RELATIONSHIP BETWEEN FEEDING TYPE AND THE OCCURRENCE OF AFLATOXIN M1 IN MILK OF HIGH YIELDING DAIRY COWS

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Abstract - The major problem associated with concentrate feeds used for feeding cattle is declining quality by contamination with Aflatoxins.
Objective: The aim of the study was to detect levels of Aflatoxin M1(AFM1) in cow milk , AFM1 levels present in milk related to different feed types and to identify the relationship between feed type and Aflatoxin M1 in milk.
Design: Cross sectional study design. Milk samples from each farm assessed for presence of AFM1 using High Performance Liquid Chromatographic method.
Setting: Ten dairy farms located in Nuwara-Eliya district were randomly selected.AFM1 analysis was done using High Performance Liquid Chromatography(HPLC).
Results: The results indicated that AFM1 was present in 50% of samples. Coconut poonac shown the most significant relationship among individual feeds having a correlation of 0.65 and P value of 0.042 . Among feed combinations, coconut poonac and beer pulp combination showed the highest correlation of 0.77 and P value of 0.05. Grasses had shown a very poor relationship with the AFM1 occurrence in milk (r=0.053, P=0.885). Relationship between overall concentrate feeds in the study and AFM1 in milk, it was clear that they had a significant relationship having correlation of 0.65 and P value of 0.042. Majority of samples lied between 0-10 ng L-1 of AFM1 and one sample exceeded above 30 ng L-1. Two samples had AFM1 concentrations between 22-32 ng L-1. One sample lied between 32-42ng L-1 , did not exceed the EU recommended level of 50 ng L-1. The presence of AFM1 in milk under various management and feeding conditions is yet to be investigated in Sri Lanka.

Keywords - Aflatoxin M1, Feed, Cow Milk

I. INTRODUCTION

Aflatoxins are naturally occurring mycotoxins which are metabolites produced by certain fungi in/on foods and feeds. They are the best known and most intensively researched mycotoxins in the world. Aflatoxins are toxic and among the most carcinogenic substances known.(Hudler, George., 1998) The toxin producing fungus was identified as Aspergillusflavus, and Aspergillusparasiticus.

.At least 14 different types of aflatoxin are produced in nature.(AOAC. 2005) Aflatoxin B1 is considered the most toxic and is produced by both Aspergillusflavus, and Aspergillusparasiticus.. Aflatoxin G1 and G2 are produced exclusively by A. parasiticus. Aflatoxins M1, M2 were originally discovered in the milk of cows which fed on moldy grain. These compounds are products of a conversion process in the animal's liver. However, aflatoxin M1 is present in the fermentation broth of Aspergillusparasiticus.

Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. Although the natural habitat of these fungi are soil, decaying vegetation, hay, and grains undergoing microbiological deterioration are more suitable for their growth. The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre harvest, storage, and/or processing periods. Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans. As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed. The United States Food and Drug Administration (FDA) has established action levels for aflatoxin present in food or feed to protect human and animal health.(Wikipedia, Free Encyclopedia).

The Joint Expert Committee on Food Additives (JECFA) has established an acceptable level of risk of 0.5 mg/kg for aflatoxin M1 in fluid milk. The International Grocery Manufacturers Associations (ICGMA) supports this assessment. In regions of the world where low levels of aflatoxins are virtually unavoidable in grains used for dairy feed, 0.5 mg/kg for aflatoxin M1 in milk is practical and allows an adequate supply of rations for milk producing animals. Even though the concentrations of AFM1 levels below 0.5mg/kg is acceptable in milk for human consumption it could have a negative public health effect by reducing the availability of a safe,

low cost food that is a good dietary source of high quality protein, calcium and other valuable nutrients. Reduction in availability of otherwise safe milk could also create an unnecessary health risk by depriving infants and young children of a dietary source of essential nutrients necessary for normal growth and development.

