Aflatoxin Contamination in Livestock Feeds and Feed Ingredients of Nepal

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Abstract

Aflatoxins are secondary metabolites produced by moulds Aspergillus flavus and A. parasiticus, which are ubiquitous and frequently contaminate most of the agricultural and livestock food products. These toxins are potent hepatotoxins and carcinogens, and mainly cause liver cancer. With limited information available on the level of aflatoxin contamination in Nepalese livestock feeds and feed ingredients, its risk to human health is not extensively investigated. In this study, aflatoxin contamination in various feed ingredients / processed livestock and poultry feeds collected from different parts of Nepal was measured. Altogether, 100 samples comprising of 25 livestock feeds, 50 poultry feeds and 25 feed ingredients were collected and processed for aflatoxin assay. The percentage of samples with aflatoxin contamination was 80%, 74% and 72% respectively for livestock feeds, poultry feeds and feed ingredients, giving an overall contamination of 75%. Aflatoxin B1 was detected in all aflatoxin positive samples either alone or in combination with aflatoxin B2. The concentration of aflatoxin in positive samples ranged from trace to 300 μg/kg in livestock feeds, trace to 500 μg/kg in poultry feeds and trace to 300 mg/kg of feed ingredients. There was non significant difference (P>0.05) in contamination of aflatoxins between various feeds / ingredients examined (χ²2df = 0.48). Such a high level of aflatoxin is of great concern to livestock, poultry and human health. The need for regular and frequent checking of these commodities before consumption is emphasized.

Keywords: Aspergillus flavus, Carcinogens, Hepatotoxins, Poultry feeds

Introduction

Aflatoxins have attracted considerable attention in the recent years because of their potent toxic nature and common occurrence under natural conditions. The condition was first recognised in 1960, when heavy losses of turkey poults were reported in Great Britain (Blount 1961). Aflatoxins are the toxic metabolites derived from certain species of the moulds, especially by Aspergillus flavus and A. parasiticus (Bilgrami 1985), which are distributed world wide in air and soil (Dieners et al. 1987) and their growth is favoured by a warm (25-30°C) and humid climate (Kumar and Sampath 1971).

Hazards of aflatoxin on both livestock and human health have been well-documented in literature since 1961. The principal biological effects of aflatoxin are carcinogenicity (Newberne and Rogers 1981), mutagenicity (Ong 1975), teratogenicity (Di Paola et al. 1967) and immunosuppression (Pier 1973). It is now generally accepted that chronic hepatitis B virus infection has got synergistic effect on the carcinogenicity of aflatoxin B1 (Beasley et al. 1981), which is the most abundant and potent toxic among all aflatoxins. Consumption of aflatoxins contaminated feeds impair livestock and poultry performance by reducing feed efficiency and rate of the growth resulting into heavy economic loss. The effect of aflatoxins on man and animals depends upon the age, sex, species, nutritional status, dosage level, frequency and composition of diet.

The presence of aflatoxin in almost all types of feeds/ingredients has been recorded from different parts of the world (Ciegler et al. 1971). Limited studies done in Nepal also indicate the common occurrence of these toxins in livestock feeds/ingredients of Nepal (Karmacharya 1988, 1994). It is the sad part especially in developing countries where inferior or damaged edibles are channelised for the consumption to the poor people.
livestock and poultry, which eventually reach to general public through the food chain. Various pathways of aflatoxicosis in human through the food chain is depicted in Figure 1.

Present investigation was undertaken to study the occurrence of aflatoxin under natural condition in various feeds / ingredients used for livestock and poultry in three areas namely Kathmandu, Chitwan and Biratnagar of Nepal during summer season of 1998.

![Figure 1. Aflatoxin in food chain, adopted from ICMR (1993).](image)

**Materials and methods**

Materials for the present investigation consisted of 100 samples of different livestock and poultry feeds, and different ingredients of ration. Samples were collected from Kathmandu (Livestock feeds = 20, poultry feeds = 34, and ingredients = 25), Chitwan (Livestock feeds = 3, poultry feeds = 10) and Biratnagar (Livestock feeds = 2, poultry feeds = 6) during summer season from various sources. Samples were collected with the permission of the feed millers, i.e., only from those factories which were willing to cooperate. Hence, the number of samples analysed from each site and within each category is variable and probably less than optimally expected. Each sample comprised of one kg representative of a 100 kg feed / ingredient lots. Samples were collected in sterile polythene bag and were brought to the laboratory for aflatoxin analysis within 48 hrs of collection. Precautions were taken during sampling to avoid erroneous result of uneven distribution. Samples were immediately dried after their arrival at the laboratory at 60°C to remove moisture and kept in deep freeze to check further fungal growth.

