

Research Article

Assessment of aflatoxin in beef cattle feed and feed ingredients in Bangladesh: A safety issue

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E-mail: hashem_as@bau.edu.bd**Article Info**Received: 21st May, 2022Accepted: 25th July, 2022Published online: 31st August, 2022**Keywords:**

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Abstract

The present study was conducted to find out the aflatoxin concentration present in commercial beef cattle feed and feed ingredients in Bangladesh. Commercial beef cattle feed and feed ingredients samples collected from four district of Bangladesh were analyzed by ELISA test kit for aflatoxin. The levels of aflatoxin varied widely in all types of feed ingredients from 6.36 to 59.18 ppb. The mean concentration of aflatoxin in feed ingredients was found to be significantly higher ($p < 0.05$) in maize (32.34 ± 24.81 ppb) and rice polish (20.33 ± 9.55 ppb) which were higher than the maximum permitted levels (MPLs) set by Food Standards Agency. The mean concentration of aflatoxin in feed ingredients was lower in de-oiled rice bran (7.97 ± 1.73 ppb). Level of mean concentration of aflatoxin in other feed ingredients such as sesame oil cake (10.28 ± 3.50 ppb), wheat bran (10.36 ± 1.82 ppb), gram bran (10.97 ± 2.54 ppb), mustard oil cake (12.74 ± 3.15 ppb), matikalai hull (9.96 ± 2.58 ppb), rapeseed meal (13.29 ± 2.87 ppb), soybean meal (9.76 ± 3.46 ppb), lentil hull (11.95 ± 3.46 ppb), cotton by-products (9.86 ± 1.95 ppb) and straw (12.05 ± 3.90 ppb) were almost similar and within the range of 9.76 ± 3.46 ppb to 13.29 ± 2.87 ppb. The levels of aflatoxin ranged in all types of commercial compound beef cattle feeds varied from 7.78 to 14.66 ppb. These levels are lower than the levels of maximum acceptable limit of aflatoxin (20 ppb) content in complete cattle feed set by Food Standards Agency.

Introduction

The role of livestock in the production of safe food is recognized worldwide and plays a vital role in promoting national economy of Bangladesh which is one of the most important sub-sectors of agriculture (Hasan et al., 2021 & 2022; Kamal et al., 2019 & 2022, Rahman et al., 1999). Food safety hazards associated with animal feed can be biological, chemical or physical (Rahman et al., 2020). The safety of livestock products directly related to feeds (Kamal et al., 2020; Kamal et al., 2016; Rahman et al., 1998). Animal feeds and forages contain a wide range of contaminants and toxins arising from anthropogenic and natural sources (Kamal et al., 2022; Hossain et al., 2016 and 1996). In this report, the distribution of aflatoxins in feed ingredients, complete feeds are presented.

Mycotoxins are fungal toxic metabolites which naturally contaminate food and feed. Aflatoxins (AFs), a kind of mycotoxins, are the main toxic secondary metabolites of some *Aspergillus* moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and the rare *Aspergillus nomius* (Ali et al., 2005; Alcaide-Molina et al., 2009). Several feed ingredients and the resulting feed produced thereof, such as compound feed, may be contaminated with mycotoxins. The consumption of mycotoxin contaminated feed has detrimental effects on animal production.

The residues of aflatoxin not only affect the public health but also cause economic losses to the livestock industry. The aim of the study was to gain insight into the contamination with aflatoxin in feed ingredients and compound beef cattle feed. The results can be used to define priorities for national monitoring plans.

Methods and Materials**Sample collection**

Nine (9) commercial compound beef feed samples and thirteen (13) feed ingredients were collected from different regions of Bangladesh. The method of analysis is according to Enzyme Linked Immune Sorbent Assay (ELISA) technique. Aflatoxin analysis was done according to the guideline or methodology supplied by Aflatoxin ELISA Kit manufacturer (Romer Labs, Singapore- www.romerlabs.com).

Sample preparation / extraction

1. Samples were ground at 2mm.
2. 10 g sample was taken in centrifuge tube.
3. 50 ml of 70% methanol was added.
4. Then the sample was shaken for 3 minutes vigorously.
5. 10 minutes were allowed for settle down.
6. Then it was filtrated through Whiteman filter paper.