Table .1 FDA action levels for Aflatoxins

Commodity	Level(ng/g)
All products, except milk designated for humans	20
Milk	0.5
Corn for immature animals and dairy cattle	20
Corn for breeding dairy cattle, swine and mature poultry	100
Corn for beef cattle	300
Cotton seed meal(As a feed ingredient)	300
All feedstuff other than corn	20

These toxins are heat stable, so neither cooking nor freezing can destroy the toxin. Aflatoxins are produce on many grains and legumes, usually grains intended for human and animal consumption. These toxins may be present in grains and legumes used for animal feed production, hence may introduce to the digestive system through the animal feed. When processed, aflatoxins get into the general food supply where they have been found in both pet and human foods as well as in feedstocks for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk products and meat when animals are fed contaminated grains.(Scientificteaching.wis.edu)

Table.2 Maximum acceptable Levels in some regions

Region	Maximum Accepteble Levels(ng/l)	Type
European Union	50	Milk
Austria	50	Milk
Argentina	50	Milk
Bulgaria	500	Milk
Germany	50	Milk
Australia	20	Children's Milk
Sweden	50	Liquid Milk Products
Netherlands	20	Butter
Switzerland	50	Milk & Milk Products
Belgium	50	Milk
USA	50	Milk

Aflatoxins present in cattle feed will be metabolized in the liver and produce toxic metabolites which subsequently excreted in milk. Aflatoxin M1 is such less toxic hydroxylated metabolite of Aflatoxin B1 The main target organ in mammals is the liver so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favourmould growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control.(Smith, Tara (June 2005)).

Higher levels of Aflatoxin M1 in milk is not good for human consumption since it is a threat to the human health. Specially, if these toxins are present in infant formulae that would be more harmful. Low levels of aflatoxin exposure require continuous consumption for several weeks to months in order for signs of liver dysfunction to appear.(Fratamico, PM et al. 2008) .

Even though there are some standard levels which suitable for human consumption, identified by some countries, no special testing methods for Aflatoxin M1 in milk for human consumption in Sri Lanka. Therefore, it is important to detect levels of aflatoxins in milk of high yielding cows fed with concentrate feeds in Sri Lanka.

MATERIALS AND METHODS

Research was conducted in two (02) phases including, field survey and sample collection and laboratory investigations.
The field survey has been conducted in Veterinary Range Ambagamuwa(Hatton DS Division) in NuwaraEliya District. Random sampling method has been used.32 farms were base on Feeding type,management system (Intensive Management),number of Animals> 10 cows, and animals regularly fed with concentrates with grasses(Cut & Fed).

Data collected using a pre-prepared questionnaire and filled for each farm according to the data provided by the farmers regarding farm, animal, feed types and quantity given and other important data where necessary. Based on the information gathered through the questionnaire, 10 most suitable farms were selected among 32 farms. The animals of those farms fed with concentrates including commercial concentrate feed, coconut poonac, beer pulp together with grasses and three farms fed animals only with chopped hybrid grasses. Feeding consistency of each farm was observed for one week and milk samples were collected thereafter for analysis of AFM1.

Sample Collection- 10 Milk samples of 60 ml in volume were collected from each farm according to the type of feed combination given. Each farm

consisted of minimum 10 milking cows. Morning milk from all milking cows in the farm was bulked and representative sample was taken. Analysis of milk samples for Aflatoxin M_1 were carried out using Official Methods of Analysis of Association of Official Analytical Chemists (AOAC) Official Liquid Chromatographic Method 986.16 - for Aflatoxins M_1 and M_2 in Fluid Milk. AFM1 concentrations has been obtained by the HPLC test. Based on the findings, evaluated the relationship between AFM1 concentrations in milk and different feed types.

HPLC analysis:

Milk mobile phase for HPLC was deionized water: isopropyl alcohol: ACN (80: 12: 8). It was filtered in a filter unit using Varian-Nylon 66 filters and degassed in an ultrasonic bath before using for HPLC analysis. Samples and standards also were filtered using WHATMAN Puradisc™ 13 mm syringe filters. Before injecting samples into HPLC, milk mobile phase had been passed through the columns for a particular time period until the system was stabilised and gave a good baseline. Samples had been injected to the sample injector followed by passing through HPLC columns at a flow rate of 1.0 mL min^{-1}. Identification of AFM_1 was based on the retention time with respect to the standard. After obtaining the chromatograms, aflatoxin M1 concentrations were calculated by following equation.

AFM1 concentration (ppb) = (H × C' × VI' × V) / (H' × VI × W)

where H and H' = peak height of sample and standard, respectively;

C' = concentration of standard (ng μL^{-1});

VI' and VI = volume injected of standard and sample, respectively;

V = final total sample volume (µL);

W = volume of milk represented by final extract (typically 20 mL).

