Effects of Feeding Pattern and Feeding Type on Occurrence of Aflatoxin M_1 in Milk of High Producing Dairy Cows

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Introduction

The dairy sector has been identified as the priority sector for the development among other livestock sub sectors in Sri Lanka as the country is currently in the process of achieving at least 50% self sufficiency in milk production by year 2015. The demand for ruminant feeds, mainly roughages and concentrates is always increasing with the increase of milk production. As there is lack of suitable lands for enough roughage production and due to their poor quality, dairy producers tend to use higher amounts of concentrate feeds, such as rice by products, maize, coconut poonac, compound animal feeds, etc. The major problem associated with concentrate feeds is declining quality by contamination with fungal toxins called "Aflatoxins".

Aflatoxins (AFs) are a group of mycotoxins produced mainly by common fungi *Aspergillus flavus, A. parasiticus* and *A. nominus* that present in foods and feedstuffs such as in cereals, nuts, etc (Zinedine *et al.,* 2007). Aflatoxin M_1 (AFM_1), a hydroxylated metabolite of Aflatoxin B_1 formed in the liver subsequently occurs in milk from lactating animals consuming AFB_1 contaminated feed. (Lin *et al.* 2004). Aflatoxins are associated with various health and production problems in lactating animals. International Agency for Research on Cancer (IARC) classifies AFM_1 as a possible human carcinogen that has comparable liver toxicity, which can reduce the immunity of infants. Humans get those toxins through AFM_1 contaminated milk and milk products. Several countries have established legislation to regulate the levels of AFB_1 in feeds and AFM_1 in milk because of their carcinogenicity (Lin *et al.,* 2004).

Though considerable numbers of studies have been conducted internationally, research performed locally on this matter is very limited. Thus, Sri Lanka does not have standards for maximum aflatoxin levels to be allowed in animal feeds and milk. It is important to know the amount of AFM_1 in locally produced milk having a special significance for infants and children. A recently conducted study by Pathirana *et al.*, (2010) to evaluate the farm gate cow milk for AFM_1, revealed that 33% of the locally collected milk contained aflatoxin M_1 with 9.2% exceeded the European Communities/Codex Alimentarius recommended limit of 50 ng L^{-1}, highlighting the significance of the problem. The study also states that the incidence of AFM_1 contamination is often high where cows are fed with high amounts of compound concentrate feeds. The occurrence of AFM_1 under various feeding practices under Sri Lankan conditions should be investigated to understand the effects of feeding type on the incidence of AFM_1 in the milk as there is no such study available currently.

Therefore, this research was conducted as a preliminary study for the objectives of investigating the relationship between feeding pattern and feed type on the occurrence of AFM_1 in cow milk collected from the up country dairy farms.

Materials and Methods

Ten dairy farms located in Government Veterinary Surgeon's area of Hatton (Abagamuwa) in Nuwara-Eliya district were randomly selected. The criteria used to select the farms were; intensive management, consisting high producing animals and feed with concentrates and grasses (cut and fed). Each farm had been visited, milk samples were collected and background information was gathered using a questionnaire. Immediately after collection, samples were transported to the laboratory packed and stored in between ice bags in a Styrofoam box. The samples were stored at -20 °C until AFM_1 analysis at the laboratory.

Analysis of the milk samples for Aflatoxin M_1, were carried out using official liquid chromatographic Method 986 of association of official analytical chemists (AOAC).16 - for Aflatoxins M_1 and M_2 in Fluid Milk. The AFM_1 was extracted to by sending 20 mL of milk diluted with 20 mL of ~80 °C hot water followed by wash solution, through a C_{18} cartridge (Sep-Pak® Classic, Waters Corporation, USA) primed with methanol and deionised water. The cartridge was reprimed by 150 µL CH_3CN and AFM_1 was purified by eluting with ether and CH_2Cl_2-alcohol (95:5) onto a silica column (Sep-Pak® Classic, Waters Corporation, USA).The eluate was driedunder a gentle stream of nitrogen and derivatised using hexane and trifluoroacetic acid. The final residue was dissolved in 2 mL of H_2O:CH_3CN (75:25). AFM_1 extract of 50 µL was analyzed by High Performance Liquid Chromatography (HPLC) (Varian Inc., California, USA).