Chemical extraction of aflatoxin from 65 samples was done in methanol : water (60 : 40 v/v) as suggested by Thomas et al. (1975) and from the remaining 35 samples in acetone : water (85 : 15 v/v) by pressure mini column method as per AOAC (1980). The methanolic and acetonate extract was finally eluted with chloroform. The qualitative estimation of aflatoxin was determined by thin layer chromatography technique as described by Scott et al. (1970). The quantitative estimation of aflatoxin B₁ was done spectrophotometrically as described by Nabney and Nesbitt (1965). The amount of aflatoxin present in the samples was calculated by the following formula:

\[
\text{AFL} \mu g / ml = D \times M \times 10^6 / e \times 1 \times 1000
\]

Where, \(D\) = optical desnity, \(M\) = molecular weight of aflatoxin, \(e\) = molar extinction coefficient, \(l\) = path length (1 cm cell was used).

The significance of contamination of aflatoxin was calculated using the chi-square test as per Snedecor and Cochran (1967).

**Results**

Characterization of different toxins was based on the fluorescence observed under ultra violet light. Aflatoxin B₁ and B₂ showed strong blue fluorescence, whereas aflatoxin G₁ and G₂ revealed green fluorescence. Analysis revealed that only 25% of the samples examined were free of aflatoxin contamination whereas the remaining 75% of feeds/ingredients were contaminated with aflatoxin (Table 1). Three different category, viz. Livestock feeds, poultry feeds and ingredients were examined in which aflatoxin contamination was 80%, 74%, and 72% respectively. Samples collected from all three locations viz. Kathmandu, Chitwan and Biratnagar had aflatoxin contamination in feeds / feed ingredients.

The percentage of feeds / ingredients with aflatoxin contamination ranged from a minimum of 66.67% to a maximum of 88.89% (Table 1). Among livestock feeds, contamination ranged from 66.67% for buffalo feed B to 88.89% for buffalo feed A (Table 1). Among the six different types of poultry feeds, aflatoxin contamination ranged from 66.67% in layer No. 1 and 2 to 81.82% in broiler No. 2. (Table 1). Within the seven different types of feed ingredients, aflatoxin contamination ranged from 66.67% (rice bran, rice polish, wheat bran and bone meal) to 80% (Maize). Seventy five percent of groundnut cake and fish meal samples had detectable level of aflatoxins (Table 1).

All aflatoxin positive samples were further analyzed for the presence of different two groups (B₁ and B₁+ B₂) of aflatoxin. It was observed that aflatoxin B₁ was present in all positive samples either alone or in combination with aflatoxin B₂. The
Table 1. Aflatoxin contamination in livestock feeds and feed ingredients of Nepal

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Name of samples</th>
<th>No. of samples analysed</th>
<th>No. of samples with aflatoxin</th>
<th>Range of concentration of aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B1</td>
<td>B1+B2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.</td>
<td>Livestock feeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cattle feed - A (Milch)</td>
<td>9</td>
<td>7(77.78)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Cattle feed - B (Dry)</td>
<td>4</td>
<td>3(75)</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Buffalo feed - A (Milch)</td>
<td>9</td>
<td>8(88.89)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Buffalo feed - B (Dry)</td>
<td>3</td>
<td>2(66.67)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td>20(80)</td>
<td>17</td>
</tr>
<tr>
<td>B.</td>
<td>Poultry feeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Layer No. 1 (Starter’s mash)</td>
<td>9</td>
<td>6(66.67)</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Layer No. 2 (Grower’s mash)</td>
<td>6</td>
<td>4(66.67)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Layer No. 3 (Finisher’s mash)</td>
<td>10</td>
<td>7(70)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Broiler No. 1 (Starter’s mash)</td>
<td>10</td>
<td>8(80)</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Broiler No. 2 (Grower’s mash)</td>
<td>11</td>
<td>9(81.82)</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Broiler No. 3 (Finisher’s mash)</td>
<td>4</td>
<td>3(75)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>50</td>
<td>37(74)</td>
<td>32</td>
</tr>
<tr>
<td>C.</td>
<td>Ingredients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Rice bran</td>
<td>3</td>
<td>2(66.67)</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Rice polish</td>
<td>3</td>
<td>2(66.67)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Maize</td>
<td>5</td>
<td>4(80)</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Wheat bran</td>
<td>3</td>
<td>2(66.67)</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Groundnut cake</td>
<td>4</td>
<td>3(75)</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Fish meal</td>
<td>4</td>
<td>3(75)</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Bone meal</td>
<td>3</td>
<td>2(66.67)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td>18(72)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Grand Total</td>
<td>100</td>
<td>75(75)</td>
<td>64</td>
</tr>
</tbody>
</table>

N.B. Figures in parentheses denote percentage

Aflatoxin B1 alone was found in 85.33% of positive samples while B1 was found in combination with aflatoxin B2 in remaining 14.67% of positive samples (Table 1). The statistical analysis showed non-significant (p>0.05) differences in proportion of various types of feeds / ingredients (livestock feeds, poultry feeds and feed ingredients) contaminated with aflatoxin ($\chi^2$ 2 df =0.48).

