
	AFG1	0.42	0.40	0.39	0.38	0.36	0.35	0.32	0.31	0.25	0.16	0.09
	AFG2	0.42	0.39	0.38	0.38	0.35	0.33	0.30	0.27	0.21	0.19	0.03
	Total	2.06	1.99	1.96	1.93	1.87	1.81	1.69	1.58	1.37	1.07	0.79

†At 11% moisture level, aflatoxin biosynthesis did not occurred at any of the tartaric acid concentrations including control.

The AFG_1 production decreased from 0.30 (control) to 0.04 ppm at 0.50% TA level. The total AFs production in control group was 1.53 ppm which reduced to 0.24 ppm at 0.50% TA. Therefore, for complete inhibition of AFs production in poultry feed containing 15% moisture, more than 0.50% TA concentration is required. Singh and Mandal (2014) also reported that at 15 moisture level in feed, more than 0.50% of fumaric acid or citric acid is required for complete inhibition of biosynthesis of aflatoxins.

At 17% moisture level (Table 1), the AFB_1 production decreased from 0.60 to 0.35; AFB_2, 0.41 to 0.22; AFG_1, 0.28 to 0.07; AFG_2, 0.24 to 0.06 and total AFs, 1.53 to 0.70 ppm at 0.50% PA concentration. In case of benzoic acid treatment at 17% moisture level (Table 2), the production of AFB_1 decreased from 0.62 to 0.34; AFB_2, 0.40 to 0.20; AFG_1, 0.31 to 0.08; AFG_2, 0.25 to 0.05 and total AF, 1.58 to 0.67 ppm at 0.50% BA treatment. With regard to tartaric acid treatment at 17% moisture level (Table 3), the production of AFB_1 decreased from 0.72 to 0.49; AFB_2, 0.50 to 0.18; AFG_1, 0.42 to 0.09; AFG_2, 0.42 to 0.03 and total AF, 2.06 to 0.79 ppm at 0.50% TA treatment. All the three acids failed to completely inhibit the synthesis of any of the four fractions of aflatoxins at 17% moisture level and 0.50% concentration of these acids. Singh and Mandal (2014) also reported that at 17% moisture level in feed, more than 0.50% of fumaric acid or citric acid is required for complete inhibition of biosynthesis of aflatoxins. On the other hand, Sauer and Burroughs (1974) reported that propionic

acid at 0.5% concentration was effective in preserving the maize containing 18% moisture. Also, Gowda *et al*. (2004) reported that propionic acid at 0.05–0.5% and benzoic acid at 0.2% concentration completely inhibited aflatoxin production in cattle feed containing 18% moisture content. The results of the present investigation indicated that on increasing the concentration of any acid, there was a decrease in the synthesis of any of the four fractions of aflatoxin. These results are in agreement with Tsai *et al*. (1984) who reported that organic acid inhibited aflatoxin formation largely through inhibition of growth of *Aspergillus flavus* and *Aspergillus parasiticus*. Further, these acids might be hindering one or the other biosynthetic pathways of aflatoxin synthesis as reported by Salunkhe *et al*. (1987) in the case of propionic acid.

In present investigation, propionic acid proved to be more efficaceous than benzoic or tartaric acid in inhibiting the synthesis of aflatoxins. Thus, the levels of organic acids for preserving feed depend on the moisture level in the feed and the type of acid used. These findings are in agreement with earlier reports available in literature. Santurio (1995) also recommended that propionic acid application in maize vary from 0.1 for grains with 11-12% moisture to 0.5% for grains with 18% moisture. Sherwood and Pederby (1974) reported that 0.1 to 1.0% formic acid, acetic acid, propionic acid and butyric acid are needed to inhibit the growth of *Fusarium* in wheat incubated at 31% moisture. Higgins and Brinkhaus (1999) also reported that valeric acid, propionic acid and butyric acid exhibited the highest efficacy against various moulds with the effective concentrations ranging from 0.05 to 0.25% in the Potato Dextrose Agar medium.

CONCLUSIONS

It is concluded that the production of aflatoxin at 13% moisture level in poultry feed can be completely inhibited by adding 0.25% propionic acid or 0.30% benzoic acid or 0.40% tartaric acid. At higher (15 and 17%) moisture level in feed, higher concentrations of the studied organic acids required for complete inhibition of aflatoxin synthesis. Furthermore, propionic acid was found to be more efficaceous than benzoic or tartaric acid in inhibiting the synthesis of aflatoxins.

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