Aflatoxin m1 excretion in the milk of tropical dairy cow fed contaminated aflatoxin b1 in the diet[1]

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ABSTRACT: This explorative trial was conducted to study the main characteristics of AFB1 excretion in Indonesian lactating dairy cows. Five Indonesian Frisian Holsteins were received a ration with base of AFB1 intake of 306.5 µg/cow/day. An AFB1 inclusion treatment was designed by crude AFB1 addition to perform levels of inclusion of 0; 50; 100; 250; and 250 µg AFB1/kg of contaminated diet. The contaminated diets were offered for 21 days. The observed parameters were AFB1 intake, AFM1 content, and the proportion of AFB1 excretion into AFM1 in milk. Results indicated that AFM1 rapidly appeared in milk and the content of AFM1 tended to increase with higher level of AFB1 inclusion, namely 44.4; 61.0; 63.2; 66.9 and 70.0 ng/kg for the treatments of 0; 50; 100; 250 and 500 µg AFB1/kg, respectively. The proportions of AFB1 excretions were ranging from 0.08 to 0.20% which was relatively low comparing to the previous studies for sub tropical cows.

Key words: AFB1 contamination, AFM1 excretion, tropical dairy cow

INTRODUCTION

High ambient temperatures and high relative humidity in tropical regions such as in Indonesia are highly favourable for the development of certain fungus to produce mycotoxins**.** Aflatoxin B1 (AFB1) produced by *Aspergillus flavus* and *A. parasiticus* was recognised as the most toxic mycotoxins and exposures of this toxin to human or animal cause chronic or acute consequences. Since AFB1 in feed can be transferred into the milk as its hydroxylated metabolites, aflatoxins M1 (AFM1), the contamination of aflatoxins in animal feeds has became more crucial for most of dairy farms. Testing in animal laboratory indicated that AFM1 has a similar toxicity and carcinogenicity to AFB1 (van Egmond, 1989). The presence of AFM1 may consumption of milk and milk products to be one of the principal ways by which aflatoxin is introduced to human diet (Galvano et al., 1998).

Previous studies on Indonesian agricultural products indicated high occurrences and levels of AFB1 contaminations (Ali et al., 1998; Goto et al., 1999). Those results were confirmed by high occurrence of AFM1 contamination in fresh milk samples surveyed by Nuryono et al. (2009). In their study, all of the samples were found contaminated by AFM1, however none of samples containing the toxin exceeded European Union regulation (50 ng/kg milk). Those findings showed that in the milk of Indonesian dairy cows, the occurrence of AFM1 contamination was supposed high but the levels of contamination was supposed low. Filaeli (2007) surveyed both feed and milk of Indonesian Frisian Holstein and found that the percentages of AFB1 excretion into AFM1 in milk were ranging from 0.08 to 2.69%. In this present study, we carried out an explorative research to determine the effect of levels of AFB1 inclusion to the proportion of AFM1 excretion in the milk of Indonesian dairy cows.

MATERIAL AND METHOD

Five Indonesian Frisian Holstein which were in the mid of lactation stage were used in the experiment. The basal diet was Napier grass and supplemented with 6 kg/cow/day of concentrate

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feed. Every cow received different levels of AFB1 inclusion. Inclusion of AFB1 in the feed was carried out by the addition of crude AFB1 source in the concentrate feed in certain amounts for making the levels of inclusion were 0; 50; 100; 250; and 500 µg/kg. Crude AFB1 was produced by *A. flavus* isolate which was inoculated in the combination of corn and ground peanut as substrate according to the procedure which was described by Ali Agus et al. (2010). The experiment was conducted in 4 weeks which were divided as inclusion period (week 1-week 3) and clearing period (week 4). Cows were milked two times a day in the morning and afternoon. Milk samples from morning and afternoon milking were pooled proportionally and sampled for AFM1 analysis by ELISA test. ELISA tests were performed using ELISA test kits for AFB1 (Ridascreen@ Aflatoxin B1 30/15 R-Biopharm AG, Germany) and AFM1 (Ridascreen@ Aflatoxin M1 30/15 R-Biopharm AG, Germany). Parameters observed in this experiment were AFB1 intake, AFM1 content in the milk, and the proportion of AFB1 excretion into AFM1 milk. Data was analysed descriptively to study the effect of levels of AFB1 inclusion in Indonesian Frisian Holstein (tropical dairy cow) comparing to similar study using sub tropical dairy cow.

