Excretion of Aflatoxin M1 in milk of goats fed diet contaminated by Aflatoxin B1

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ABSTRACT - An experiment was carried out to study the excretion of aflatoxin M1(AFM1) in milk of three goats fed a single dose (0.8mg/head) of pure aflatoxin B1 (AFB1). The values of AFM1 concentration excreted in milk was highly variable among goats, even if the pattern of excretion over time was very similar among the three animals. AFM1 was first detected at the milking performed 1h after the AFB1 administration. The highest values of AFM1 concentration in milk were reached 3 and 6h after the AFB1 intake. The trend of clearance of AFM1 in milk over time was expressed by a decreasing exponential equation. AFM1 concentration was below the EU maximum allowed level (50 ng/L) in milk collected 36 h after the AFB1 administration.

Key words: Goat, Aflatoxin B1, Aflatoxin M1, Milk.

Introduction - The International Agency for Research on Cancer of WHO (IARC, 2002) includes aflatoxins (AFs) among the substances which are carcinogenic for humans. AFs are the most studied family of secondary metabolites produced by molds, especially *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1) is considered the most toxic compound produced by these moulds and aflatoxin M1 (AFM1), which is its main oxidized metabolite, is excreted in milk when lactating animals are fed AFB1-contaminated feed (De Iongh, 1964).

The European Community has regulated the maximum allowable levels of AFB1 in feed and AFM1 in milk and dairy products. Many studies have dealt with the transfer of AFB1 in milk as AFM1 when lactating animals ingested contaminated feed continuously, especially in cows (Veldman *et al.*, 1992; Masoero *et al.,* 2007). In contrast, little research has been conducted on the transfer of AFM1 into milk as a result of a single assumption of AFB1. From a practical standpoint, the use of highly contaminated feed by dairy farmers is unlikely, however, a single accidental feeding of contaminated feed may happen and can lead to milk AFM1 content above tolerance levels.

The aim of this work was to study the relationship between a single-dose intake of AFB1 and AFM1 excretion in goat's milk.

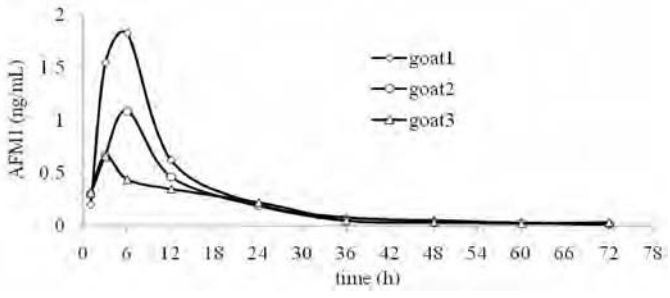
Material and methods - Three multiparous Saanen goats in mid-lactation (average 120 DIM) were used. Daily milk yield and body weight (BW) averaged 3.35±0.47 kg/d and 59.7±0.75 kg (mean ±SD), respectively. Goats were fed 1.5 kg/d per head of a commercial concentrate for lactating small ruminants and grass hay and water *ad libitum* throughout the trial. After the morning machine-milking of the 1st day of the experimental period, the animals were stimulated by a 1-IU i.v. oxytocin

injection, and hand milked to empty the udder. After that, each animal received 0.8 mg of AFB1 in a single dose, *per os*. Pure AFB1 was dissolved in methanol and a concentrate pellet was used as the carrier for aflatoxin (SIGMA®, A-6636, Sigma-Chemical Co, St. Louis, MO). Milk yield was recorded and individual milk samples were collected at 0, 1, 3, 6, 12, 24, 36, 48, 60 and 72h from the administration of the single dose of AFB1. Milk samples were stored at –18°C until the analyses for AFM1 were performed. The extraction of AFM1 from milk was done using an immunoaffinity technique and its concentration was determined by a HPLC chromatograph equipped with a fluorescence detector as described in Battacone *et al.* (2003). The equation of the calibration curve was used to compute the AFM1 content in sample extracts.

Kinetics of AFM1 over time was determined separately for each goat. A simple exponential regression model was used to describe the relation between individual AFM1 concentration in milk and time (h) after the AFB1 administration.