AFM_1 working standard of 500 ppb were daily prepared in Benzene: CH_3CN (90:10) using AFM_1 stock standard (SIGMA, Steinheim, Germany) from *Aspergillus flavus* of 10 ppm. The working standard was derivatized prior injection into HPLC system. Milk mobile phase for HPLC was deionised water: isopropyl alcohol: ACN (80: 12: 8). Samples were injected to the sample injector followed by passing through HPLC columns (Microsorb™-MV; L×ID : 150 × 4.6 mm) at a flow rate of 1.0 mL min^{-1} and AFM_1 was identified based on the retention time (about 12.0 min.) with respect to the standard. Correlation coefficient of AFM_1 to different feeding and production parameters were analysed using Minitab 15.0 and Microsoft Excel 2007. Respective graphs were prepared and statistical values including the graph formula were indicated.

Results and Discussion

Detected levels of AFM1 contamination in milk samples were shown in Figure 1.

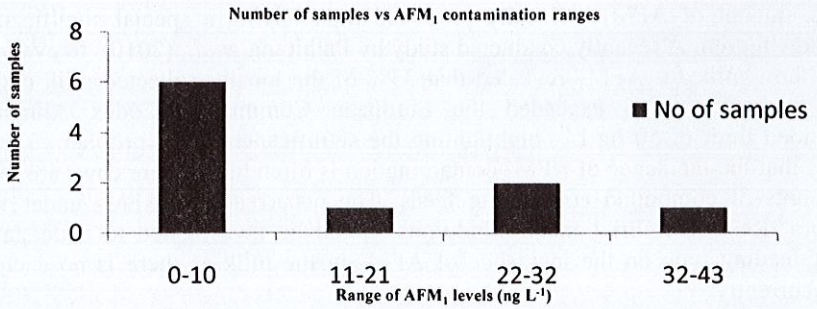

Figure 1: Number of AFM_1 contaminated samples in relation to contaminated ranges

Mean level of AFM_1 contamination in the samples was 11.1 ng L^{-1}. None of the samples exceeded European Communities/Codex Alimentarius recommended level of 50 ng L^{-1}. Furthermore, lowest contamination level was 8 ng L^{-1} while the highest contamination level was 36 ng L^{-1}.

There are many studies in the world on Aflatoxin M_1 contamination in milk and milk products in different markets. But few studies have been conducted to investigate relationship between AFM_1 occurrence and feed type. Zinedine *et al.,* (2007) indicated a seasonal variation of AFM_1 contamination in milk, showing less occurrence of AFM_1 in milk as cows receive less concentrated feed in summer when they are grazing where the animals tend to consume more forage, roughage and pasture. Sinprasopchai *et al.* (2006) had shown that there was a trend of considerable positive correlation between total aflatoxin in concentrated cow feed and aflatoxin M_1 in milk.

In this study, there was no strong correlation found between the milk production and AFM_1 content in milk, despite some literature indicate an association between decreased milk production and AFM_1 contamination. Studies reveal that appearance of AFM_1 in milk is directly related to that in the feed, so that, quantity of milk produced would not have any effect. Normally, consumption of aflatoxin contaminated feed for long periods can result in decreased milk production Zinedine *et al.,* (2007) but as feed was not analysed for basic aflatoxins, the study is unable to justify that.

In this study, the major feed types given to the cows in the selected farms were concentrates (compound cattle feed, coconut poonac and beer pulp) and grasses. The correlations of milk production/day to individual feed types and different concentrate feed type combinations with detected AFM_1 concentrations are provided in table 1.

