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7 Estimation of Aflatoxin M1 Exposure through Consumption of Various Dairy Milk Products in Yogyakarta, Indonesia

*(ESTIMASI PAPANAN AFLATOKSIN M1 MELALUI KONSUMSI
BERBAGAI PRODUK SUSU DI YOGYAKARTA, INDONESIA)*

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22 ABSTRACT

This study was conducted to investigate the occurrence of aflatoxin M1 (AFM1) in various milk products marketed in Yogyakarta Province (Indonesia) and to estimate the exposure of aflatoxin through contaminated milk consumption. Fresh milk (n=20), pasteurized milk (n=16), and recombined milk products (n=6) were sampled for AFM1 concentration testing by a competitive ELISA test using ELISA kit for AFM1 assay. A survey was conducted to interview consumers (n=88) on milk consumption habit (milk type and amount of consumption). ELISA assays showed 92.5% of samples were contaminated with AFM1 in a range of 24-570 ng/L (average: 216 ng/L). The highest average AFM1 concentration was detected in pasteurized milk sample (244 ng/L), followed by fresh milk (219 ng/L), and the lowest was in recombined milk sample (131 ng/L). However, 100% of recombined milk samples had AFM1 concentration >50-500 ng/L. Thus, recombined milk product was most likely the main source of AFM1 intake due to its high daily consumption in all age groups. Based on AFM1 levels found in milk and consumption of corresponding milk sample, it was estimated that the overall AFM1 exposure ranges from 1.23 ng/kg body weight/day (in 6-15 year-old children) up to 5.26 ng/kg body weight/day (in 3-5 year-old children). In conclusion, this study revealed high occurrences of AFM1 dairy milk marketed in Yogyakarta. Although levels of AFM1 contamination were in Indonesian regulatory limit, high exposure of aflatoxin found in all age groups of consumer. Thus, this preliminary study provides evidence that AFM1 contaminated milk is a serious public health hazard in Indonesia.

Key words: aflatoxin M1; aflatoxin transfer; aflatoxin exposure; dairy milk

ABSTRAK

Penelitian bertujuan untuk mengetahui tingkat kejadian cemaran aflatoxin M1 (AFM1) pada berbagai produk susu sapi perah yang dipasarkan di Kabupaten Sleman dan Kulomprogo serta Kotamadya Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia, serta estimasi paparan AFM1 melalui konsumsi susu terkontaminasi tersebut. Sampling dilakukan pada susu segar (n=20), susu pasteurisasi (n=16) dan susu rekombinasi (n=6) untuk diuji kandungan AFM1-nya dengan metode kompetitif ELISA menggunakan ELISA kit untuk analisis AFM1. Selain itu, wawancara juga dilakukan pada konsumen susu (n=88) untuk mengetahui kebiasaan minum susu (jenis dan jumlah konsumsi). Analisis ELISA memperlihatkan 92,5% dari sampel susu terdeteksi mengandung AFM1 dengan kisaran 24-570 ng/L (rerata 216 ng/L). Konsentrasi AFM1 tertinggi ditemukan pada sampel susu pasteurisasi (244 ng/L), diikuti susu segar (219 ng/L) dan susu rekombinasi (131

ng/L). Meskipun demikian, susu rekombinasi memiliki tingkat kejadian cemaran AFM1 100% dengan tingkat cemaran antara 50-500 ng/L. Berdasarkan data konsentrasi AFM1 sampel susu, konsumsi susu sampel, berat badan dan kelompok umur konsumen, diestimasikan paparan AFM1 melalui susu berkisar dari 1,23 ng/kg bobot badan/hari (kelompok usia 6-15 tahun) hingga 5,26 ng/kg bobot badan/hari (kelompok usia 3-5 tahun). Disimpulkan, bahwa kejadian cemaran AFM1 tinggi pada susu yang dipasarkan di Yogyakarta. Meskipun tingkat cemaran AFM1 masih dalam batas yang diperbolehkan, namun tingkat paparan yang tinggi ditemukan pada semua kelompok umur. Oleh sebab itu, studi awal ini menegaskan bahwa susu yang tercemar aflatoksin merupakan masalah serius dalam kesehatan masyarakat di Indonesia.