RESULTS AND DISCUSSION

AFB1 Intake

ELISA test for AFB1 in the samples of concentrate feed showed that concentrate used in the experiment containing AFB1 51.08 µg/kg. Therefore, consumption of offered concentrate (6 kg/cow/day) contributed on intake of AFB1 equal to 306.5 µg/cow/day. Table below shows the average of AFB1 intake during the inclusion period. The amount of AFB1 intake was counted up from the concentrate and the source of crude AFB1 (inoculated corn-ground peanut).

Table 1. The average of absolute intake of AFB1 in the inclusion period (µg/cow/day)

	Level of AFB1 inclusion (µg/kg)				
Sources of AFB1	0	50	100	250	500
Inoculated corn-ground peanut	0.0	15.2	30.4	75.9	150.9
Concentrate feed	306.5	306.5	306.5	306.5	306.5
Total	306.5	321.7	336.9	382.4	457.3

Excretion of AFM1 in the Milk

AFM1 appeared in the first milk sample (the 2nd day of inclusion period) and still detected until 5 days after AFB1 was removed from the diet. The figure 1 below shows the pattern of AFM1 excretion in milk.

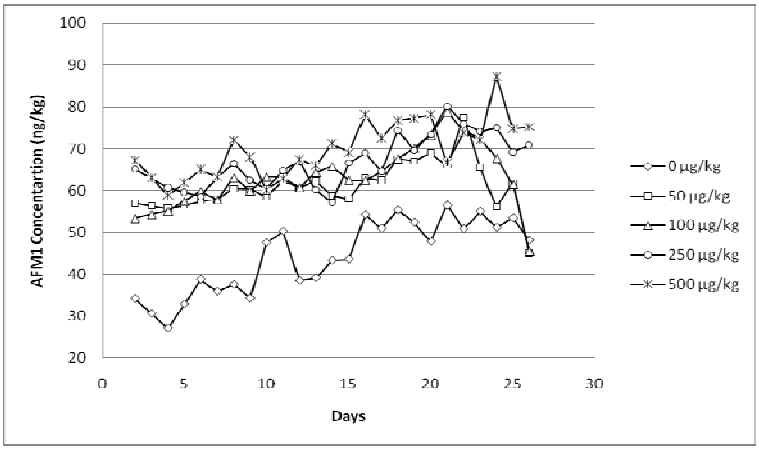

Figure 1. AFM1 excretion in the milk of cows treated with different levels of AFB1 inclusion.

The averages of AFM1 content were 44.4; 61.0; 63.2; 66.9 and 70.0 ng AFM1/kg milk for the levels of AFB1 inclusion of 0; 50; 100; 250 and 500 µg AFB1/kg contaminated feed respectively.

Proportion of AFB1 excretion into AFM1 in milk

The proportions of AFB1 excretion into AFM1 in milk were ranging from 0.08 to 0.2%. The comparison of these percentages between treatments is presented in the following figure:

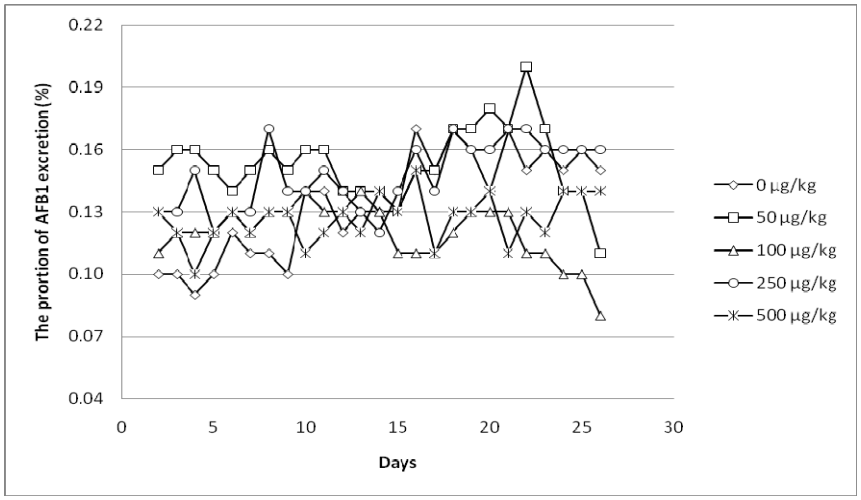

Figure 2. The proportion of AFB1 excretion into AFM1 in milk of cows treated with different levels of AFB1 inclusion

ELISA test showed that the concentrate feed which was used in the trial containing 51.08 µg AFB1/kg and resulted in a base of AFB1 intake of 306.5 µg per day for each cow. This study revealed a difficulty to obtain an AFB1 free diet for dairy cows in Indonesia and might lead to high occurrence of AFM1 contamination in fresh milk sample, such as in survey shown by Nuryono et al. (2009).

AFM1 was detected in the first milk sample after cows consumed contaminated diet. This result was in according to previous studies which indicated that AFM1 appears in the first milk samples after AFB1 ingested by the cow (Diaz et al., 2004; Masoero et al., 2007). Due to its low molecular weight, aflatoxins are rapidly absorbed through membranes by a passive mechanism (Yiannikouris and Jouany, 2002), and could be detected in the plasma as soon as 15 minutes after AFB1 intake (Moschini et al., 2008).

There was no steady state of AFM1 concentrations observed and the concentrations tended to slightly increase during the inclusion period. This result was conflicting to previous studies that showed a steady state of AFM1 concentration was reached in 2-3 days after treatment of AFB1 (van Egmond, 1989).

A higher level of AFB1 inclusion seemed to result in a higher AFM1 concentration in milk. Among the treatments of AFB1 inclusion, only the 0 µg/kg treatment resulted in a concentration of AFM1 (44.4 ng/kg) below the threshold for AFM1 in dairy products regulated by EU (50 ng/kg). Furthermore, this result illustrated a practical situation in Indonesian dairy farming, especially for small holder farmer, which is supposed used feedstuffs contaminated by AFB1 and produces milk contaminated by AFM1.

The proportion of AFB1 excretion into AFM1 in milk in this study was ranging between 0.08-0.2%. Numerous studies had been carried out for sub tropical dairy cow and revealed that the carry over of AFB1 feed into AFM1 in milk to range from 1% to 3% in lactating dairy cows and to be principally affected by milk yield (Diaz et al. 2004; Van Eijkeren et al. 2006; Masoero et al. 2007), with a reported maximum value of about 6% (Veldman et al., 1992). Some cow variables affect on the carry over rate of AFB1 as follows: levels of contamination (van Egmond, 1989), stage of lactation

(Veldman et al., 1992), milk yield (Masoero et al., 2007), species differences (Battacone et al., 2003) and individual variability (van Egmond, 1989).

The cows used in this study were in the mid of lactation period with milk production between 5-11 kg/cow/day. The differences in cow variables and a long experienced in AFB1 exposure seemed to be the main factor in low carry over rate of AFB1 for Indonesian dairy cow.

CONCLUSION

This study confirmed a rapid excretion of AFB1 metabolite in milk after AFB1 ingestion by the cows. A higher AFB1 intake clearly resulted in a higher AFM1 concentration in milk. However, this study indicated a low of the proportion of AFB1 excretion into AFM1 in milk. Differences in cow variables and long term experience in AFB1 exposure might be the reason for low carry over of AFB1 in Indonesian Frisian Holstein compare to the results in sub tropical dairy cow.

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