RESULTS AND DISCUSSION

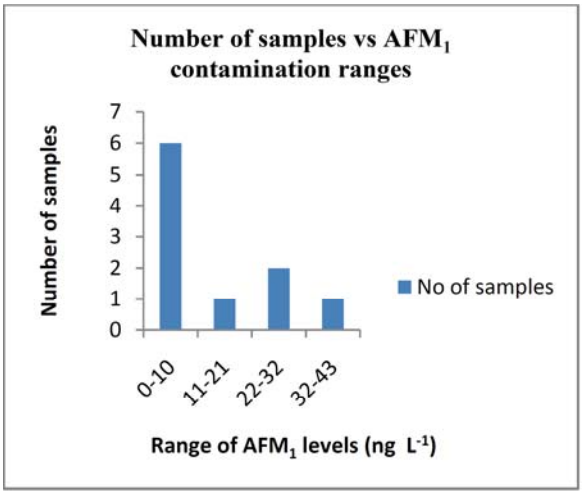


Figure 1. Graph showing number of AFM_1 contaminated samples in relation to contaminated ranges

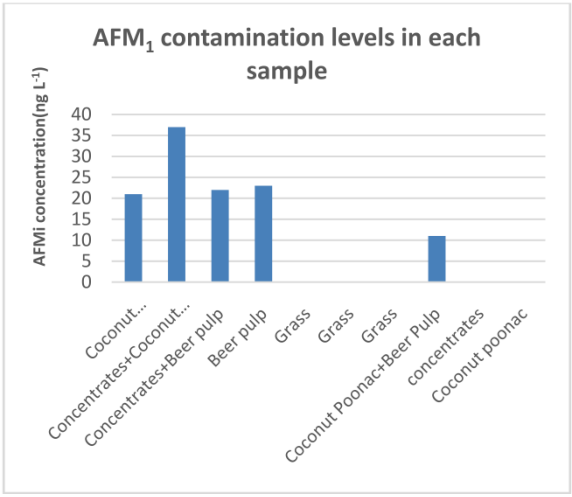


Figure 2. Graph showing contamination levels of AFM_1 in milk samples

The results indicated that AFM1 was present in 50% of milk samples which which indicated the extent of AFM1 contamination. When compared individual feed types, coconut poonac was considered to have the most significant relationship with the AFM1 occurrence having a correlation of 0.65 and P value of 0.042 . Among the feed type combinations, coconut poonac and beer pulp combination had showed the highest correlation of 0.77 and P value of 0.05. Grasses had shown a very poor relationship with the AFM1 occurrence in milk (r=0.053, P=0.885). However, when consider the relationship between overall concentrate feeds present in the study and AFM1 contamination in milk, it was clear that they had a significant relationship having correlation of 0.65 and P value of 0.042. Most number of the samples lied between 0-10 ng L-1ofAFM1 concentrations and one sample exceeded above 30 ng L-1. Two samples had AFM1 concentrations between 22-32 ng L-1. Only one sample lied between 32-42ng L-1 did not exceed the EU recommended level of 50 ng L-1.

Most number of the samples lied between 0-10 ng L-1ofAFM1 concentrations. Only one sample exceeded above 30 ng L-1. Two samples had AFM1 concentrations between 22-32 ng L-1. None of the samples exceeded above the EU recommended level of 50 ng L-1.

According to this study, overall conclusion was that, concentrated cattle feed (concentrates) was not significantly responsible for the occurrence of AFM1 in milk here, but relationship observed when considered individual samples, and accordingly;there was a relationship between coconut poonac and AFM1 concentration, a strong relationship between AFM1 concentration and concentrates+coconutpoonac , a significant relationship between coconut poonac+beerpulp+concentrates and AFM1

concentration, and no relationship between grasses and AFM1 concentration.

CONCLUSION

Based on results obtained, it can be concluded that concentrate feeds have a significant relationship on the AFM1 contamination in cow milk, indicating coconut poonac has the highest risk of contamination among the individual feed types whereas coconut poonac and beer pulp combination showed the highest contamination among feed combinations. Grasses have no effect on the incidence. These kinds of studies would be beneficial for local dairy producers to understand the nature of aflatoxin contamination in feed and relevant authorities to take necessary precautions to protect the dairy product consumers in Sri Lanka. Further,concentrated feeds should be stored properly to minimize contamination by aflatoxin like fungi. However, these findings were preliminary and further investigations would be essential for conclude on AFM1 contamination in milk and relationship of feeding types with the incidence of AFM1 and presence of AFM1 in milk under various management and feeding conditions is yet to be investigated in Sri Lanka.

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