ISSN : 0974 - 7435 Volume 7 Issue 9

BioTechnology
An Indian Journal

SHORT COMMUNICATION

BTAIJ, 7(9), 2013 [334-336]

Determination of aflatoxin B_1 in animal feed in Mashhad, Iran

Mohamad Sadegh[1], Ali Mohamadi Sani[1]*, Reza Ghiasvand[2]
[1]Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, (IRAN)
[2]Food Security Research Center, Isfahan University of Medical Sciences, (IRAN)
E-mail: mohamadisani@yahoo.com

ABSTRACT

This research examines the existence of aflatoxin B_1 ($AFB_{1)}$ in samples of feedstuff. In this study a total of 90 animal feed samples consisting of barley, wheat bran, wheat pulp, canola meal, safflower meal, cottonseed meal and sunflower meal were assessed, using the HPLC technique. The results revealed that 36.67% of the feed stuff samples were contaminated but the contamination level was under the permissible level of AFB_1 accepted by national standard. The range of contamination was from 0.34 to 5.81 ppb. It was also found out that the contamination level was the highest in cottonseed and then in sunflower meal samples. The mean concentration of AFB_1 in the mentioned samples was, 3.47 and 0.21 ppb, respectively.

KEYWORDS

Feedstuff;
AFB_1;
HPLC.

INTRODUCTION

The problem of feed contamination with toxigenic moulds especially *Aspergillus* species is of current concern and has received a great deal of attention during the last three decades. Aflatoxins are toxic secondary metabolites produced by these fungi found on agricultural commodities directly in the field or during storage[1]. Humans and animals can be exposed to aflatoxins both directly and indirectly. An accumulating number of studies have demonstrated that aflatoxins cause multiple forms of toxic damage including hepatotoxic, carcinogenic, and mutagenic effects. Aflatoxin B_1 (AFB_1) is the most highly toxic and predominant form present in various foods and feedstuffs[2]. When animals eat foodstuffs containing AFB_1, these toxins will be metabolized and excreted as AFM_1 in milk and this is the only route for transformation of AFB_1 to AFM_1[3,4]. Direct consequences of consumption of mycotoxin-contaminated animal feed include: reduced feed intake, poor feed conversion, diminished body weight gain, increased incidence of disease (due to immune-suppression) and reduced reproductive capacity[5,6]. The Food and Agricultural Organization (FAO) 2004 report on mycotoxins revealed that, at least 99 countries worldwide had regulations in place for permitted mycotoxin levels in food or feed, and have set limits for AFB_1. The maximum permissible level for AFB_1 in feed was set at 20ppb[7]. Consequently, the aim of the present study was to analyze the presence of AFB_1 in animal feed in Iran by high performance liquid chromatography (HPLC) method.

MATERIALS AND METHODS

Materials

Samples of animal feed were purchased from the markets (Mashhad, Iran). Samples including barley, wheat bran, wheat pulp, canola meal, safflower meal, cottonseed meal and sunflower meal.

Chemicals

AFB_1 standard solution was prepared from Sigma with purity of 98%; standard stock solutions were prepared in acetonitrile according to the AOAC method[8]. All solvents used for the experiments (methanol, acetonitrile and deionized water) were HPLC grade supplied by Merck. Aflatest immunoaffinity columns (IAC) were purchased from VICAM Company, HPLC column (C_{18}) was used.

Sample preparation

For minimizing the sub-sampling error in AF analysis, all the samples were grinded with miller and collected in a plastic bag. Finally, 50 g of test portion from the ground samples was taken for analysis.

AF standard

Working standard dilutions were prepared from aflatoxin standard purchased. These standards were used to prepare mixed working standards for HPLC.

HPLC determination

In brief 5 g of homogenized samples was extracted with 0.5 g NaCl and 30 ml methanol: water, (2: 8) by high – speed blender. (In fatty samples n – Hexan was added in order to remove fat) and then filtered through a Wathman filter paper No. 4. Five milliliters of extract was diluted with 95 ml of phosphate buffered saline (PBS, pH 7.4). The immunoaffinity column was conditioned with 10 ml of PBS and 50 ml of the diluted filtrate were applied to the column at a flow rate of 3 ml/min. After the clean-up step the column was washed with 20 ml of water and air was forced through the column prior to eluate aflatoxins by applying 1.75 ml of methanol. The eluate was diluted with 3.25 ml of water to give a total volume of 4.50 ml and 100 µl of eluate was injected onto HPLC system. The mobile phase consisted of MeCN: MeOH: water (17:29:54, v/v/v) with a flow rate of 1 ml/min. AF was quantitated by reverse-phase HPLC and fluorescence detector[9,10,11]. The aflatoxins were detected at the excitation and emission wavelengths of 365 and 435 nm, respectively. The employed column was a C_{18} 150 * 4.6 mm, 5 µm.

Statistical analysis

Results are presented as means (±SD). Variable means for measurements showing significant differences in the ANOVA were compared using the least significant difference procedure. Values were judged to be significantly different if $P < 0.05$. All experiments were carried out as triplicates. SPSS.16 software was used for data analysis.

RESULT AND DISCUSSION

As it can be clearly seen in TABLE 1, among a total of 90 samples, the incidence of AFB_1 was 36.67% within the range of 0.34-5.81ppb, and the mean concentration of AFB_1 in feedstuff samples was 2.311[12]. revealed, AFB_1 was detected by ELISA in most of the corn samples in two regions, and average levels were 17.3 and 23.7 ppb[12].