Kata-kata kunci: aflatoksin M1; transfer aflatoksin; paparan aflatoksin; susu sapi

INTRODUCTION

Aflatoxin is a secondary metabolite generally produced by toxigenic strains of fungi, *Aspergillus flavus* and *A. parasiticus*. Among the group of mycotoxin, aflatoxin B1 (AFB1) is the most toxic and carcinogenic (IARC, 2002). Temperature and moisture in the tropical climate favor the growth of and aflatoxin production by toxigenic strains of molds. A 3-year survey conducted by Rodrigues and Naehrer (2012) indicated high aflatoxin contamination in feedstuffs and feed on the equatorial region.

Aflatoxin was present in 82% of corn samples collected from South Asia and in 71% of samples collected from South-East Asia. Surveys on aflatoxin contamination in feedstuffs have been conducted in Indonesia and reveal high occurrence and levels of aflatoxin contamination (Pranowo *et al.*, 2013; Sumantri *et al.*, 2017).

In lactating animals, consumption of AFB1-contaminated feed will result in excretion of aflatoxin metabolites into milk, especially aflatoxin M1 (AFM1), which is similar to AFB1 and is classified as a human carcinogen by IARC (Kos *et al.*, 2014). The carry-over of aflatoxin from feed and metabolite transfer into milk are very worrying because milk constitutes the main food of babies and children, the two age groups that are most vulnerable to the negative impacts of aflatoxin exposure (Mohammadi, 2011). Aflatoxin exposure on human will cause teratogenicity, immunotoxicity, hepatotoxicity, and mortality. In children, aflatoxin exposure can result in impaired growth (Quintana *et al.*, 2012).

Because of this risk, many countries have imposed regular aflatoxin checks of dairy feed to prevent aflatoxin residues entering the food chain (Viel *et al.*, 2011). Indonesia National Agency of Drug and Food Control (BPOM

has established the maximum AFM1 concentration in milk, namely 500 ng/L (BPOM, 2009). However, only a few surveys were conducted to investigate aflatoxin contamination in dairy milk products in Indonesia and none assessed on the AFM1 intake.

Expanding of middle class in Indonesia leads to strong growing of dairy milk consumption, which is reported 15% increases (GAIN, 2016). Therefore, current survey was conducted to investigate the presences and levels of AFM1 in dairy milk products marketed in Yogyakarta (Indonesia). Also, to estimate AFM1 intake and describe the consumer risk of aflatoxin exposure through consumption of contaminated milk. Yogyakarta city is important in this study due to dairy milk consumption is significantly influenced by age and education level of the consumer (Widiati *et al.*, 2013). This preliminary study on aflatoxin exposure will provide evidence the potential hazard of aflatoxin contaminated milk on food safety in Indonesia.

RESEARCH METHODS

Milk Samples Collection

The sampling was carried out in Yogyakarta Province, namely in Sleman, Yogyakarta, and Kulon Progo Districts. Milk samples were obtained randomly from dairy cooperatives, milk retailers and milk bar in Yogyakarta, that were classified as fresh milk (n=20) and pasteurized milk (16). As a reference, samples of recombined milk products (n=6) from different brands were analysed for the possibility of AFM1 contamination in commercial milk products. All of milk products were produced in Indonesia. As much as 500 mL pasteurized milk samples were homogenized, and 5 mL samples were poured into 5 mL tubes and stored in the freezer before analysis. Recombined milk samples were collected as follow: a packed of re-

combined milk is poured into Erlenmeyer then shaken gently. Five millilitres of homogenized samples were poured into 5 mL tubes and stored in the freezer.

Quantification of AFM1 in Milk Sample

AFM1 concentrations in milk were analysed using Enzyme-Linked Immunosorbent Assay (ELISA) test. Previously, milk samples were defatted by centrifugation at 3000 x g for 10 min at 4° C. As much as 0.4 mL of skim milk was mixed with 0.1 mL of 100% methanol. ELISA assay for AFM1 was performed using ELISA kit AgraQuant® Aflatoxin M1 Sensitive 25/500 (Romer Labs, Singapore).

ELISA was performed as described in the ELISA kit protocols. The absorbance values for standards and samples were measured using an ELISA reader, and AFM1 concentrations were calculated based on a semi-logarithmic equation derived from the standard curve.