Test procedure

1. Appropriate number of antibody coated microcells was placed in a microwell strip holder.
2. Unused microcells were returned to the foil pouch with the desiccant packet and reseal pouch.
3. 200 μ l conjugate solution was pipetted into dilution wells by single pipette.
4. 100 μ l of each standard or sample extract was added into the dilution wells by single pipette.
5. Then the wells were mixed and transferred 100 μ l from dilution wells into anti body coated wells by 8/12 digit pipette.
6. Incubated at room temperature for 15 minutes.
7. Then the wells were washed 5 times with distilled or de-ionized water.
8. Wells were washed by tap dry.
9. 100 μ l substrate solutions were pipetted into antibody coated wells.
10. Incubated at room temperature for 5 minutes.
11. 100 μ l stop solution was pipetted into antibody coated wells.
12. The wells were read with ELISA reader using 450nm and 630nm different filter.

Method validation

Table 1. Calibration curve

Std. Level	Abs.	B/Bo	Log(Conc.)	Logit B/Bo
0 ppb =	2.189			
4 ppb =	1.729	0.79	0.60	0.58
10 ppb =	0.869	0.40	1.00	-0.18
20 ppb =	0.398	0.18	1.30	-0.65
40 ppb =	0.17	0.08	1.60	-1.07

R² =	-0.9973
Slope =	-1.6506
Intercept =	1.5254657
50% Inhibition =	8.42

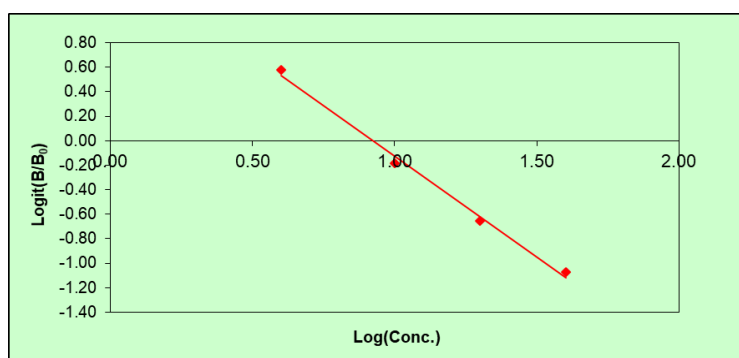


Figure 1. Calibration Curve

Statistical analysis

The data were statistically analyzed using the software, IBM SPSS Statistics, version 20 to find the mean and standard deviation among the various samples in triplicate. A one-way ANOVA was performed to test significance and $p < 0.05$ was considered as significant. Duncan's multiple-range test was done to compare the mean differences among the samples.

Results and discussion

Concentrations of Aflatoxin in feed ingredients

The levels of aflatoxin in different feed ingredients are summarized in Table 2. The levels of aflatoxin varied significantly ($p < 0.05$) in all types of feed ingredients and found within the range from 6.36 to 59.18 ppb.

The mean concentration of aflatoxin in feed ingredients was found to be higher in maize (32.34 ± 24.81 ppb) and rice polish (20.33 ± 9.55 ppb) which were higher than the maximum permitted levels (MPLs) set by Food Standards Agency (<https://www.food.gov.uk>). The mean concentration of aflatoxin in feed ingredients was lowest in de-oiled rice bran (7.97 ± 1.73 ppb).

According to Food Standards Agency all feed ingredients except maize and rice polish were in safe level for aflatoxin. In a study Becha *et al.* (2013) found higher aflatoxin content in maize than other feed ingredients which is similar to the findings. But the aflatoxin content of maize (122 ± 53.36 ppb) was much higher than this experiment. Kotinagu *et al.* (2015) reported 62 ppb and 50 ppb mean concentration of aflatoxin in maize and soybean cake, respectively. Alshawabkeh *et al.* (2015) found 9.42 ± 4.29 ppb and 13.88 ± 2.48 ppb aflatoxin by ELISA in soybean and wheat bran, respectively, which was similar to this result. However, Mohammadi *et al.* (2012) reported that among 152 samples of rice analyzed, 75% showed levels of aflatoxin B1 contamination with the mean of 0.671 ppb, which was lower than our findings.

