Determination of Aflatoxin B1 Levels of the Feedstuffs in Traditional and Semi-industrial Cattle Farms in Amol, Northern Iran

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ABSTRACT

The study was conducted in order to measure the levels of Aflatoxin B1 (AFB1) in feedstuff by ELISA used by some of cattle farms in Amol (northern Iran). Out of 96 samples at 45 traditional and 51 semi-industrial cattle farms ones (concentrated feed, beet pulp and cotton meal) were selected in random basis during 3 different seasons of winter, spring and summer 2009. Out of 45 traditional samples, 20 samples (44.4%) and out of 51 semi-industrial samples, 22 samples (43.1%) showed AFB1 contamination more than the limit accepted by EU (5 µg kg⁻¹). The contamination levels in concentrated feed, beet pulp and cotton seed were 58.8, 43.7 and 26.7%, respectively. The contamination level in winter, spring and summer were 56.4, 38.7 and 34.4%, respectively. Feedstuffs should be tested regularly in terms of AFB1 and if they were contaminated, it would be better not to use them.

Key words: Aflatoxin B1, feedstuff, traditional cattle farm, semi-industrial cattle farm

INTRODUCTION

Aflatoxins (AfS) are a group of secondary metabolites which are produced by Aspergillus flavus, A. parasiticus and A. nomius fungi on different kinds of food and feed (Richard, 2007; D’Mello and MacDonald, 1997). Human and animals contact with these toxins via oral, inhaling and direct contact and these toxins have various disorders. The molecular weight of this toxins are as low as other mycotoxin and for this reason, they are non-immunogenic. The main kinds of Aflatoxin are B1, B2, G1 and G2 which Aflatoxin B1 (AFB1) is the most important and toxic. These toxins have toxic effects such as acute and chronic toxicity, teratogenicity, mutagenicity, immunosuppression and carcinogenicity in mammals (Binder et al., 2007). If the feedstuff is being contaminated with AFB1, the toxin is metabolized inside livestock body and Aflatoxin M1 (AFM1) will enter into the milk (Iheshiolor et al., 2011; Deshpande, 2002). The European Union (EU) determined the permissible limit of AFB1 in feedstuff 5 µg kg⁻¹. Iran also follows this limit (Richard, 2007; European Commission Directive, 2003).

Numerous countries were successful to achieve toxin levels control in food and feed by suitable methods but many countries such as Iran have not yet reached to the standard limits. Prabakaran and Dhanapal (2009) detected Aflatoxins in some poultry feed that was in Kampu (220 ppb for AFB1 and 45 ppb for AFB2) and in Saamai (18 ppb for AFB1). In Serbia toxigenic fungi and mycotoxins were isolated from animal feed. The frequent incidence of animal feed contamination were Fusarium spp., Aspergillus spp. and Penicillium spp. Natural occurrence
of ochratoxin A is more frequent than incidence of aflatoxins (Krnjaća et al., 2009). In Ankara, Turkey the levels of AFB1 were reported between 0.08-1.61 µg kg⁻¹ in foodstuff and feedstuff (Baydar et al., 2005). In South Africa, aflatoxins were detected in animal feeds in 17 samples with levels ranged between 0.84±0.2 and 156±8 µg kg⁻¹ (ppb) (Mngadi et al., 2008). In India 23 different species of Penicillium in poultry feeds and cattle feeds were isolated (Rao et al., 2011). The AFB1 in 19 animal maize samples were measured with contamination ranging from 0.90 to 32.30 µg kg⁻¹ (Oruc et al., 2003). The feedstuff samples were analyzed at European, Mediterranean and Asian supermarkets for mycotoxins. 33% of Asian feedstuffs were positive which mostly contained deoxynivalenol, zearalenone, fumonisin and aflatoxin (Binder et al., 2007). During 2003 to 2004 fungi were isolated in cereal types, legumes, fatty seeds and animal feeds in Libya. The contaminated animal feeds (35%) were over than the diet (30.63%) (Attitalla et al., 2010). In a study by El-Maraghy fungi were found in 25 samples of sheep, cattle and camel feedstuffs collected in Libya. Aspergillus, Penicillium and Fusarium were the most common genera in the three substrates tested (El-Maraghy, 1996). In Belgium AFB1 was detected in cattle farms feed with contamination levels 1.2-3.6 µg kg⁻¹ that was less than the limit accepted by EU (5 µg kg⁻¹) (Stroka et al., 2003). In brewers grain used to feed dairy cattle Aspergillus was reported with the most frequently (42.5%), followed by Penicillium, Mucor, Rhizopus and Fusarium (Simas et al., 2006). In some studies co-occurring mycotoxins and fungal contamination in animal feeds were found (Mngadi et al., 2008).

