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Efficacy of fumaric and citric acids in preventing biosynthesis of aflatoxins in poultry feed with variable moisture content

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ABSTRACT

A poultry feed was prepared using conventional feed ingredients free from aflatoxins. The moisture content of the feed was adjusted at 11, 13, 15 and 17%. The feeds with each level of moisture were then mixed with fumaric or citric 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%. Sample (100 g) from each preparation was taken in duplicate, inoculated with fresh spores of aflatoxins producing mould (*Aspergillus parasiticus* NRRL 2999), incubated at room temperature for 1 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, none of the 4 aflatoxins were recorded in any of the treatments. However, with the increase in moisture in feed from 11 to 17%, oproduction of aflatoxins increased. The concentrations of AFB_1 were 40–60 ppb and total AF 110–170 ppb even at 13% moisture level after 1 month storage of such feed. The biosynthesis of any of the aflatoxins was completely inhibited with 0.20% fumaric acid or 0.45% citric acid in feed containing 13% moisture. However, fumaric or citric acid at 0.50% concentration, failed to completely inhibit synthesis of any of the 4 fractions of aflatoxins in feeds containing 15 and 17% moisture level, though with increased concentrations of acids, biosynthesis of total as well as individual fractions of aflatoxins decreased. It is concluded that storage of feed for 1 month with 13% moisture was not safe. Moreover, the production of AF at 13% moisture level can be completely inhibited by adding fumaric acid @ 0.20% or citric acid @ 0.45%. However, at 15 and 17% moisture level in feed, more than 0.50% of fumaric acid or citric acid is required for complete inhibition of biosynthesis of aflatoxins.

Key words: Aflatoxin, Citric acid, Fumaric acid, Poultry feed

All feeds (raw material and finished products) are suitable media for growth of a wide variety of moulds, provided moisture and temperature requirements are met. The moulds in feed may cause problems in two ways (i) the moulds itself use the feed nutrients for their growth, and (ii) the moulds can produce mycotoxins in feed. Reduction in the metabolisable energy value (Bartov 1982), and reduced fat content, dry matter and protein retention, metabolisable energy and performance of broilers were reported when fed on mouldy diet (Bartov 1983). Significant positive correlations were also found between the length of storage period and fat content in diets containing mould- free maize and mouldy maize (Bartov 1985). The mycotoxins of most concern due to their toxicity and occurrence are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin and T–2 toxin (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). Major aflatoxins produced in feeds are 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 (Smith and Ross 1991). Significant reduction in body weight and feed efficiency was reported in broiler chicken fed diet with AFB_1 (Afzal and Saleem 2004, Denli and Okan 2006). Aflatoxicosis in poultry also causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo *et al*. 2000), anaemia (Oguz *et al*. 2000), reduction in immune function (Oguz *et al*. 2003), hepatotoxicity, haemorrhage (Ortatatli and Oguz 2001), teratogenesis (Sur and Celik 2003), carcinogenesis and mutagenesis are associated with aflatoxicosis (Basmacioglu 2005). Aflatoxins could be eliminated by growth inhibition of the fungus-producing strain. Therefore, inhibition of mycotoxin producing fungus during storage of feed is of great importance. The aim of this communication was to assess the efficacy of fumaric and citric acids as mould growth inhibitor in poultry feed.

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MATERIALS AND METHODS

Prepararion of fungus inoculum: Lyophilized preparation of *Aspergillus parasiticus* NRRL 2999 was obtained from U.S. Department of Agriculture, Peoria, Illinois (USA). This lyophilized preparation was revived on Potato Dextrose Agar (PDA) medium and maintained in mycotoxin laboratory of CARI, Izatnagar. To get fresh spores of the fungus, the culture was sub-cultured on PDA medium slants and stored at 5°C.

Feed preparation: Poultry feed (broiler starter) was prepared from aflatoxin-free feed ingredients as per the following composition.

Ingredients	Broiler starter (%)
Yellow maize	56.00
Soybean meal	32.00
Sunflower cake	1.625
Rapeseed meal	4.50
Fish meal	3.00
Limestone	0.80
Di-calciun phosphate	1.40
Common salt	0.20
DL-methionine	0.07
Lysine	0.03
TM premix1	0.11
Vit. premix1	0.15
B complex	0.015
Ch. chloride	0.05
Coccidiostat	0.05

The moisture content of the feed was adjusted at 4 levels of 11, 13, 15 and 17%. Feeds having these moisture levels were mixed separately with fumaric acid or citric 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%. 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. The flasks were cotton plugged and incubated at room temperature for a period of one month. The minimum and maximum temperature during the study period varied from 15°C to 38°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 to a constant weight for the estimation of aflatoxins. The aflatoxins content was extracted using aqueous acetone. The estimation of aflatoxins was undertaken as per the method of Pons *et al.* (1966).

