小規模酪農農家における飼料および飼料管理が牛乳中のアフラトキシンM1に与える影響

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Feed and feed storage factors in relation to aflatoxin M_1 contamination in bulk milk of smallholder dairy farms

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Abstract

The aim of the study was to determine feed and feed storage factors associated with aflatoxin M_1 (AFM_1) contamination in bulk milk of dairy farms. The study was conducted from May to July 2016, at all smallholder farms in Mae Wang dairy cooperative, Chiang Mai, Thailand. Data on feed and feed storage factors were collected from the farmers using interviews and observations. For feed, we included type of roughage and physical appearance of concentrated feed, and for feed storage factor, we included storage method of roughages. AFM_1 concentration was measured using the Charm® ROSA® MRLAFMQ (aflatoxin M_1) Test. Fisher's exact chi-square test was used to determine the association of feed and feed management factors with AFM_1 contamination. From a total of 67 farms, 50 farms were included in the analysis. AFM_1 contamination was observed in 46% of the samples. Farms using factory-corn silage had a significantly higher percentage of AFM_1 contamination (62.5%) than farms that did not use factory-corn silage (30.8%). AFM_1 contamination in farms that used concentrates with cracked pellets was significantly higher (64.3%) than in those that did not (22.7%). For feed storage, roughage stored in piles within the barn was associated with significantly higher AFM_1 contamination than that stored outside (61.5% and 29.2%, respectively). In addition, AFM_1 contamination for roughage piles with mold on the surface was higher (60%) than that for roughage piles without mold (25%). Our results indicate that type of feed and feed storage factors are associated with AFM_1 contamination in bulk milk.

Introduction

Aflatoxin M1 (AFM_1) is a mycotoxin found in milk, which is metabolized from aflatoxin B_1 (AFB_1) consumed by cattle through their feeds, and is classified by the International Agency for Research on Cancer as a group 2B human carcinogen[1)]. Owing to the high consumption of milk and milk products by humans, AFM_1 contamination of milk is one of the greatest public health concerns, especially in children[2),3)]. Problems due to AFM_1 contamination in milk have been reported in many countries, including Thailand[2),3)].

The main factor determining AFM_1 concentration in milk is the level of AFB_1 concentration in cattle feeds[4),5)]. Findings reported by Suriyasathaporn and Nakprasert[2)] suggest that dairy farm mismanagement causes AFM_1 contamination in commercial pasteurized milk. Improper environment in feed storage rooms, including temperature and humidity, facilitates fungal infection and promotes AFB_1 production in dairy cow feeds[6),7),8),9)]. AFM_1 contamination in milk samples is also related to improper environment of feed storage rooms[10)]. In addition, use of commercial concentrates with abnormal characteristics, such as having cracked pellets or having a positive result to fluorescent light, increased the risk of AFM_1 contamination[11)].

Many countries, including Thailand, use mixed rations of roughage and concentrates[10),11)]. Most studies have investigated AFM_1 or AFB_1 contamination in concentrates and related raw materials due to their long duration of storage[10),11),12)]. A recent study showed that storing roughage for a short time also increased the risk of AFB_1 contamination[11)]. However, it is necessary to know the characteristic factors of roughage related to AFM_1 contamination in milk. Therefore, the objective of this study was to determine the factors of abnormal feed characteristics, type of roughage and feed storage method associated with AFM_1 contamination in bulk milk.

Materials and Methods

Study design and data collection

An observational cross-sectional study was performed from May to July 2016, at 67 smallholder dairy farms from Mae Wang dairy cooperative, Chiang Mai Province, Thailand. Most farms had, along with milking cows, five to sixty animals, and fed their cows with a mixed ration of roughage and commercial concentrates. Data on feed and feed storage method were collected on the same day of milk collection. The majority of commercial concentrates were purchased from the cooperative. Types of roughage originated from post-harvest agricultural plants, including grass, hay, and maize, or were by-products from corn processing factories, including dry ground outer layer of corn seed (corn dust), dry corn cover, and boiled corn cobs with husks (factory-corn silage). Feed and feed storage factors included characteristics of commercial concentrates (with or without cracked pellets), keeping roughage in piles within cow barns (yes or no), and having mold on the surface area of roughage piles (yes or no).

Milk collection and AFM_1 measurement

At each farm, 30 ml of bulk tank milk was collected in a plastic tube, stored on ice, and immediately transported to the Faculty of Veterinary Medicine, Chiang Mai University. Concentrations of AFM_1 were determined by an immunoreceptor assay utilizing Charm® ROSA® (Rapid One Step Assay) lateral flow technology, using the Charm® MRL aflatoxin M1 quantitative test (MRLAFMQ) (Charm Sciences Inc., Lawrence, MA, USA). Test kits were from kit lot 016 (Exp. 10/2016). Samples were kept at 0°C to 7°C and mixed well using vortex mixer before testing. A milk sample (300 µl) was added to the MRLAFMQ test strip and incubated at 40°C on the ROSA® incubator for 15 min. The test strips were removed and inserted into the Charm EZ READING instrument for 1 min, to determine AFM_1 concentrations in the milk samples. Samples with AFM_1 concentrations greater than 40 ppt were considered contaminated with AFM_1.