In Northern Iran AFB1 was found in animal food from 10.4 to 68.8% (Moalem et al., 2010). Also, in the same area Aflatoxin M1 (AFM1) was found in 100% of the pasteurized yogurt and local yogurt samples in autumn 2009 with concentrations of 2.1-61.7 ng L⁻¹ and 7-83 ng L⁻¹, respectively (Barjesteh et al., 2010).

In Uganda was reported maize aflatoxin levels mean 13.0-7.7 ppb (Bigirwa et al., 2006). Aflatoxins were detected in food in Jordan by ELISA with a contamination of 3% (Salem and Ahmad, 2010). Fusarium and Aspergillus were isolated at high levels in maize grains produced in Karnataka (India) for the post harvest (Sreenivasa et al., 2011). The AFB1, was detected in 45.3% of the maize samples that 12.3% of them exceeding 5 µg kg⁻¹ (Matumba et al., 2009). Lutfullah and Hussain (2012) analyzed total aflatoxins in rice (25%), broken rice (15%), wheat (20%), maize (40%) barley (20%) and sorghum (30%), while in red kidney beans they were (20%), split peas (27%), chick pea (10%), cow pea (20%) and soybean (15%). Soleimany reported the occurrence of mycotoxins in cereal samples in morocco; 70, 40, 25, 36, 19, 13, 18 and 16% for aflatoxins, OTA, ZEA, DON, FB1, FB2, T2 and HT2-toxin, respectively (Soleimany et al., 2012). In India AFB1 in wheat was determined = 5 ppb in 40.3% of samples (Toteja et al., 2006).

The study was performed in order to measure the levels of AFB1 in feedstuff used by some of traditional and semi-industrial cattle farms in Amol (Northern Iran) and the results were compared with permitted limits.

MATERIALS AND METHODS

Samples: Ninety six samples were selected from 3 different feedstuff ingredients (34 concentrated feed, 32 beet pulp and 30 cotton meal) at 2 kinds of cattle farms (45 traditional and 51 semi-industrial) in random basis in Amol during 3 different seasons of winter, spring and summer 2009. Twenty to forty grams of each sample were taken and immediately entered bags.

Materials: For determining AFB1 levels ELISA kit was used (RIDASCREEN AFB, 30/15, R-Biopharm AG, Darmstadt, Germany).
Sample preparation: The samples after transferring to laboratory firstly were grinded then 5 g of grinded sample mixed with 25 cc of 70% methanol then were shaken for 3 min with hand or shaker. Obtained extracts were refined with filter paper Whatman No. 1 and 1 cc of refined solution was used to evaluate toxin levels (Aksoy et al., 2009).

AFB₃ analysis by ELISA: Fifty microliter of this solution was poured into every micropipette ELISA well. At first, with pipettes, standard samples and conjugated solutions and anti-Aflatoxin antibodies, were added to each well. After the first incubation, using substrate in wells, its red color was converted into blue and by using the stop solution its blue color was changed into yellow. AFB₃ concentration and absorbance was read by ELISA Reader at 450 nm. Data were analyzed by ANOVA utilizing SPSS software package. The results were analyzed by comparing them with standard limits (Moalem et al., 2010; Ozaslan et al., 2011; Salem and Ahmad, 2010; Jayeola et al., 2011).

RESULTS
Out of 96 samples, 42 samples (43.7%) showed contamination more than the limit accepted by EU (5 µg kg⁻¹). Three different kinds of feedstuffs concentrated feed, beet pulp and cotton seed revealed 58.8, 43.7 and 25.7% contamination rate, respectively (Table 1). The differences among concentrated feed and beet pulp (p = 0.012) and concentrated feed and cotton seed (p = 0.01) contamination were statistically significant (t-test) but beet pulp and cotton seed (p>0.05) contamination revealed no statistically significant difference. The contamination level between concentrates in winter, spring and summer were found 56.7, 54.4 and 54.4%, respectively (Fig. 1).