Estimation of AFM1 Exposure through Milk Consumption

To estimate the mean intake of AFM1 (ng/kg body weight/day) through milk consumption, an interview using questionnaire was done. The consumers (n=88) were interviewed to obtain information on milk preference, amount of milk consumption per day, body weight, and age. AFM1 intake was estimated by multiplying daily milk consumption with AFM1 content in the milk sample consumed by the respondent. Respondents were between 3-55 years old and selected randomly from consumers in milk bar and milk retailer who agree to be interviewed in this study.

Data Analysis

Milk sampling and respondent interviews were randomly obtained. Data were statistically analysed using IBM SPSS Statistics software version 21.0 (IBM Corp., USA). Mean concentrations of AFM1 between milk types and also mean of AFM1 intakes and AFM1 exposures between age groups were compared using Kruskal-Wallis test considering the samples are heterogenous.

RESULTS AND DISCUSSION

AFM1 Contamination in Various Milk Products

Results indicated 92.5% of milk samples are contaminated with AFM1, with the content ranging from 24 to 570 ng/L (average: 216 ng/L) (Table 1). This demonstrates the high occurrence of aflatoxin residue in dairy milk marketed in Yogyakarta. According to Indonesian national standard, only 2.5% of sample surpassed the maximum regulatory limit (500 ng/L). Mean AFM1 concentration in milk in this study (216 ng/L) was higher than that reported in a previous survey by Nuryono *et al.* (2009), who found the mean AFM1 concentration in fresh milk samples collected from Yogyakarta to be 8.5 ng/L, with no samples with AFM1 levels >50 ng/L.

Although recombined milk products were produced in Indonesia, the dried milk for recombined milk production is most likely imported milk product because there is no local nonfat dry milk production in Indonesia (GAIN, 2017). Refer to the European Union Commission Regulation (EC), 97.5% of the positive sample contained AFM1 > 25 ng/L and 92.5% of positive samples contained AFM1 >50 ng/L (Table 2).

The concentration of AFM1 in milk shows variations according to geographical region, season, environmental conditions, level of development, dairy farming system, and consumption of feed concentrates (Becker-Algeri *et al.*, 2016). In Iran, AFM1 levels in milk were decreased at the end of summer due to more consumption of grass than concentrate feed (Darsanaki *et al.*, 2013). In Thailand, AFM1 concentration in pasteurized milk collected in rainy season is higher than milk samples collected in summer (Ruangwises and Ruangwises, 2009). Study of Widiastuti and Anastasia (2018) on small holder dairy farmer in Lampung and West Java Provinces of Indonesia found that the occurrences of AFM1 contaminations in fresh milk are higher in dry season (40%) than in rainy season (2%) because the use of concentrate feed when fresh grass is limited in dry season.

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Table 1. Occurrence of aflatoxin M1 (AFM1) in various milk products

Milk type	Tested <i>n</i>	Positives <i>n</i> (%)	AFM1 (ng/l)		<i>p</i> -value
			Range	Mean	
Fresh milk	20	90.0	24-449	219	0.224
Pasteurized milk	16	100	100-570	244	
Recombined milk	6	100	99-161	131	
Total	42	92.5	24-570	216	

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Table 2. Frequency distribution of aflatoxin M1 (AFM1) in positive milk samples

Milk Type	Positives <i>n</i>	Frequency <i>n</i> (%)			
		< 25 ng/L	25-50 ng/L	>50-500 ng/L	>500 ng/L
Fresh milk	18	5.6	11.1	83.3	0.0
Pasteurized milk	16	0.0	0.0	93.7	6.3
Recombined milk	6	0.0	0.0	100	0.0
Total	40	2.5	5.0	90.0	2.5

This study revealed that AFM1 concentrations in pasteurized milk served in milk bar and the restaurant was higher than in fresh milk collected from dairy cooperatives. Although recombined milk had the lowest AFM1 concentration compare to fresh and pasteurised milk samples, recombined milk was the most likely source of AFM1 for the consumer as the AFM1 level in all recombined milk samples exceeded 50 ng/L and higher consumption of recombined milk than pasteurised milk in all of respondent groups.