RESULTS AND DISCUSSION

Aflatoxin contents (total and fractions) of poultry feed as influenced by different concentrations of fumaric acid and citric acid at four moisture levels are depicted in Table 1 and 2, respectively. At 11% moisture level, none of the aflatoxins (B_1, B_2, G_1 and G_2) were recorded at any of the fumaric acid or citric acid concentrations including control indicating that moisture level is the first limiting factor for the biosynthesis of aflatoxins in feed. Jones (2005) also reported that moisture is the single most important factor in determining how rapidly moulds will grow in feeds. Gosh *et al*. (1996) on the other hand, reported that aflatoxin biosynthesis occurred even at 10 percent moisture when groundnut cake was inoculated with pure culture of *Aspergillus flavus*. However, Lopez and Christensen (1976) reported that *Aspergillus flavus* did not invade maize samples even at 17.5% moisture level, however, extensive growth appeared at 18.5% moisture.

In the present study, at 13% moisture level, the biosynthesis of AFB_1 reduced from 0.04 ppm (control) to 0.03 ppm at 0.15% fumaric acid (FA) concentration, whereas, the biosynthesis of AFB_2 reduced from 0.03 ppm (control) to 0.02 ppm at the same concentration of FA. Neither AFB_1 nor AFB_2 content was recorded at 0.20% or higher concentrations of FA. The biosynthesis of each AFG_1 and AFG_2 decreased from 0.02 ppm (control) to 0.01 ppm at 0.10% FA concentration. The complete biosynthesis of both G fractions was inhibited by 0.15% or higher levels of FA. Biosynthesis of total AF decreased from 0.11 ppm (control) to 0.05 ppm at 0.15% FA. Complete inhibition of aflatoxins production at 13% moisture level was achieved at 0.20% FA concentration.

With regard to citric acid treatment and at 13% moisture level (Table 2), the biosynthesis of AFB_1 and AFB_2 reduced from 0.06 ppm (control) to 0.01 ppm at 0.40% citric acid (CA) concentration. The production of both B fractions was completely inhibited by 0.45% or higher concentrations of CA. The biosynthesis of AFG_1 decreased from 0.03 ppm (control) to 0.01 ppm at 0.30% CA, whereas, that of AFG_2 decreased from 0.02 ppm (control) to 0.01 ppm at the same concentration (0.30%). The complete inhibition of both G fractions was achieved by 0.35% or higher levels of CA. Total AF production decreased from 0.17 ppm (control) to 0.02 ppm at 0.40% CA. Complete inhibition of all the aflatoxin fractions production at 13% moisture level was achieved at 0.45% CA concentration. The contents of total aflatoxins as well as individual fractions decreased with the increasing levels of both the acids. Growth of *Aspergillus flavus* and aflatoxin production in maize containing 13% moisture was also reported by Sauer and Burroughs (1974).

At 15% moisture level (Table 1), the production of AFB_1 reduced from 0.39 (control) to 0.18 ppm and that of AFB_2, 0.22 (control) to 0.03 ppm at 0.50% fumaric acid. Whereas, AFG_1 and AFG_2 biosynthesis reduced from 0.20 (control) to 0.04 ppm and 0.18 (control) to 0.03 ppm, respectively at 0.35% FA concentration. Complete inhibition of AFG_1 and AFG_2 biosynthesis was recorded at 0.40% FA. In case of total AF, the biosynthesis of total AFs reduced from 0.99 ppm (control) to 0.21 ppm at 0.50% FA. In case of citric acid treatment at 15% moisture level (Table 2), the AFB_1 and AFB_2 production decreased from 0.48 (control) to 0.10 ppm and 0.40 (control) to 0.02 ppm, respectively at 0.50% CA treatment, indicating that 0.50% CA level could not completely inhibit the synthesis of B aflatoxins. However, the synthesis of G aflatoxins was completely inhibited at

this (0.50% CA) concentration. The total AFs production in control group was 1.40 ppm which reduced to 0.12 ppm at 0.50% CA. Therefore, for complete inhibition of AFs production in poultry feed containing 15% moisture, more than 0.50% FA or CA concentration is required.