<table>
<thead>
<tr>
<th>Feedstuffs cattle farm</th>
<th>Concentrated feed</th>
<th>Beet pulp</th>
<th>Cotton meal</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Traditional</td>
<td>16</td>
<td>10</td>
<td>62.5</td>
<td>15</td>
</tr>
<tr>
<td>Semi-industrial</td>
<td>18</td>
<td>10</td>
<td>55.6</td>
<td>17</td>
</tr>
<tr>
<td>Sum</td>
<td>34</td>
<td>20</td>
<td>68.8</td>
<td>32</td>
</tr>
</tbody>
</table>

N: Number of samples, P: Positive (over than 5 µg kg⁻¹)

![Bar chart showing contamination levels of feedstuffs to AFB₃ according to the type of cattle farm](image)

![Bar chart showing contamination levels of samples to AFB₃ according to season](image)

Fig. 1: The contamination levels of samples to AFB₃ according to season
The contamination between 10-15 ppb was 12.3% (Fig. 2). In general, max, min and mean of the concentration were 1, 15 and 5.3 µg kg⁻¹, respectively (Fig. 3). The difference for contamination level in winter and summer was significant (p = 0.001) but between spring and summer and winter and spring was not significant (p>0.05). AFB₁ contamination level in traditional, semi-industrial cattle farms were 44.4 and 43.1%, respectively and no significant difference (p>0.05) was found between them (Table 1).

DISCUSSION

Contamination to aflatoxins in different kinds of food and feed is inevitable. This toxin has various impacts on human and animals specialist carcinogenicity. Contaminated feedstuffs is AFB₁, too metabolized inside livestock body and AFM, will enter into the milk. With due attention to milk and other dairy products used by human vastly, it is necessary to monitor consumer, health especially sensitive persons such as children and the old. Therefore, much research is done for determining this toxin in food and feed (Hell and Mutege, 2011; Farombi, 2006).

In Kenya detected 53.2% of Aflatoxin contamination at feedstuff over than 20 ppb (Lanyasunya et al., 2005). Reddy and Salleh in Malaysia collected animal feeds (corn grain) in order to determine AFB₁ by ELISA. AFB₁ were detected in 81.2% samples ranging from 1.0-135 µg kg⁻¹ (Reddy and Salleh, 2011). Whereas our study 43.7% of samples was contaminated over than the limit accepted by EU (5 µg kg⁻¹) and 56.3% were under 5 µg kg⁻¹. In Sudan, 64.29% of the samples of animal feed were contaminated with Aflatoxins at average concentration of
130.63 μg kg⁻¹ (Elzipir et al., 2009). Whereas, in our study we found max, min and mean of the concentration 1, 15 and 5.3 μg kg⁻¹, respectively. In Turkey AFB₁ and total aflatoxin contamination of the crop were reported 43 and 32%, respectively (Alptekin et al., 2009). The occurrence of AFB₁ at animal feed in Turkey by ELISA method was found to be 95% (Aksoy et al., 2009). In Southern Vietnam AFB₁ levels in feeds were 7.5 μg kg⁻¹ (Thieu et al., 2008).

Charoenpornsook and Kavisarasai in Thailand found out 25 samples of feedstuff, 23 samples (92%) contained AFB₁ with mean concentrations 7.56 μg kg⁻¹ (Charoenpornsook and Kavisarasai, 2009). In Kenya AFB₁ was isolated in 98.6% of animal feeds (Kangethe et al., 2007). In north Italy feedstuffs such as corn and concentrated feed were to have by ELISA. Out of 541 samples in 2004, 8.1% were contaminated with AFB₁ and in 2005 no sample was contaminated (Decastelh et al., 2007). In Bangladesh poultry feed samples were reported to have from 0 to 98 ppb AFB₁. The maximum of incidence was at June, July and August, while from November to February it was not detected in any feed. Similar to our study higher amount was observed in rainy season (Khan et al., 2005). In Iran AFB₁ was detected in 43.6% maize samples (Karami-Osboo et al., 2012). In this research unfortunately due to humidity in north of Iran, 43.7% of samples were over than standard limit. Due to harmful effects of this toxin on human body especially its carcinogenicity and immunosuppression in children; it is very hazardous for dairy products users, therefore it requires much attention (Shrif et al., 2008; Iheshiulor et al., 2011; Richard, 2007).

To decrease and reach to permissible limit of AFB₁ levels in human used livestock products and with due attention to this point that prevention is always better than remedy, only effective method is controlling contamination of feedstuffs and use of healthy feedstuffs. To minimize AFM, in milk and other dairy products and in order to ensure consumer health feedstuffs ingredients should be tested regularly in terms of AFB₁ and fungi growth should be prevented in order to have healthy feedstuffs for animal feeding.

CONCLUSION
Considering various effects of AFB₁ in human and livestock and the fact that a lot of livestock products such as milk and other dairy products used as main food for human, it is necessary to check AFB₁ levels in food and feed.

REFERENCES