TABLE 1 : Mean, standard deviation, range of aflatoxin B_1 contamination in animal feed samples.

Mycotoxin	Positive sample (%)	Range (µg/kg)	Mean±SD*
AFB_1	33(36.67%)	0.34-5.81	2.311±2.14

*** standard deviation**

According to Figure 1, cottonseed and sunflower meal samples are the most contaminated feeds to AFB_1 in which the mean concentration of AFB_1 were 3.47 and 0.21 ppb, respectively. But the mean contamination

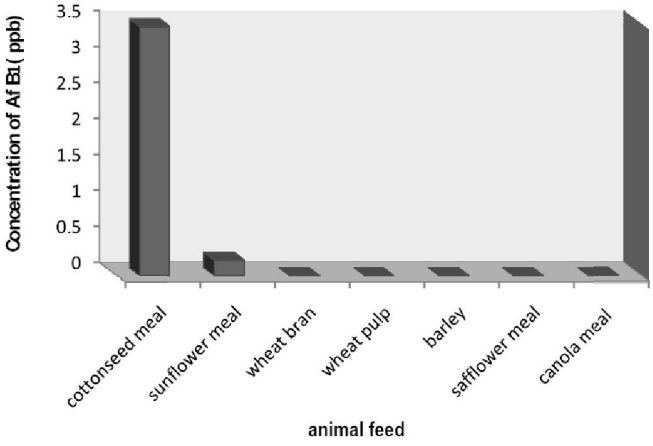

Figure 1 : Incidence of AFB_1 in different animal feed samples

SHORT COMMUNICATION

TABLE 2 : Range and percentage of AFB_1 contamination in animal feed samples

Sample category	Number of sample	Number of contaminated sample	Percentage of contaminated sample	Contamination range (ppb)
Cottonseed meal	18	18	100%	0.46-5.81
Sunflower meal	28	11	39.28%	0.34-0.9
Wheat bran	12	0	0%	-
Wheat pulp	16	0	0%	-
barley	9	0	0%	-
Safflower meal	5	0	0%	-
Canola meal	2	0	0%	-

level was much lower than maximum tolerated level (20 ppb) of AFB_1 accepted by Iranian standard organization[13].

According to the TABLE 2, AFB_1 contamination in 7 kind of feedstuff is expressed distinctly. All cottonseed meal samples were reported contaminated. The range of contamination was from 0.46 to 5.81 ppb and 11 out of 28 sunflower samples were manifested contaminated to AFB_1 in extent of 0.34 to 0.9 ppb.

According to the results of [14]. AFB_1 concentration by HPLC in different feedstuff including Alfalfa, Straw, Rapeseed, Cottonseed, Corn silage and Soybean meal was 0.38, 0.39, 1.54, 34.96, 0.45 and 0.65, respectively[14]. It is clear from the data given that cottonseed had highest level of contamination even higher than maximum tolerated level.

Stated cottonseed samples were the most contaminated samples from different feedstuff in two season (winter and summer). The mean concentration for the mentioned seasons reported 5.13 and 5.16 ppb respectively [15].

CONCLUSION

This study demonstrates that AFB_1 level in feedstuff samples was below the acceptable limit concentration for livestock consumption**.** But it is necessary to study the toxin concentration on more kinds of feedstuffs in other geographic regions and in different season to reach a logical conclusion. In short, aflatoxins continue to pose a health concern via livestock exposure to contaminated feedstuff. Routine controls and survey researches have to be performed for the detection of aflatoxin contaminations in animal feed.

REFERENCES

[1] R.Krska, A.Molinelli; Anal.Bioanal.Chem., **387**, 145 **(2007)**.
[2] B.D.Roebuck; J.Cell.Biochem., **91**, 243 **(2004)**.
[3] I.Bakirci; Food Cont., **12**, 47 **(2001)**.
[4] C.Lopez, L.Ramos, S.Ramadan, L.Bulacio, J.Perez; Int.J.Food Microb., **64,** 211 **(2001)**.
[5] L.Fink Gremmels, H.Malekinejad; Anim.Feed Sci.Technol., **137**, 326 **(2007)**.
[6] D.P.Morgavi, R.T.Riley; Anim Feed Sci.Technol., **137**, 201 **(2007)**.
[7] Worldwide regulations for mycotoxins in food and feed. (FAO); Food and Nutrition, Report of the Food and Agriculture Organization of the United Nations: Rome, 81 **(2004)**.
[8] International Agency for Research on Cancer (IARC). Summary of data reported and evaluation, **82**, **(2002)**.
[9] J.Stroka, E.Ankle, U.Jorissen, I.Gilbert; J.AOAC Int., **83**, 320 **(2000)**.
[10] J.Stroka, C.Von Holst, E.Anklam; J.AOAC Int., **86**, 1179 **(2003)**.
[11] ISIRI 6872; National Stand., 6872 **(2004)**.
[12] Y.Ueno, K.Iijima, S.D.Wang, Y.Sugiura, M.Sekijima, Y.Tanaka, C.Chen, S.Z.O.Yu; Food Chem.Toxicol., **35**, 1143 **(1997)**.
[13] ISIRI 5925; National Stand. 5925 **(2002)**.
[14] A.Pirestani, S.N.Tabatabaei, M.H.Fazeli, M.Antikchi, M.Baabaei; J.Res.Agr.Sci., **7**, 71 **(2011)**.
[15] S.H.Hadizadeh Moalem, I.Gholampour Azizi, M.Azami; Global Vet., **4**, 144 **(2010)**.