At 17% moisture level (Table 1), the biosynthesis of AFB_1 decreased from 0.55 to 0.30; AFB_2, 0.37 to 0.16; AFG_1, 0.22 to 0.07; AFG_2, 0.20 to 0.06 and total AFs, 1.34 to 0.59 ppm at 0.50% FA concentration. With regard to citric acid treatment at 17% moisture level (Table 2) the production of AFB_1 decreased from 0.69 to 0.48; AFB_2, 0.48 to 0.09; AFG_1, 0.39 to 0.03; AFG_2, 0.39 to 0.10 and total AF, 1.95 to 0.61 ppm at 0.50% CA treatment. Both the acids failed to completely inhibit the synthesis of any of the four fractions of aflatoxins at 17% moisture level and 0.50% concentration of either acid. However, Sauer and Burroughs (1974) reported that propionic acid at 0.5% concentration was effective in preserving the maize

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

		Fumaric acid concentration (%)										
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
Moisture 11%	AFB1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFB2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13%	AFB1	0.04	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFB2	0.03	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG1	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG2	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.11	0.10	0.07	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15%	AFB1	0.39	0.39	0.39	0.39	0.38	0.39	0.36	0.37	0.32	0.22	0.18
	AFB2	0.22	0.22	0.21	0.22	0.22	0.22	0.19	0.18	0.11	0.06	0.03
	AFG1	0.20	0.17	0.19	0.18	0.17	0.11	0.10	0.04	0.00	0.00	0.00
	AFG2	0.18	0.17	0.17	0.13	0.17	0.12	0.09	0.03	0.00	0.00	0.00
	Total	0.99	0.95	0.96	0.92	0.94	0.84	0.74	0.62	0.43	0.28	0.21
17%	AFB1	0.55	0.55	0.52	0.54	0.53	0.54	0.51	0.50	0.48	0.41	0.30
	AFB2	0.37	0.37	0.32	0.35	0.31	0.30	0.27	0.26	0.19	0.18	0.16
	AFG1	0.22	0.20	0.20	0.20	0.20	0.17	0.13	0.10	0.09	0.08	0.07
	AFG2	0.20	0.18	0.18	0.19	0.18	0.16	0.13	0.10	0.09	0.07	0.06
	Total	1.34	1.30	1.22	1.28	1.22	1.17	1.04	0.96	0.85	0.74	0.59

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

		Citric acid concentration (%)										
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
Moisture 11%	AFB1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFB2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	AFG2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13%	AFB1	0.06	0.05	0.06	0.05	0.05	0.05	0.03	0.02	0.01	0.00	0.00
	AFB2	0.06	0.06	0.06	0.04	0.05	0.04	0.02	0.01	0.01	0.00	0.00
	AFG1	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00
	AFG2	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00
	Total	0.17	0.16	0.16	0.13	0.14	0.13	0.07	0.03	0.02	0.00	0.00
15%	AFB1	0.48	0.48	0.48	0.46	0.42	0.42	0.40	0.33	0.29	0.13	0.10
	AFB2	0.40	0.36	0.35	0.33	0.31	0.30	0.29	0.21	0.15	0.08	0.02
	AFG1	0.26	0.26	0.26	0.23	0.21	0.19	0.18	0.10	0.09	0.03	0.00
	AFG2	0.26	0.24	0.23	0.24	0.22	0.20	0.17	0.09	0.03	0.00	0.00
	Total	1.40	1.34	1.33	1.26	1.16	1.11	1.04	0.73	0.56	0.24	0.12
17%	AFB1	0.69	0.69	0.69	0.67	0.65	0.63	0.61	0.55	0.54	0.50	0.48
	AFB2	0.48	0.47	0.47	0.46	0.45	0.38	0.36	0.35	0.29	0.13	0.09
	AFG1	0.39	0.36	0.36	0.35	0.33	0.25	0.24	0.23	0.21	0.15	0.03
	AFG2	0.39	0.35	0.34	0.35	0.33	0.25	0.25	0.24	0.17	0.12	0.01
	Total	1.95	1.87	1.86	1.83	1.76	1.51	1.46	1.37	1.21	0.90	0.61

containing 18% moisture. The results of the present investigation indicated that on increasing the concentration of fumaric or citric acid, there was a decrease in the synthesis of any of the four aflatoxins. Tsai *et al.* (1984) reported that organic acid inhibited aflatoxin formation largely through inhibition of growth of *Aspergillus flavus* and *Aspergillus parasiticus*. It may also be possible that these acids might be hindering one or the other biosynthetic pathways of aflatoxin synthesis as reported by Salunkhe *et al.* (1987) in propionic acid. In present investigation, fumaric acid appeared to be more efficaceous than citric 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. 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. 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. Higgins and Brinkhaus (1999) also reported that valeric acid, propionic acid and butyric acid displayed the highest efficacy against various moulds with the effective concentrations ranging from 0.05 to 0.25% in the Potato Dextrose Agar medium.

It is concluded that storage of feed for a month with 13% moisture was not safe. The production of AF at 13% moisture level can be completely inhibited by adding fumaric acid @ 0.20% or citric acid @ 0.45%. However, at 15 and 17% moisture level in feed, more than 0.50% of fumaric acid or citric acid is required for complete inhibition of biosynthesis of aflatoxins. Again fumaric acid was more efficient to prevent mould growth in comparison to citric acid.

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