The concentration of aflatoxin B1 in positive samples was estimated. The frequency distribution of livestock feeds, poultry feeds and ingredients according to their range of aflatoxin B1 concentration is presented in Figure 2.

**Figure 2.** Frequency distribution of livestock feeds, poultry feeds and ingredients according to their range of aflatoxin concentration (μg/kg)

**Discussion**

Under natural condition there are three factors that contribute towards the aflatoxin contamination of food and feed substrate. These three factors are: presence of a toxigenic strain of *Aspergillus flavus*, congenial climate and a suitable food or feed
substrate. Dienert and Davis (1969) opined that moisture and relative humidity of the surroundings are the important factors for the growth and aflatoxin production by *Aspergillus flavus*. It is evident from Table 1 that only 25% of feed / ingredient samples examined during summer season were free of aflatoxin. The remaining 75% samples had aflatoxin contamination, in which the range of aflatoxin B1 concentration ranged from trace to 500 μg/kg feed. The broiler feed No. 2 had the highest amount of aflatoxin B1 (up to 500 μg/kg).

Though the present study was done only during the summer season and with a limited number of samples, the findings presented here clearly indicates the problem of aflatoxicosis in Nepalese feeds and their ingredients. The findings of the present study are similar those reported by Singh et al. (1997) in India, where 65% of 67 dairy cattle feeds were positive for aflatoxins, with a concentration of 50-400 μg per kg of feed. However, a higher proportion of aflatoxin positive feeds was detected in the present study when compared with that reported earlier from Nepal by Karmacharya (1994), where only 60% feeds were aflatoxin positive. This difference is likely to be due the time of sampling as their samples were collected year round in contrast to the only summer season sample collection in this study.

A high level of aflatoxin contamination in livestock / poultry feeds and their ingredients in the present study may be due to association of toxigenic strain of *Aspergillus flavus* along with the favourable climatic conditions prevailing in these areas during sample collection. Pre-monsoon and monsoon rain are heavy in these areas. Moreover, harvesting, storage and marketing facilities are poor, which greatly stimulate the aflatoxin occurrence (Sinha et al. 1999). All these conditions might have contributed to high moisture level in the feed/ingredient samples which become a fertile base for mould growth and consequently aflatoxin elaboration (Bilgrami 1986). This indicates the importance of year round sample collection for aflatoxin analysis.

In the present study, 35 of 100 feed/ingredient samples were processed by pressure mini column (PMC) method (AOAC 1980). The results obtained by pressure mini column are comparable to Thomas et al. (1975) method (Kathuria et al. 1993). The PMC method, however, had several advantages over other methods, in that it considerably saves time, labour and reagents, and is without loss of sensitivity. Thus detection of aflatoxin in feeds/ingredients by PMC method is highly suggestive for screening purposes, and will be easily applicable under Nepalese conditions.

The level of aflatoxin in more than half of the feed/ingredient samples exceeded 50 μg/kg (Table 1) which is more than the tolerance level fixed by HMG/Nepal (1995/96). This limit is higher than 30 μg/kg tolerance level fixed in India and 15 μg/kg tolerance level accepted internationally (ICMR 1993). Consumption of aflatoxin contaminated feed will not only affect the health of livestock and poultry but conversion of feed into animal protein is also reduced (Bhat and Miller 1991). Consumption of aflatoxin contaminated feeds is of a great public health concern as aflatoxin M1 secretion in milk of animals depends on the aflatoxin B1 in feed (Galvano et al. 1996).

It is difficult to get rid of the aflatoxin problem of the animal feed, especially in the tropical countries where, most of the months moisture, high humidity and suitable temperature causes favourable growth of the aflatoxigenic fungi and subsequently aflatoxin productions. Therefore a good harvesting, storage and marketing practices play a significant role in reducing these problems and should be advocated to educate farmers, feed/ingredient handlers and people involved in feed milling. Furthermore, it is essential to have rigorous monitoring programme for aflatoxin, so that better feed management can be done for the livestock and poultry. Provision of aflatoxin free feeds to livestock and poultry will also ensure a healthy food for human consumption and will reduce the risk of aflatoxicosis in the population.

**Conclusion and Recommendations**

1. The prescribed permissible level of aflatoxin fixed by HMG/Nepal is 30 ppb (μg/kg) in cereal grains and 50 ppb (μg/kg) for milch cattle feed respectively. Thus, proper rules and regulations have to be framed for monitoring of human food and animal feed, so that proper rejection of foods and feeds having aflatoxins level beyond the tolerance level can be done.

2. Farmers should be properly educated in the food and feed harvesting and storage practices.

3. For enhancing safety and minimizing losses from aflatoxicosis, farmers, grain handlers and marketing people should be educated from time to time.

4. Animals should not be fed with feeds and the fodder infested with moulds especially with *A. flavus*, as there is no curative treatment available...
for aflatoxicosis.

5. Routine screening of feeds / ingredients for aflatoxin by pressure mini column method should be adopted to reduce the losses from this problem.

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