Table 1: Correlations of AFM_1 concentration with milk production (L/day), concentrates (kg/day) and grasses (kg/day)

	Milk production(L/day)	Concentrates (kg/day)							Grasses (kg/day)
		A	B	C	A+B	A+C	B+C	A+B+C	
r value with AFM_1 concentration (ng L^{-1})	0.37	0.52	0.65	0.41	0.66	0.52	0.77	0.65	0.053
R^2 (Coefficient of determination (%)	13.7	27.1	42.2	16.4	42.9	27.2	58.8	42.3	0.02

df (Degree of freedom) = 8; Critical r value = 0.632; r - Coefficient of correlation
A-Compound cattle feed; B-Coconut poonac; C-Beer pulp

When compared the variations of aflatoxins in individual concentrate feed components fed, in the study, coconut poonac was considered to have the most significant relationship with the AFM_1 occurrence having a positive correlation of 0.65. Among the feed type combinations, coconut poonac and beer pulp combination had showed the highest correlation of 0.77. Overall, almost all concentrate feed components had given positive correlations above 0.5 with AFM_1 contamination in milk except beer pulp due to its lower quantities given in few farms. Grasses had shown a very poor relationship (r=0.053) with the AFM_1 occurrence in milk. However, when considered the relationship between

concentrate feeding (Compilation of the amounts of compound cattle feed, coconut poonac and beer pulp fed) and AFM_1 contamination in milk, it was clear that they had a significant positive relationship. Studies show that the main factor, which determines the occurrence of AFM_1 in milk, is the amount of aflatoxins in the concentrate feed but not the quantity fed, which is mainly affected by weather, storage conditions and other factors. In the current study, in some farms where milk samples were obtained, poor storage conditions of concentrates had been observed. Therefore, concentrated feeds should be stored properly to minimize contamination by aflatoxin producing fungi. However, these findings were preliminary and further investigations would be essential for conclude on AFM_1 contamination in milk and the relationship of feeding types with the incidence of AFM_1 in milk in Sri Lanka.

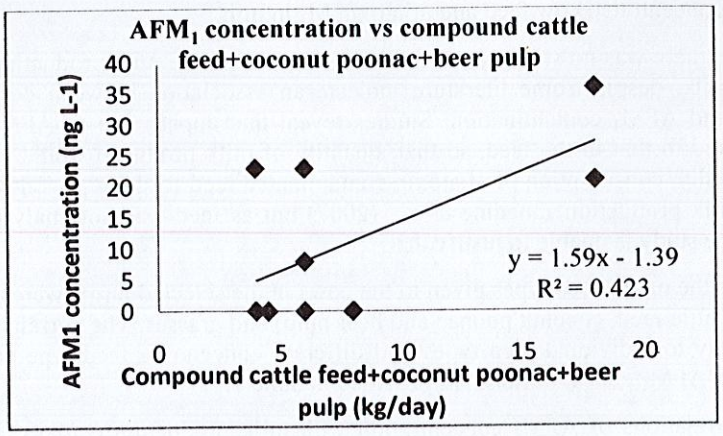

Figure 2: Relationship between compound cattle feed+beer pulp+coconut poonac (kg/day) and AFM_1 concentration (ng L^{-1})

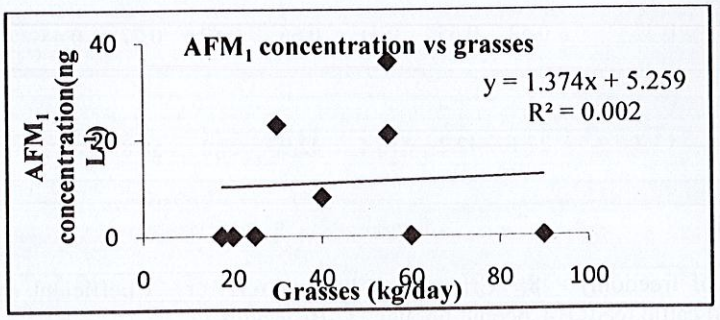

Figure 3: Relationship between Grasses (kg/day) and AFM_1 concentration (ng L^{-1})

Conclusion

Based on the results obtained, it can be concluded that concentrate feeds have a significant relationship on the AFM_1 contamination in cow milk, while 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 particular feed types and relevant

authorities to take necessary precautions to protect the dairy product consumers in Sri Lanka. The presence of AFM_1 in milk under the various management systems, seasonal and feeding conditions are yet to be investigated in Sri Lanka.

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