Statistical analysis

Data on feed and feed management factors were expressed as percentages. The association of feed and feed management factors with AFM_1 contamination was analyzed using Fisher's exact chi-square test (SAS, 1997). The significant level was set at $P < 0.05$ for all analyses.

Results

Out of the 67 farms, 17 were excluded due to incomplete data collection. Twenty-three (46%) of the 50 milk samples in the final analysis were contaminated with AFM_1 (Fig. 1).

The distribution of feed used is shown in Table 1. Twenty-eight farms (56%) used commercial concentrates with cracked pellets. Regarding type of roughage, dry corn cover (78%) was used in most farms, whereas corn dust was used in only six farms (12%). Due to the high incidence of AFM_1 contamination in the milk samples (46%), all feed had high levels of AFM_1 contamination ranging from 22.7% to 64.3% for farms using and not using concentrates with cracked pellets. Farms using grass had a lower percentage of AFM_1 contamination than farms using another type of roughage. Comparing all feed types, farms that used factory-corn silage (62.5%) or concentrates with cracked pellets (64.3%) had significantly higher percentages of AFM_1 contamination than those that did not (30.8% and 22.7%, respectively) (*P = 0.046, P = 0.005*).

AFM_1 contamination in bulk milk based on facility and feed storage factors are reported in Table 2. Our results show that farms that had fewer than 15 milking cows exhibited significantly lower rates of AFM_1 (23.5%) contamination than larger farms (57.6%) (*P = 0.035*). As for feed storage method, roughage stored in piles within the cow barn was associated with significantly greater AFM_1 contamination than roughage stored outside (61.5% and 29.2%, respectively) (*P = 0.027*). In addition, roughage piles with mold on the surface were associated with higher percentages of AFM_1 contamination than those without (60% and 25%, respectively) ($P = 0.021$).

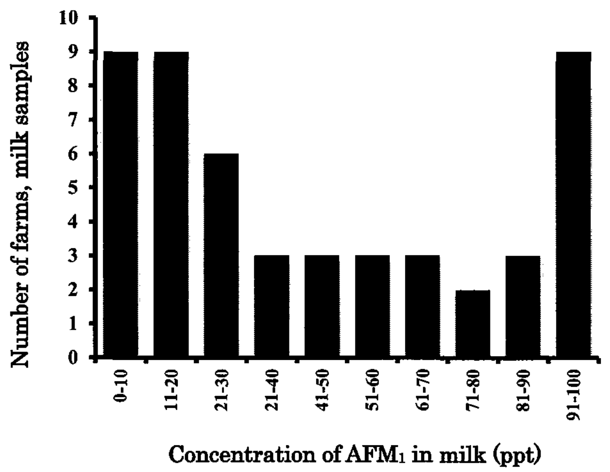

Fig. 1 Distribution of AFM_1 contamination in smallholder dairy farms in Mae Wang dairy cooperative, Chiang Mai, Thailand.

Table 1 Percentage of AFM_1 contaminated milk in relation to feed type and presence.

Factor	Yes			No			P-value (α = 0.05)
	Total (n)	AFM_1 positive (n)	%	Total (n)	AFM_1 positive (n)	%	
Concentrates with cracked pellets	28	18	64.3	22	5	22.7	0.005
Corn	14	7	50.0	36	16	44.4	0.760
Grass	21	8	38.1	29	15	51.7	0.398
Hay	32	16	50.0	18	7	38.9	0.559
Factory-corn silage	24	15	62.5	26	8	30.8	0.046
Dry corn cover	39	19	48.7	11	4	36.4	0.515
Corn dust	6	3	50.0	44	20	45.5	1.000

Table 2 Percentage of AFM_1 contaminated milk in relation to facility and feed storage factors.

Factor	Yes			No			P-value (α = 0.05)
	Total (n)	AFM_1 positive (n)	%	Total (n)	AFM_1 positive (n)	%	
Milking cows ≤15	17	4	23.5	33	19	57.6	0.035
Keeping roughage pile within barns	26	16	61.5	24	7	29.2	0.027
Keeping roughage in plastic bags outside barns	11	7	63.6	39	16	41.0	0.305
Using plastic sheets for roughage	20	11	55.0	28	10	35.7	0.242
Mold found on surface area of roughage	30	18	60.0	20	5	25.0	0.021

Disscusion

This study analyzed AFM_1 contamination using Charm® ROSA® MRLAFMQ, a rapid method for detection of AFM_1 in milk. This is a routine screening method for periodic checks of AFM_1 contamination in milk before sending it to factories, especially in European countries. Based on the test guidelines, milk samples with AFM_1 concentration of 40 ppt or greater are considered contaminated. According to the EU regulations, samples with concentrations exceeding the European Union maximum residue levels (EU MRL) (> 0.05 ppb or > 50 ppt) were considered AFM_1-contaminated. The difference in the cutoff values for the Charm® ROSA® MRLAFMQ guidelines and the EU MRL might be due to limitations of the test. However, this study uses cutoff value of the test in order to minimize this limitation.