Results and conclusions – In the milk sampled before the AFB1 administration no traces of AFM1 were detected (LOD of analysis method=0.04 ng/mL). The AFM1 presence was already detected in the milk samples collected at the first milking (i.e. 1h after the AFB1 administration) (Figure 1). This suggests that the absorption of AFB1 through the gastrointestinal and its oxidation were very quick processes, as previously found in dairy cows by Moschini *et al.* (2007), who detected AFB1 and AFM1 in plasma as soon as 15 minutes after the ingestion of feed contaminated by AFs. The AFM1 concentration reached its highest value in milk sampled 3 and 6h after the AFB1 ingestion. However, individual variability for the AFM1 concentration in milk sampled within the first 12h after administration was high, even if goats had been given the same amount of AFB1. Previously, a high individual difference on AFM1 concentration was also observed in milk from ewes fed a much higher dose of AFB1 (2 mg/head) (Battacone *et al.,* 2003). Gallo *et al.* (2008) also reported high standard deviation values for AFM1 concentration in plasma of lactating cows submitted to AFs administration through the vaginal implant of a contaminated cotton wad. The large variability observed among goats of the AFM1 concentration in milk sampled within the first 12h could be due to their different individual responses regarding the activation of liver biotransformation pathways of the absorbed AFB1.

Figure 1. Individual excretion pattern of AFM1 concentration in milk of goats fed a single oral dose of AFB1.

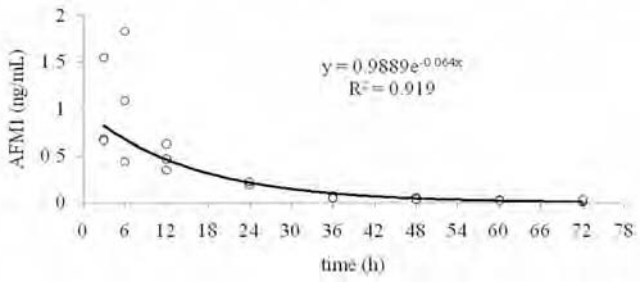


In the three goats, the AFM1 concentration in milk collected at 36h after dosing was below the European Community maximum allowed levels (50 ng/kg). The contamination level in milk sampled at 72h after dosing was near the LOD value of our analytical method. The clearance of toxin in milk was as fast as that reported in a previous study carried out with lactating ewes (Battacone *et al.,* 2003).

From the moment at which the peak of AFM1 concentration was reached, the pattern of its excretion in milk was represented by a decreasing exponential function (Figure 2). This trend was in accordance with the results reported by Battacone *et al.* (2003) for ewes fed a single dose of AFB1 (2 mg/head).

The mean percentage ratio of AFM1 excreted in milk within the 72h to the amount of AFB1 ingested was 0.26%, with a high individual variability. This ratio was markedly higher than that previously observed in lactating ewes (0. 032%; Battacone et al., 2003). This discrepancy could be partially due to

Figure 2. Relationship between the AFM1 in goat milk and the time (h) of milking during the clearance period (3h after the ingestion of single dose of AFB1).



differences in metabolism of AFB1 by the liver microsomal enzymes between these two species (Guengerich *et al.,* 1998).

In conclusion the data of our experiment demonstrated that the AFB1 ingested by lactating goats is quikly transferred to milk as AFM1. The maximum concentration of AFM1 was reached 3-6h after a the single oral administration of pure AFB1. The clearance of AFM1 in milk showed a negative exponential trend and the toxin was no longer detected after 72h from administration. Therefore, an occasional oral assumption of AFB1 can lead to a transient contamination of AFM1 in goat's milk.

REFERENCES - **Battacone,** G., Nudda, A., Cannas, A., Cappio Borlino, A., Bomboi, G., Pulina, G., 2003. Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B1. J Dairy Sci. 86:2667-2675. **De Iongh,** H., Vles, R. O., Van Pelt, J. G., 1964. Milk of mammals fed an aflatoxin-containing diet. Nature, 202:466. **Gallo,** A., Moschini, M., Masoero, F., 2008. Aflatoxins absorption in the gastro-intestinal tract and in the vaginal mucosa in lactating dairy cows. Ital. J. Anim. Sci. 7:53-63. **Guengerich,** F. P., Johnson, W.W., Shimada, T., Ueng, Y.F., Yamazaki, H., Langoueot, S., 1998. Activation and detoxication of aflatoxin B1. Mut. Res. 402:121–128. **IARC,** 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation. IARC Monographs on the evaluation of the carcinogenic risk to humans. Vol. 82. International Agency for Research on Cancer, Lyon, France. **Masoero,** F., Gallo, A., Moschini, M., Piva, G., Diaz, D., 2007. Carryover of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts. Animal, 1:1344–1350. **Moschini,** M., Masoero, F., Gallo, A., Diaz, D., 2007. Mucosal absorption of aflatoxin B1 in lactating dairy cows. Ital. J. Anim. Sci. 6 (suppl.1):324-326. **Veldman,** A., Meijs, J.A.C., Borggreve, G.J., Heeres-van der Tol, J.J., 1992. Carry-over of aflatoxin from cows' food to milk. Anim. Prod. 55:163-168.