This finding indicated there were no significant different of AFM1 concentration between milk type. Highlight was should be addressed to recombined milk (branded milk products) due to high frequent of AFM1 contamination. However, only six samples of branded milk products were analyzed in this study that is not sufficient to give general conclusion. High frequent of AFM1 contamination in branded milk products was also reported by Wijaya *et al.* (2017) who found AFM1 contaminations in 85% of powdered milk samples and 68% of sweetened condensed milk samples that were collected in several cities in Java. Their survey indicated 55% of powdered milk samples contain AFM1 between 50-200 ng/kg. This evidence was worrying because the main consumer of recombined milk in this

survey was children, an age group that is most vulnerable to aflatoxin exposure (Mohammadi, 2011).

Estimation of Aflatoxin Exposure

Here in, 88 respondents belonged to different age groups, between 3 to 55 years old. The consumers were interviewed to obtain information on milk preference, amount of milk consumption per day, body weight, and age. Estimation of AFM1 exposure for each group of the consumer was calculated based on the amount of daily milk consumption (Table 3) multiplied with an AFM1 concentration in the corresponding milk samples and divided by body weight (Table 4). Calculations indicated high aflatoxin exposure to all of the age groups of milk consumer, especially in age groups 3-5 years old, which had AFM1 exposure more than 5 ng/kg body weight/d (Table 4).

Our calculations indicated high AFM1 exposure to all of the age groups of milk consumer, especially in age groups 3-5 years old, which had AFM1 exposure more than 5 ng/kg body weight/d. High AFM1 intakes were found due to high recombined milk consumption, especially in the age groups of 3-5 year-old. Several works have suggested that dairy milk could be regarded as a hazardous source of AFM1 for children due to

Table 3. Average milk and aflatoxin M1 (AFM1) consumption in different age groups

Age Group (years)	Respondent (n)	Milk consumption(L/d)		AFM1 intake(ng/d)	
		Pasteurized	Recombined	Pasteurized	Recombined
3-5	7	0.0	0.61	0.0	79.6
6-15	11	0.0	0.29	0.0	37.3
16-25	56	0.13	0.37	31.7	48.3
26-55	14	0.20	0.39	49.4	51.2

Table 4. Estimation of aflatoxin M1 (AFM1) exposure through milk consumption in different age groups

Age Group (years)	AFM1 intake (ng/d)		Average body weight(kg)	AFM1 exposure(ng/kg body weight/d)	
	Average	p-value		Average	p-value
3-5	79.6	0.004	15.1	5.26	0.000
6-15	37.3		30.5	1.23	
16-25	80.0		54.7	1.46	
26-55	100.6		64.0	1.57	

the high regular intake of milk in this age group (El-Tras *et al.*, 2011).

Because of the carcinogenic potential of aflatoxins, no admissible aflatoxin daily intake has been specified [27] international expert committees. However, it was concluded that daily aflatoxin exposure of as little as <1 ng/kg body weight contributed to an increased risk of liver cancer (Duarte *et al.*, 2013). Regular intake of a low dose of AFB1 was reported as responsible for the development of liver cancer, kwashiorkor, and impairment of child growth (Bhat *et al.*, 2010).

Exposure to AFM1 poses a threat to human health because its carcinogenic potential is similar to [37] that of AFB1 (IARC, 2002). AFM1 is resistant to heat treatment and acidic conditions in fermented milk; thus AFM1 is found in dairy products (yogurt, cheese, cream or butter) made from contaminated milk (Kos *et al.*, 2014). Several ways have been developed to reduce aflatoxin transfer from feed into the milk. Maki *et al.* (2016) reported feeding adsorbent could reduce AFM1 excretion and concentration in milk.

Annual milk consumption per capita in Indonesia is low (14.3 liters), but considering to income growth and increasing middle class, milk consumption in Indonesia is increasing higher than other regional countries (GAIN, 2016). Therefore, aflatoxin exposure could be higher

than this preliminary study if there is no strict inspection and control in a dairy production system to prevent a high level of AFM1 concentration in milk.

CONCLUSIONS

The frequent occurrence of AFM1 contamination was found in this study, especially in recombined milk which containing AFM1 >50 ng/L. The survey also indicated high consumer exposure to aflatoxin through the consumption of contaminated milk in Yogyakarta. This study could be evidence that contaminated milk is a substantial hazardous source of carcinogenic substance in Indonesia. Therefore, increased effort should be directed toward preventing the AFB1 transfer from dairy feed into milk, as well government and consumer awareness of the aflatoxin risk.

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