Overall, 46% of bulk milk was AFM_1-contaminated, with concentrations ranging between 0 ppt and 100 ppt (Fig. 1). Ruangwises and Ruangwises[3] reported that in central Thailand, 47.5% of milk samples contaminated with AFM_1 had concentration levels exceeding the EU MRL value. In addition, AFM_1 in commercial pasteurized milk throughout Thailand ranged from 4 ppt to 293 ppt[2], which is a similar range as reported in the present study. Feed-related factors, such as feed characteristics and feed positive to blue-greenish yellow fluorescence test related with AFM_1 contamination in milk, have been previously reported[5),10)]. This study found that farms using concentrated feeds with cracked pellets had a higher percentage of AFM_1 contamination in milk than did farms using concentrated feeds without cracked pellets. This finding is supported by a previous study showing that cracked particles of concentrated feeds increased the risk of AFM_1 contamination in raw bulk milk[10),13)]. Broken or fine pellets indicate low quality of raw ingredients[12)], which increases risk of contamination with fungi and AFB_1[14),15)].

Among the six types of roughage, hay, factory-corn silage, dry corn covers, and corn dust had long durations of storage time outside barns. In contrast, corn and grass were generally transported to dairy farms immediately. Out of the four types of roughage with longer storage duration, factory-corn silage was the only wet roughage with very high humidity. It is well documented that in areas of high humidity, high temperature, and improper storage conditions, fungal growth is favored and there is an increased amount of mycotoxins produced in silage[8),16)]. Therefore, the characteristic wetness of factory-corn silage might greatly increase fungal growth and aflatoxin presence[8),10),11)].

Farms with greater than 15 milking cows had a higher percentage of AFM_1 contamination. This might relate to the duration of feed storage times due to the high amount needed for farm maintenance. Storing factory-corn silage for 2 weeks increased AFM_1 contamination by approximately twofold compared to the contamination before storage[11)]. This might be due to increased levels of fungi and aflatoxin contamination in maize[17),18)]. The high risk of AFM_1 contamination in farms storing roughage in piles within barns might be explained by the high humidity within barns, which is favorable for mold growth and increased AFB_1 production[19),20),21)]. Increased risk of AFM_1 in milk because of increased mold growth is also supported by our result showing that farms using roughage piles that have mold on the surface had higher percentages of AFM_1 contamination than farms using roughage piles without mold (Table 2). Other studies have also found that mold on the surface increases the amount of AFB_1 in the feed[8),16)]. These criteria might be used as a tool for monitoring the risk of AFM_1 contamination in dairy farms.

Conclusion

Feed and feed management, which includes type of roughage (factory-corn silage), farm size, cracked pellets of concentrated feed, mold appearing on the surface of feeds, and roughage stored in piles within barns are associated with AFM_1 contamination in bulk milk.

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小規模酪農農家における飼料および飼料管理が牛乳中のアフラトキシンM_1に与える影響

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本研究は，飼料と農場の牛乳中AFM_1汚染に関連する飼料管理因子を同定するために行った．全ての小規模酪農農家を対象に，2016年5月から6月の間に実施した．飼料と飼料管理のデータは集められた．大部分の農場では，粗飼料は草，干し草，とうもろこしを含む収穫後の農作物や，製粉後の外皮乾燥物（トウモロコシフスマ）や鞘と一緒に軸をゆでて乾燥させたもの（サイレージ）を含む地元のトウモロコシ工場からの副産物由来であった．市販の濃厚飼料は共同農場からの購入がほとんどであった．飼料管理因子は以下の項目を含む．市販濃厚飼料について：挽き割り粒子の有無，牛舎内への粗飼料パイルの保管：有無，粗飼料パイル表面へのかび付着：有無，牛乳中AFM1量はCharm EZ system（Charm sciences Inc., USA）を使用してCharm ROSA MRLAFMQ（Aflatoxin M_1）Testにより測定した．Fisherのカイ二乗検定により，飼料と，AFM_1汚染された飼料管理因子の交互作用を解析した．総計67農場のうち，最終分析できたのは50農場であった．サンプルの46%がAFM_1に汚染されていることが結果から分かった．サイレージが使われている農場（62.5%）が，そうでない農場（30.8%）よりAFM_1汚染率が有意に高かった．挽き割り粒子のある濃縮飼料が使われている農場（64.3%）でのAFM_1汚染は$P<0.01$で挽き割り粒子なし（22.7%）よりも含有率が高かった．飼料管理について，牛舎内で（61.5%対29.2%）パイルとして保存されている粗飼料ではAFM_1汚染含有率が高いのが特徴的であった．さらに，表面にかび付着があった粗飼料パイルは（60%対20%）AFM_1汚染率が高かった（$P=0.02$）．以上より，飼料と，飼料保管管理因子の両方が，牛乳中のAFM_1汚染に関連していることが明らかとなった．

キーワード：アフラトキシンM_1，汚染，牛乳，飼料管理