Table 2. Concentrations of Aflatoxin in feed ingredients (parts per billion-ppb)

Feed ingredients	No. of samples	Mean \pm SD	Minimum	Maximum
Maize	3	32.34 ^a \pm 24.81	10.25	59.18
Rice polish	3	20.33 ^{ab} \pm 9.55	9.67	28.11
Wheat bran	3	10.36 ^b \pm 1.82	8.48	12.11
Gram bran/hull	3	10.97 ^b \pm 2.54	8.13	13.02
Matikalai hull	3	9.96 ^b \pm 2.58	8.02	12.89
Lentil hull	3	11.95 ^b \pm 3.46	8.34	15.23
Soybean meal	3	9.76 ^b \pm 3.46	6.15	13.05
Mustard oil cake	3	12.74 ^b \pm 3.15	9.20	15.23
Sesame oil cake	3	10.28 ^b \pm 3.50	6.36	13.09
Rapeseed meal	3	13.29 ^b \pm 2.87	10.46	16.19
De-oiled rice bran	3	7.97 ^b \pm 1.73	6.22	9.68
Straw	3	12.05 ^b \pm 3.90	8.29	16.08
Cotton by-products	3	9.86 ^b \pm 1.95	8.22	12.02
p-Value		0.041		

* Mean in a column with different superscripts are significantly different ($p < 0.05$)

Aflatoxin concentrations in different commercial compound beef feed

The concentrations of aflatoxin were found in all types of commercial compound beef cattle feeds from 7.78 to 14.66 ppb (Table 3). The mean concentration of aflatoxin in commercial complete feed (Gain feed, ACI, Care feed, Teer, Saudia, United, Provita, Index and IFAEL) were found 10.64 ± 1.44 , 11.92 ± 3.29 , 13.08 ± 2.20 , 10.38 ± 1.13 , 10.24 ± 0.65 , 9.93 ± 2.16 , 9.93 ± 0.59 , 12.33 ± 0.79 and 10.64 ± 1.44 ppm, respectively.

These levels are lower than the levels of maximum acceptable limit of aflatoxin (20 ppb) content in complete cattle feed set by Food Standards Agency (United Kingdom). Becha et al. (2013) found 26.90 ± 9.2 ppb mean concentration of aflatoxin in cattle feed which was higher than this study. Kotinagu et al. (2015) found 32 ppb aflatoxin in cattle feed which was also higher than this study. In another study, Martins et al. (2007) reported that levels of aflatoxin B1 above the maximum limit established in Portugal (5 ppb) for dairy cattle feed samples were observed in 62 samples (6.2%) with levels ranging from 5.1 to 74 ppb. Anjum et al. (2012) reported the overall incidence of 6% of aflatoxin B1 with average and maximum contamination levels of 37.62 and 56 ppb, respectively.

Aflatoxins can occur before harvest on starchy cereal crops (corn, cottonseed, and peanuts) or after harvest on stored commodities. Strains of *Aspergillus flavus* mainly produce aflatoxin B1, which is considered the most toxic and carcinogenic (cancer-causing) of the aflatoxins. Feeding trials were conducted with beef cattle and dairy cattle to determine adverse effects, if any, of graded levels of aflatoxins in rations. In addition, samples of meat and milk from these animals were analyzed chemically to determine if aflatoxin was transmitted into these products. No toxic effects were observed at levels of 300 ppb or lower in cross-bred beef steers fed aflatoxin rations for 4.5 months. In dairy cows, weekly intakes of 67 to 200 mg of aflatoxin B1 per cow produced 70 to 154 ppb aflatoxin M1 in lyophilized milk (Keyl and Booth, 1971).

Table 3. Aflatoxin in commercial compound beef feed

Name of Commercial beef feed	No. of samples	Mean \pm SD (ppb)	Minimum (ppb)	Maximum (ppb)
Gain feed	3	10.64 ± 1.44	9.56	12.28
ACI	3	11.92 ± 3.29	8.28	14.66
Care feed	3	13.08 ± 2.20	10.55	14.58
Teer	3	10.38 ± 1.13	9.11	11.29
Saudia	3	10.24 ± 0.65	9.59	10.88
United	3	9.93 ± 2.16	7.78	12.09
Provita	3	9.93 ± 0.59	9.51	10.60
Index	3	12.33 ± 0.79	11.75	13.23
IFAEL	3	10.64 ± 1.44	11.59	14.46
Over all			7.78	14.66
p-Value		0.331		

Conclusions

The results revealed low average aflatoxin concentration than the permissible levels, for livestock compound beef feed samples and feed ingredients except maize. The results of the study showed a higher incidence and contamination of aflatoxin in maize and rice polish.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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